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Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.01/A1

Topic: A.03. Stem Cells and Reprogramming

Support: NIH RF1MH128695

Title: Boma app: a user-friendly web-app for brain and organoid manifold alignment

Authors: *P. KUMARAGE[1], X. HUANG[1], S. SANDOVAL[1,2], X. ZHAO[1,3], D. WANG[1,4]; [1]Waisman Center, Univ. of Wisconsin-Madison, Madison, WI; [2]Neurosci. Training Program, Univ. of Wisconsin-Madison, Madison, WI; [3]Dept. of Neurosci., [4]Departments of Biostatistics and Med. Informatics, Sch. of Med. and Publ. Health, Univ. of Wisconsin-Madison, Madison, WI

Abstract: Organoids have emerged as powerful tools for dissecting the intricate cellular and molecular mechanisms governing brain development. Despite their promise, questions persist about whether organoid gene expression patterns closely recapitulate those in the human brain. To address this, we developed BOMA (Brain and Organoid Manifold Alignment, boma.daifengwanglab.org), a web application designed to elucidate developmental trajectories by comparing gene expression between the brain and organoids. BOMA first employs a global alignment across all developmental gene expression data from both brains and organoids and then refines this using advanced manifold learning techniques at a local level. Supporting both single-cell and bulk RNA sequencing data, BOMA not only facilitates the comparison and analysis of gene expression data from diverse sources, such as human/non-human primate brain regions and organoids, but also significantly enhances the efficiency of identifying both conserved and divergent developmental patterns. BOMA app offers an intuitive user interface. Users can either utilize pre-loaded datasets or upload their own, which must be pre-formatted and include a consistent set of genes relevant to the study. Upon dataset loading, users select algorithms and parameters, then initiate alignment using options like dynamic time warping (default) or K-nearest neighbors for global alignment, and various manifold techniques for local refinement (nonlinear manifold alignment is default). Pre-selected defaults simplify use for novices, while still providing customization for experts. Post-alignment, the results are visualized as 3D interactive plots showing the latent space and alignment score, with additional data clustering and distance heatmaps available. Aligned datasets can be downloaded for deeper analysis. BOMA's design streamlines the analysis workflow, empowering researchers to derive valuable insights from their comparative studies of brain and organoid gene expression data.

Disclosures: P. Kumarage: None. **X. Huang:** None. **S. Sandoval:** None. **X. Zhao:** None. **D. Wang:** None.

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.02/A2

Topic: A.03. Stem Cells and Reprogramming

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Title: Rigor and reproducibility of cellular and electrophysiological analysis in human brain organoid research: Metabolic aspects

Authors: *K. KRUTH[1], G. CAPPUCCIO[2], S. O. SANDOVAL[3], X. ZHAO[4], M. MALETIC-SAVATIC[5], A. J. WILLIAMS[6];
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Abstract: In recent years, stem cell-derived brain organoid models have dramatically advanced our ability to investigate the human brain on a molecular level in 3D. However, their use has been hindered by high variability and a lack of reproducibility from one organoid to the next, and even more so between different investigators and protocols. In addition, many studies have reported elevated markers of stress, such as oxidative stress and ER stress, in control organoids, indicating that current culture methods may not be optimal. As a means to address these issues, the multi-institution consortium of Intellectual and Developmental Disabilities Research Centers (IDDRCs) has recently published an in-depth review of suggested methods to improve and standardize culture conditions and quality control methods for brain organoid research. Here, we present a summary of the issues specifically affecting metabolic health in organoids, including medium composition and oxygen levels, and we provide suggestions for monitoring and improving metabolic health in brain organoid cultures. Furthermore, we emphasize that wide

variation in medium composition between protocols likely contributes significantly to the variability and reproducibility issues observed across studies. We therefore propose that brain organoid culture methods should be standardized in the field at large, ideally mimicking physiological conditions, as much as is practically possible. By doing so, we will improve the health, reproducibility, and usefulness of brain organoid culture as an investigative tool for researchers across all fields of neuroscience.

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Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: A.03. Stem Cells and Reprogramming

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Kellett Mid-Career Award
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Title: Rigor and reproducibility of cellular and electrophysiological analysis in human brain organoid research

Authors: *S. O. SANDOVAL[1], K. KRUTH[2], A. J. WILLIAMS[3], M. MALETIC-SAVATIC[4], X. ZHAO[5];
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Abstract: Cortical organoids derived from human induced pluripotent stem cells (iPSCs) have become increasingly useful in the study of human brain development and neurodevelopmental disorders because they resemble aspects of *in-vivo* human brain formation. The most common parameters used to assess functional maturation of cortical organoids include cell proliferation, death, differentiation, lineage specification, morphology, and electrophysiology. Most of the techniques and quantitative framework applied to the analysis of cortical organoids have been derived from studies in embryonic brain tissue or iPSC-derived neuronal cultures. However, unlike the human brain, organoids display vast heterogeneity across iPSC lines, differentiation methods, and across and within differentiation batches, and unlike homogenous 2D neuronal cultures, organoids represent a more complex 3D structure with distinct cell types. With the increasing applicability of organoids in neuroscience research, it is crucial to establish a standardized quantitative framework that allows for rigor and reproducibility within and across laboratories. To address this challenge, together with the IDDRC consortium, we have conducted an in-depth analysis of published quantitative methods for brain organoids. We have recommended minimum and ideal standards for reproducible quantification of organoids. Finally, we have applied some of these methods and recommendations to assess cortical organoids differentiated from iPSCs of individuals diagnosed with fragile X syndrome, the most commonly inherited neurodevelopmental disorder. With an established recommended quantitative framework for organoid research, we expect to increase reproducibility across laboratories, increasing the usefulness of organoids in the study of brain diseases.

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Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: A.03. Stem Cells and Reprogramming

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DOD IIRA grant
Jenni and Kyle Professorship
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Title: Loss of RACK1 regulation by FMRP contributes to electrophysiological and mitochondrial deficits in human Fragile X Syndrome neurons

Authors: C. L. SIROIS[1], M. SHEN[1], ***Y. GUO**[1], Q. DONG[1], N. MENDEZ-ALBELO[1], Y. GAO[1], S. SANDOVAL[1], Z. XU[1], J. E. LEVINE[2], A. M. SOUSA[1], Q. CHANG[1], A. BHATTACHARYYA[1], D. WANG[1], D. M. WERLING[1], X. ZHAO[1];
[1]UW-Madison, Madison, WI; [2]Wisconsin Natl. Primate Res. Ctr., Madison, WI

Abstract: Regulation of hundreds of different mRNAs by Fragile X messenger ribonucleoprotein 1 protein (FMRP) is essential to normal cortical development and function, the loss of which contributes to the etiology of Fragile X Syndrome. While much effort was spent identifying and validating FMRP's many mRNA targets in animal models and non-neuronal cell types, identification of human-specific FMRP targets in disease-relevant subtypes is important for the discovery of translational therapeutic targets. Here, we show that FMRP is important for human and macaque prenatal brain development. FMRP-deficient neurons and FXS patient stem cell-derived neurons exhibit mitochondrial dysfunctions and hyperexcitability. Using multiomics analyses, we have identified both FMRP-bound mRNAs and FMRP-interacting proteins in human neurons. We demonstrate that FMRP interaction with CCR4-NOT transcription complex subunit 1 (CNOT1) maintains the levels of receptor for activated C kinase 1 (RACK1), a species-specific FMRP target. Genetic reduction of RACK1 leads to both mitochondrial dysfunction and hyperexcitability, resembling FXS neurons, and restoration of RACK1 rescues deficits of human FXS neurons. Finally, enhancing mitochondrial functions using a small molecule rescues deficits of FMRP-deficient cortical neurons, demonstrating targeting mitochondrial dysfunction as a potential therapeutic target in FXS.

Disclosures: C.L. Sirois: None. **M. Shen:** None. **Y. Guo:** None. **Q. Dong:** None. **N. Mendez-Albelo:** None. **Y. Gao:** None. **S. Sandoval:** None. **Z. Xu:** None. **J.E. Levine:** None. **A.M. Sousa:** None. **Q. Chang:** None. **A. Bhattacharyya:** None. **D. Wang:** None. **D.M. Werling:** None. **X. Zhao:** None.

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

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Topic: A.03. Stem Cells and Reprogramming

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Autism Science Foundation Postdoctoral Fellowship (to C.L.S.)
UW SCRMC Postdoctoral Fellowship (to C.L.S.)

Title: CGG repeats in the human *FMR1* gene regulate mRNA localization and cellular stress in developing neurons

Authors: *C. L. SIROIS[1], Y. GUO[2], S. SANDOVAL[3], J. LEE[2], M. SHEN[2], A. M. SOUSA[4], A. BHATTACHARYYA[5], X. ZHAO[4];
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Abstract: The human genome has many short tandem repeats, yet the normal functions of these repeats are unclear. The 5' untranslated region (UTR) of the Fragile X messenger ribonucleoprotein 1 (*FMR1*) gene contains polymorphic CGG repeats, the length of which has differing effects on *FMR1* expression and human health, including the neurodevelopmental disorder Fragile X Syndrome. We deleted the CGG repeats in the *FMR1* gene (0CGG) in human stem cells and examined the effects on differentiated neurons. 0CGG neurons have altered subcellular localization of *FMR1* mRNA and protein, and differential expression of cellular stress proteins compared to neurons with normal repeats (31CGG). In addition, 0CGG neurons have altered responses to glucocorticoid receptor (GR) activation, including *FMR1* mRNA localization, GR chaperone HSP90 expression, GR localization, and cellular stress protein levels. Therefore, the CGG repeats in the *FMR1* gene are important for the homeostatic responses of neurons to stress signals.

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Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

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Topic: A.03. Stem Cells and Reprogramming

Support: National Science Foundation CAREER Grant 1749772
R01MH11392
R01NS135763
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Title: Uncovering the structural dynamics of transplanted human neural progenitor cells (NPCs) on log space and time scales with in vivo imaging

Authors: *C. DONEGAN[1], K. PADMANABHAN[2];
[1]Univ. of Rochester Sch. of Med., Rochester, NY; [2]Neurosci., Univ. of Rochester, Rochester, NY

Abstract: Stem cell transplantation therapies are emerging as some of the most promising methods for treating an array of neurological and neurodegenerative disorders where the primary cause of pathology is cell death. Among the most exciting of these approaches is the transplantation of human Neural Precursor Cells (hNPCs) that are derived from human induced Pluripotent Stem Cells (hiPSCs) to replace neuronal loss. For targeted stem cell therapies to be successful, programmed hNPCs must undergo a complex and dynamic set of processes *in vivo* after transplantation, including proliferation, migration, and integration into the existing circuit. Although it is accepted that these processes are critical to the success of hNPC transplantation therapies very little is known about their dynamics, either in patients or in animal models. The ability to track the changes that hNPCs undergo once in the neocortex, *in vivo* over the lifetime of the animal, will provide key insights into the mechanisms governing transplant integration, as well as the functional impact that transplants have on existing circuits. To address this challenge, we transplanted GFP (LV-CMV-GFP) labeled hNPCs and prepatterned human Neurons (hNeurons) reprogrammed from 2 individuals (M = 34 y.o, F = 51 y.o) into P40-P55 SCID mice in the primary visual cortex (R.C: -4mm, M/L: 2.5 mm, D/L = 200-400 um) resulting in transplant of between 15000-20000 cells/animal (N=6 animals). We acquired Z-stacks of engrafted hNPCs at intervals between 24 hours and 1 week/animal (N=15 sessions/animal). We aligned raw Z-stack data across imaging sessions using a rigid body transform of the original image into a rotated and translated image by maximizing the correlation between the image and the image taken in the previous session . We observed substantial plasticity across imaging sessions, including observing cell migration, cell division, and neurite extensions and retractions. We quantified the distance traveled by the cells (0.7 ± 0.7 um/day, N = 51 cells) and observed that hNPCs engrafted into the adult traveled as much as 3.4 um/day. Furthermore, when we imaged individual neurites (N=15) over multiple days, we say extension and retraction events of 150 um/day up to 24 days post transplantation (DPT). Taken together, these data suggest that a critical feature for evaluation of the success of transplantation as a therapy is understanding the structural dynamics of individual transplants; Our multi-photon long term imaging method offers one powerful way to address this question.

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Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

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Topic: A.03. Stem Cells and Reprogramming

Support: Adelson Medical Research Foundation
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Title: Pten and mtor pathway regulation of human neurite outgrowth

Authors: *M. C. CONDRO[1], K. TESSEMA[1], L. ELAHI[2], S. IRVIN[2], R. KAWAGUCHI[2], J. E. LE BELLE[3], D. SAREEN[4], J. MARTINEZ[2], M. F. WELLS[5], R. DAMOISEAUX[2], H. I. KORNBLUM[6];
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Abstract: A challenge in understanding and treating Autism Spectrum Disorder (ASD) is the diversity in underlying genetic and functional mechanisms involved. While no single gene or pathway is responsible for all cases, the PI3K-AKT-mTOR pathway is one that has been linked to ASD, macrocephaly, abnormal neuronal connectivity, and behavior. PTEN is a negative regulator of PI3K activity, and therefore activates mTOR downstream. PTEN mutations have been associated with ASD and macrocephaly, though the mechanism(s) by which this occurs has not yet been determined. Inhibition or deletion of PTEN in rodent models has been shown to increase neurite outgrowth, and was recently reported in human neurons[1]. Here, we used an NGN2 overexpression paradigm to rapidly differentiate human embryonic and induced pluripotent stem cells into neurons. We measured neurite outgrowth by automated image acquisition and analysis and compared neurite outgrowth in cells with both PTEN deletion and mutations. We generated hESC lines in which PTEN is stably knocked out via CRISPR editing. NGN2-induced differentiation in these lines in increased neurite outgrowth on both permissive and inhibitory substrates compared to controls. This increase in neurite outgrowth from deletion of PTEN was also observed in neurons generated from brain organoids. In both *in vitro* systems, the effect of increased neurite outgrowth was abrogated by treatment with rapamycin, indicating that PTEN inhibits neurite outgrowth through the mTOR pathway. Since it is unknown whether monoallelic ASD-associated mutations in PTEN result in complete loss of function, we also examined the role of endogenous *de novo* PTEN mutations in induced neurons derived from iPSC lines from patients diagnosed with ASD and macrocephaly. These naturally occurring mutations also resulted in enhanced neurite outgrowth, an effect that was reversed in isogenic, CRISPR-corrected controls. Our results thus far suggest that PTEN mutations could contribute to ASD at least in part due to increased neurite outgrowth via the mTOR pathway, that may result in aberrant connectivity. [1]Dhaliwal, N. K., et al. 2024. Synergistic hyperactivation of both mTORC1 and mTORC2 underlies the neural abnormalities of PTEN-deficient human neurons and cortical organoids. *Cell reports*, *43*(5), 114173. https://doi.org/10.1016/j.celrep.2024.114173

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Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

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Program #/Poster #: PSTR377.08/A8

Topic: A.03. Stem Cells and Reprogramming

Support: NIH R01HD106197
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NIH P50HD105353

Title: Human stem cell models reveal transcriptional and phenotypic consequences of trisomy 21 in neurogenesis

Authors: *J. G. PICIW[1,2,3], M. CROCKETT[4], J. L. MARTINEZ[1,3], A. BHATTACHARYYA[5,3];
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Abstract: Down syndrome (DS, trisomy 21, T21), the most common genetic cause of intellectual disability, is characterized by reduction in neuron number in the cortex. The fact that fewer neurons are apparent at and before birth suggest impaired neurogenesis is a key feature in DS. However, the mechanisms by which T21 leads to altered neurogenesis are not well understood. We tested the hypothesis that T21 affects human neural development at multiple early stages, with a focus on two key transition states: (1) neural induction, the transition from pluripotency to restricted neuroepithelial state and (2) neurogenesis, the asymmetric division from neural progenitor (NPC) to neuron. To identify the effect of T21 on neural induction, we performed bulk RNA sequencing on isogenic trisomy 21 and euploid iPSCs at the iPSC stage and day 6, 10 and 17 during neural induction. Gene expression analysis revealed many differentially expressed genes in T21 compared to isogenic controls of which a small percentage (<5%) were HSA21 encoded, indicating that T21 causes early genome-wide changes. Analysis reveals early patterns of metabolic dysfunction and heterochronic gene development during neural induction. These results suggest that alterations in key pathways linked to neural fate, including metabolism, WNT, and Notch, may cause altered temporal dynamics. To test the impact of T21 on initial neurogenesis, trisomy 21 and euploid iPSCs were differentiated to early

neural progenitor cells and the balance between progenitor cell maintenance and differentiation was monitored using immunofluorescence at day 1, 3, 5, 7, and 30. Analysis reveals a reduction in SOX2 positive neural progenitor cells across time, with T21 cells showing a steeper rate of reduction. These results suggest an early and significant effect of T21 on the temporal maintenance of neural progenitors during neurogenesis. Results from both from neural induction and early neurogenesis support altered timing of key developmental pathways and processes, suggesting that T21 causes heterochronic neural development ultimately resulting in reduced neuron number.

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Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

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Topic: A.03. Stem Cells and Reprogramming

Support: R01HD106197
P50HD105353

Title: Transcriptomic Analysis of Trisomy 21 Dorsal Cortical Organoids Across in Vitro Development

Authors: *M. JANDY[1], S. KNAACK[2], S. SPIEGEL[2], M. P. CROCKETT[3], M. HOSSEINI[4], K. HANTHANAN ARACHCHILAGE[5], S.-C. ZHANG[6], D. WANG[7], A. M. SOUSA[8], A. BHATTACHARYYA[9];
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Abstract: Individuals with Down syndrome have trisomy of chromosome 21 (T21) and exhibit reduced cortical volume. The cortex is responsible for higher-order processing and memory; therefore, it is believed that this reduced volume leads to the intellectual disability observed in many individuals with T21. As this anatomical phenotype is observed prenatally, the cellular and/or molecular mechanism(s) must be active in one or more cell populations during their development. However, it is not known which cell type(s), when, and how they are affected during corticogenesis. As T21 human prenatal samples are static, induced pluripotent stem cell

models of human development allow us to model corticogenesis *in vitro*. Here we differentiated three pairs of isogenic control and T21 iPSC lines to organoids modeling dorsal cortical development. Organoids were harvested for transcriptomic and immunohistochemical (IHC) analysis at days 30, 60, and 90, representing early to mid-fetal cortical development. Bulk sequencing and single nuclear RNA sequencing identified global and cell type specific transcriptomic changes throughout development, respectively. IHC analyses demonstrate both T21 and control cortical organoids are capable of producing neurons in the expected deep-to-superficial layer sequence, followed by the production of astrocytes. Results reveal genome wide transcriptomic dysregulation in many cell types. We then defined the developmental stage and trajectory of our organoids using the Brain Organoid Manifold Alignment (BOMA) machine learning framework, which maps transcriptomic analyses to in vivo developmental data. These data will inform further investigation into how T21 affects the differentiation of specific cell types, which chromosome 21 genes are dosage sensitive, and how specific developmental pathways are dysregulated to gain a better understanding of how anatomical and cognitive phenotypes arise in T21.

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Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

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Title: High-resolution analysis of synaptic architecture in human trisomy 21 stem cell-derived neurons

Authors: *M. L. RUSSO[1], M. CROCKETT[1], N. WEST[1], S.-C. ZHANG[2], A. M. M. SOUSA[2], A. BHATTACHARYYA[1];
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Abstract: Down syndrome (DS) or trisomy 21 (T21) is marked by intellectual disability that emerges within the first months of life. These cognitive deficits are likely driven by alterations to early neurodevelopment, but the biological substrates that disrupt neurodevelopment in DS are

currently unknown. Prior work suggests that impaired synaptogenesis plays a crucial role in DS-related intellectual disability, as proxies of synapse number are reduced in the brains of infants with DS. However, detailed analysis of synapse development in human T21 neurons has yet to be performed. To fill this gap, we utilized human induced pluripotent stem cells (iPSCs) to model and monitor synaptic development *in vitro*. Using isogenic iPSC lines, which are genetically identical aside from the presence or absence of a third copy of chromosome 21, we tested the hypothesis that synaptogenesis is impaired in T21 neurons using a combination of immunofluorescence and electron microscopy (EM). Neurons were sparsely labeled with lenti-mCherry and immunolabeled for synapsin-1 and PSD95. Neurite length and synaptic density, defined by synapsin-1 and PSD95 co-localization along mCherry-labeled neurites, were quantified using Imaris software. Synaptic ultrastructure was assessed in electron micrographs using ImageJ. We found that T21 and isogenic control neurons develop structurally mature synapses, marked by PSD95 expression and well-defined post-synaptic densities and active zones, by 4 weeks in culture. Preliminary analysis revealed that T21 neurons have reduced neurite length relative to isogenic controls, but we have not observed differences in synaptic ultrastructure or synapse density at this stage. It is possible that synapse deficits are present at earlier and/or later *in vitro* timepoints, and we plan to investigate this possibility with an expanded timecourse analysis using our established pipelines.

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Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

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Topic: A.03. Stem Cells and Reprogramming

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Donation from Agilebio

Title: Network size affects the complexity of activity in human iPSC-derived neuronal populations

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Abstract: Multi-electrode recording of neuronal activity in cultures offer opportunities for understanding how the structure of a network gives rise to function. Although it is hypothesized that network size is critical for determining the dynamics of activity, this relationship in human neuronal cultures remains largely unexplored. By applying new methods for analyzing neuronal activity to human iPSC derived cultures across a range of densities, we uncovered the significant impacts that neuron number has on the individual physiological properties of these cells (such as firing rates), the collective behavior of the networks these cultures formed (as measured by entropy), and the relationship between the two. As a result, simply changing the densities of neurons generated dynamics and network behavior that differed not just in degree, but in kind. Beyond revealing the relationship between network structure and function, our findings provide a novel analytical framework to study networks including those from iPSC-derived neuronal cultures of patient populations where network function may be affected.

Disclosures: R. Santos: None. **C. Marchetto:** None. **K. Padmanabhan:** None.

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.12/A12

Topic: A.03. Stem Cells and Reprogramming

Support: Forska Utan Djurförsök, 2023-0007

Title: Rapid assaying local field potentials and network spiking of human iPSC-brain cells in 96-well format

Authors: *S. ILLES[1,2], E. ARTHURSSON[3], N. JAISUPA[1], T. LYCKENVIK[1], S. THEISS[4];
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Abstract: Combining human induced pluripotent stem cell (hiPSC)-derived brain cells with Microelectrode Array (MEA) technology offers a powerful approach to investigate the electrophysiological properties of human brain cells at both the single-cell and population levels. While spike detection and analysis are the primary readouts in such hybrid sensor systems, we introduces an human iPSC brain cell in vitro model that enables recording of synchronous spiking and local field potentials (LFPs) in short time period and allowing for the detection of distinct compound effects on LFP properties and oscillatory activity (see [1]).

In this poster, we demonstrate the establishment of a 96-well platform capable of measuring and analyzing spiking, LFPs, and oscillatory activity from hiPSC-derived brain cells in a high-capacity format with short experimental timelines. We outline assay quality criteria and provide insights into its utility for assessing physiological and pathophysiological spiking and LFPs in human brain cells. Our electrophysiological hiPSC-based brain cell platform opens new avenues for understanding the mechanisms underlying human brain LFPs and oscillatory activity (see also poster [2, 3]). Additionally, it facilitates the identification of potential harmful impacts of compounds on human brain activity and aids in establishing preliminary drug dosages for clinical trials (see also poster [4].
We provide a translational bridge between animal and human electrophysiology which improves understanding of disease processes, target roles, and compound effects on the full range of the electrophysiological function of human brain cells in a high-capacity format.
References (1) Izsak *et al*. Ontogeny of oscillatory slow-wave and neuronal population activity in human iPSC-3D cortical circuits, 2022, BioRxiv (2) Arthursson, Illes. How ion-concentrations regulate synchronous spiking and local field potential activity in human iPSC-brain cells, poster at SFN-meeting 2024.(3) Lyckenvik, Illes. BrainPhys media causes acute epileptiform activity in human iPSC-brain cell models, poster at SFN-meeting 2024. (4) Jaisupa, Illes. High-capacity assessment of compound effects on modulating epileptiform local field potentials in human iPSC-brain cells, poster at SFN-meeting 2024.

Disclosures: S. Illes: A. Employment/Salary (full or part-time):; Oscillution AB, Gothenburg, Sweden. **E. Arthursson:** None. **N. Jaisupa:** None. **T. Lyckenvik:** None. **S. Theiss:** None.

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.13/A13

Topic: A.03. Stem Cells and Reprogramming

Support: Rosamund Stone Zander Translational Neuroscience Center
BCH ECRAC grant

Title: Machine learning-based approach for quantitative imaging in induced pluripotent stem cell-derived neuronal models of neurodevelopmental disorders

Authors: *Z. YANG[1], F. GASPAROLI[2], N. A. TEANEY[1], R. CHEN[3], K. D. WINDEN[1], W. AFSHAR SABER[1], M. SAHIN[1];
[1]Neurobio., The Rosamund Stone Zander Translational Neurosci. Ctr. (RSZ TNC) - The F.M. Kirby Neurobio. Ctr. – Harvard Med. Sch. – Boston Children’s Hosp., Boston, MA; [2]CITE-Core for Imaging, Technol. & Educ., Harvard Med. Sch., Boston, MA; [3]Human Neuron Core, Boston Children's Hosp., Boston, MA

Abstract: Neurodevelopmental disorders (NDDs) are a group of disorders that affect a child's brain development and cause neurological dysfunction that can lead to lifelong disability or even early mortality. Human induced pluripotent stem cells (hiPSCs) have become a popular model for studying disease mechanisms of NDDs *in vitro*. Calcium Imaging is a functional assay to characterize neuronal activity and network connectivity at a single-cell level and in a non-invasive manner that allows chronic live-cell imaging. We developed a high-throughput platform that combines calcium imaging and machine learning to investigate the neuronal activity of iPSC-derived 2-dimensional (2D) culture. To overcome variability in expression, we generated an iPSC line stably expressing GCaMP6s using the safe harbor locus (AAVS1) and differentiated the iPSCs using the NGN2 differentiation method. We recorded the activity every 10 days, starting at 40 days *in vitro* (DIV) to DIV 90. The image acquisition was accomplished through a combination of the Perfect Focus System with the Nikon Eclipse Ti-2E microscope and a program that automatically selects multiple fields of view in different wells based on the user input, achieving a hands-off, low-effort recording session. The somas in each recording were segmented using a machine learning-based approach. We trained and compared the performance of a UNet2DS model and a Cellpose model with human-labeled calcium images of neurons, and the Cellpose model was preferred in terms of the training efforts, computational demands, and segmentation output. A Python program then quantified the neuronal activities of each labeled cell in different parameters (e.g., calcium transient amplitude, event frequency, and global connectivity, etc.) based on the calcium traces converted from the ΔF/F signal. We tested several algorithms for peak detection to characterize the calcium transients, including the spike library approach and the find_peaks function in the scipy module, and we chose the latter method for its generalized application. Future work includes connecting the automated acquisition and analysis systems more smoothly to establish a high-throughput, objective, and rapid imaging-analysis pipeline. We hope to use this platform to investigate the disease mechanisms of NDDs and perform drug screening for potential therapeutic candidates.

Disclosures: Z. Yang: None. **F. Gasparoli:** None. **N.A. Teaney:** None. **R. Chen:** None. **K.D. Winden:** None. **W. Afshar Saber:** None. **M. Sahin:** None.

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.14/A14

Topic: A.03. Stem Cells and Reprogramming

Support: NRF-2022R1A2C1002925
4120200313623

Title: Ngn2-ipsc based lnss disease modeling by regulation of kras stability

Authors: *E.-J. KIM[1], C. AHN[2], Y. KIM[3], H.-E. LEE[3], S. BAEK[4];
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Abstract: Background: Linear Nevus Sebaceous Syndrome (LNSS) is one of the RASopathies, a condition characterized by cerebral defects and epilepsy and it is caused by somatic mutations in the *KRAS* gene of the RAS/MAPK signaling pathway. In a previous study, we identified major pathological defects that occur during embryonic brain development and neural differentiation caused by $KRAS^{G12V}$ mutation. However, the onset mechanisms of epilepsy, one of the most major clinical symptoms in LNSS patients, remain unknown at the cellular, molecular, and organismal levels and particularly effective treatments beyond surgery still do not exist. Methods and Results: To identify epileptogenic mechanism in LNSS, here 1) First, we introduced Neurogenin-2 (Ngn2) induced pluripotent stem cell (iPSC) which can directly differentiate stem cells to mature neurons with Doxycycline (Dox). These cell lines differentiated into neural progenitor-like cells (NPCs) within 1-2 days of Dox treatment, and into neurons was confirmed on the day 4 of neural induction using MAP2, a mature neuronal marker. Also, These Ngn2-iPSC-derived neurons showed mature regular firing patterns and synchronization within 3 weeks of starting differentiation by measuring using Multielectrode arrays (MEAs). These electrophysiological properties at the network level will enable efficient observation of electrical changes related to epilepsy at the cellular level. 2) Next, we introduced the $KRAS^{G12V}$ gene fused with destabilizing domain (DD), the stability of which is regulated by Trimethoprim (TMP), into Ngn2-iPSCs, allowing the mutation to be reversibly regulated at the protein level. We confirmed the regulation of the pathogenic protein stability in a stabilizing TMP-dependent manner and increase of pERK, a downstream of the RAS/MAPK pathway. Conclusion: Our Ngn2-based LNSS cell model could be efficiently utilized for epileptogenic mechanisms and effective therapeutic strategies of epilepsy in LNSS and other related RASopathies.

Disclosures: E. Kim: None. **C. Ahn:** None. **Y. Kim:** None. **H. Lee:** None. **S. Baek:** None.

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.15/A15

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant NS126399

Title: Progress toward a comprehensive cellular phenotype of CHD2 epileptic encephalopathy

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Abstract: Mutations in the chromatin regulator *Chromodomain helicase DNA-binding 2 (CHD2)* are increasingly found in neurodevelopmental and neuropsychiatric conditions, including epilepsy, intellectual disability and autism spectrum disorders. To accurately recapitulate the early developmental stages of synaptic and neuronal network dysfunction in these patients, direct access to patient neurons is indispensable. Here, we describe a stem cell-based platform for phenotyping *CHD2*$^{+/-}$ human CA1-like hippocampal neurons across genomic, anatomical, electrophysiological and behavioral domains. To identify optimal conditions for producing CA1-like neurons from human induced pluripotent stem cells (hiPSCs), we exposed developing neural progenitors to 13 different combinations morphogens, based on prior efforts to generate human-derived hippocampal neurons (i.e., granule cells and CA3 pyramids). We then screened the cultures for markers of medial pallium progenitors over 15 weeks of neural development after neuron plating. Resulting neural progenitors expressed PAX6, SOX2, FOXG1, POU3F1, DCX and had a significant ~2-fold increase in the CA1-specific transcripts ZBTB20, LEF1 and OTX2, as compared to pan-neuronal neural progenitor cells. By 15 weeks in vitro or following transplantation into dorsal hippocampus CA1 of adult P45 NOD SCID recipients, the resulting neurons expressed markers of CA1, such as CAMKIIa, FIBCD1, CTIP2, OCT6 and WFS1. Expression of calbindin (mouse CA1), GRIK4 (CA3), PCP4 (CA2), PROX1 (DG), GABA (interneurons) or NKX2-1 (medial ganglionic eminence) was very low or absent. Transplanted hCA1 neurons did not migrate far from the injection site, but they integrated into mouse CA1 with anatomical features that are strikingly similar to native born mouse neurons. We next used CRISPR-Cas9 engineering to produce isogenic iPSC lines (iControl, *CHD2*$^{+/-}$ and *CHD2*$^{-/-}$). Detailed transcriptomic, anatomical and electrophysiological analysis of *CHD2* mutations in hCA1-like neurons are currently underway. Together, our work provides an innovative pre-clinical strategy for generating a highly specialized type of human neuron that can be used to identify disease mechanisms and test new therapies.

Disclosures: S. Yoon: None. **R.F. Hunt:** None.

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.16/A16

Topic: A.03. Stem Cells and Reprogramming

Support: ANR-PRC (ANR-22-CE17-0035)
National Institutes of Health (Grant No. R01AG05651)
National Cooperative Reprogrammed Cell Research Groups (NCRCRG;

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National Cancer Institute (Grant No. P30 CA014195)

Title: Bipolar patients-derived neurons are hyperexcitable and show dysregulation in Wnt/β-catenin signaling pathway

Authors: *F. RENARD[1], S. B. LINKER[2], A. MENDES[3], L. RANDOLPH-MOORE[3], C. MARCHETTO[4], F. H. GAGE[5], R. SANTOS[1];
[1]Inst. of Psychiatry and Neurosci. of Paris, Paris, France; [2]Lab. of Genet., Salk Inst. For Biol. Studies, La Jolla, CA; [3]Salk Inst., La Jolla, CA; [4]LOGG, Univ. of California San Diego, La Jolla, CA; [5]LOG-G, Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Bipolar disorder (BD) is a psychiatric mood disorder characterized by manic and depressive episodes that affect 2% of the world population. Lithium (Li) is the first-line long-term treatment for mood stabilization; however, only less than 30% of the patients are responsive to it. The Wnt/β-catenin pathway is essential for hippocampal embryonic development and adult neurogenesis and it was observed that BD patients have consistently smaller volumes of the hippocampus and dentate gyrus (DG) in imaging studies. Using induced pluripotent stem cells (iPSC) derived from BD patients, we generated DG-like neurons. The neurons from Li responsive (LR) and Li non-responsive (NR) patients showed hyperexcitability; however, Li treatment reversed hyperexcitability only in LR neurons, indicating that DG neuronal hyperexcitability correlates with patient clinical information and drug response. Moreover, we found that the activity of Wnt/β-catenin signaling pathway was severely affected, and LEF1 gene expression progressively downregulated over time during neuronal differentiation in NR neurons. Treatment of NR neurons with valproic acid, a drug used to treat patients non-responsive to lithium, upregulated LEF1, increased the transcriptional activity of Wnt/β-catenin signaling pathway and reduced neuronal excitability. Our results suggest that LEF1 may be implicated in lithium resistance in NR neurons.

Disclosures: F. Renard: None. **S.B. Linker:** None. **A. Mendes:** None. **L. Randolph-Moore:** None. **C. Marchetto:** None. **F.H. Gage:** None. **R. Santos:** None.

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.17/A17

Topic: A.03. Stem Cells and Reprogramming

Title: Assay development for the characterization of spine formation and network maturation of iPSC-derived neurons

Authors: *W. LIN[1], K. MURAMATSU[1], S. KUDO[2], S. SHIOMOTO[1], S. NAKAJIMA[1], T. HAZAMA[1], R. YAMOTO[1], T. HOSOYA[1];
[1]Biomed. Business Ctr., Ricoh Company, Ltd., Kawasaki, Japan; [2]Digital Technol. Develop. Ctr., Ricoh Company, Ltd., Ebina, Japan

Abstract: Induced pluripotent stem cell (iPSC)-derived neurons hold much promise for *in vitro* disease modeling and drug discovery. Recent transcription factor (TF)-based differentiation methods achieve accelerated and synchronized neurogenesis, offering the opportunity to address further progression into the late maturation stages of neuronal development. We have previously shown that TF-induced iPSC-derived neurons reached advanced synaptic maturation and spinogenesis in about 70-80 days of culture. These neurons gained postnatal-like features as revealed by RNA-sequencing and immunocytochemistry (ICC), suggesting that they undergo an intrinsic maturation program after neurogenesis. Such mature neurons would be valuable as models to study synapse formation and alterations predictive of drug responses. For this purpose, a further challenge is to establish quantitative methods to assess functional maturation and phenotypic changes in cell-based assays. We report herein two approaches of functional characterization made possible by using mature iPSC-derived neurons.
First, we optimized the mature neuron culture conditions in 96- and 384-well plates for the ICC visualization of proteins enriched in postsynaptic spines such as drebrin and CamKIIα, and we developed an artificial intelligence-based imaging assay of synaptic marker density to facilitate the automated screening of confocal images. Using this method, we demonstrated the feasibility of establishing a dose-response curve to a 10-minute glutamate exposure by detecting the delocalization of drebrin, which is indicative of spine functional maturation.
Second, we cultured iPSC-derived neurons onto high-density multielectrode arrays (HD-MEA) to monitor their activity from week 2 to week 12. The correlation of changes in network burst profiles with an increase in axonal propagation from around week 9, which coincides with the typical period when dendritic spines start to be detected by ICC, allowed us to extract distinctive electrophysiological features associated with network maturity.
Overall, this work contributes insights into the potential of mature iPSC-derived neurons for developing powerful assays that are relevant to human brain functions and cognitive disorders.

Disclosures: W. Lin: A. Employment/Salary (full or part-time):; Ricoh Company, Ltd. **K. Muramatsu:** A. Employment/Salary (full or part-time):; Ricoh Company, Ltd. **S. Kudo:** A. Employment/Salary (full or part-time):; Ricoh Company, Ltd. **S. Shiomoto:** A. Employment/Salary (full or part-time):; Ricoh Company, Ltd. **S. Nakajima:** A. Employment/Salary (full or part-time):; Ricoh Company, Ltd. **T. Hazama:** A. Employment/Salary (full or part-time):; Ricoh Company, Ltd. **R. Yamoto:** A. Employment/Salary (full or part-time):; Ricoh Company, Ltd. **T. Hosoya:** A. Employment/Salary (full or part-time):; Ricoh Company, Ltd..

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.18/A18

Topic: A.03. Stem Cells and Reprogramming

Title: Clinically related rare neurodevelopmental disorders display distinct functional phenotypes in human iPSC-derived cortical organoids

Authors: *V. ALSTAT, A. LACROIX, N. COUNGERIS;
AxoSim, Inc., NEW ORLEANS, LA

Abstract: Rett syndrome (RTT) and CDKL5 deficiency disorder (CDD) are rare X-linked neurodevelopmental disorders with overlapping phenotypic features including cognitive deficits, developmental delays, and seizures. There are currently no disease-modifying treatments available for either disorder. Furthermore, recapitulating pathophysiology *in vitro* has proven difficult for complex disorders like RTT and CDD, as there is a lack of scalable, physiologically relevant screening platforms on the market. We address this need by utilizing our high-throughput screening (HTS)-capable microBrain™ organoid platform to build disease models from CDD and RTT patient-derived induced pluripotent stem cells (iPSCs). We observed striking differences in each disease model in terms of their spontaneous calcium bursting activity profiles; CDD organoids show a hyperexcitability phenotype (increase in calcium peak frequency), while RTT organoids exhibit aberrant calcium oscillations (non-uniform peak heights and peak shape). We verified the reproducibility of these distinct phenotypes across independent batches of organoid plates, showing consistency in the phenotypic fingerprint and strength across organoids, plates, and batches. On this strong foundation, we developed an approach to identifying disease-modifying therapeutic candidates using the high throughput calcium imaging FLIPR assay. Through this work, we screened over 5000 compounds and identified promising biological targets and molecules that rescued the functional phenotypes in each model through unique mechanisms of action. Intriguingly, we saw no overlap in the molecules that rescued each disease phenotype, indicating that our organoid model reflects the clinical independence of the two conditions. Further screening was used to narrow down the top targets and drug candidates and assess any adverse effects in control organoids. Together, this data not only indicates that our microBrain™ cortical organoid platform is amenable to modeling neurodevelopmental diseases, but also suggests the capacity for this organoid technology to accelerate drug discovery and generate compelling preclinical human efficacy data.

Disclosures: V. Alstat: A. Employment/Salary (full or part-time):; AxoSim, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AxoSim, Inc. **A. LaCroix:** A. Employment/Salary (full or part-time):; AxoSim, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AxoSim, Inc. **N. Coungeris:** A. Employment/Salary (full or part-time):; AxoSim, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AxoSim, Inc..

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.19/A19

Topic: A.03. Stem Cells and Reprogramming

Title: Integrative Approaches for Prime Editing Drug Discovery in Repeat Expansion Diseases Using Stem Cell-Derived Neuronal Models

Authors: *N. HAIDER[1], S. KYRYCHENKO[2], S. HERNANDEZ[2], V. CHOU[2], H. HARTLEY[2], J. REICHERT[2], H. GETACHEW[2], H. YANG[2], L. FASCHING[2], Y. ZHOU[2], C. SUN[2], E. ZHENG[3], J. TAY[2], J. DUFFIELD[2];
[1]Prime Medicine, Inc, Arlington, MA; [3]neuromuscular, [2]Prime Med., Cambridge, MA

Abstract: Prime Editing (PE) enables precise correction of upwards of 90% of all human pathogenic mutations, including mutations associated with repeat expansion diseases (REDs). REDs comprise more than 40 disorders, many of which affect the nervous system including Huntington's Disease and Amyotrophic lateral sclerosis (ALS). Prime Editing's ability to precisely make large edits without double strand breaks represents a potential curative approach for patients suffering from REDs. Most REDs lack adequate preclinical models, and the models that are available often do not fully recapitulate disease phenotypes. To advance the development of Prime Editing therapies for REDs, we have established robust physiologically relevant high-throughput *in vitro* neuronal models that recapitulate disease pathology. Induced pluripotent stem cell (iPSC) derived neuronal systems are powerful tools to model neurologic disease. Using patient iPSC lines for three REDs: C9orf72-mediated ALS (n = 3), Friedreich's ataxia (FRDA) (n = 5), and Fragile X syndrome (FXS) (n = 3), we established a set of robust iPSC-derived neuronal models to support PE drug discovery: ChAT+ and ISL1+ motor neurons (iMNs), TUJ1+ and PRFN+ dorsal root ganglia organoids (iDRGs) and FOXG1+, CTIP2+ and vGLUT2+ cortical neurons (iCN). We quantified repeat length, mRNA expression, protein expression for each relevant protein in the respective disease iPSC lines (FXN for FRDA, FMR1 for FXS, and C9orf72 for ALS) and differentiated the iPSCs into relevant neuronal models and demonstrated that these *in vitro* models recapitulate hallmarks of disease pathophysiology including reduced mRNA transcript expression, increased DNA methylation (FRDA and FXS), impaired protein expression and cellular morphology such as axonal growth. To generate preclinical proof of concept models, we generated PE-corrected isogenic lines from patient iPSCs and demonstrated rescue of disease phenotype across 3 different REDs (ALS, FRDA, FXS). For ALS, excision of pathological GGGGCC hexanucleotide repeats in *C9orf72* gene rescued disease pathology (polyGP dipeptide and RNA foci). For FRDA, PE excision of expanded GAA repeats within the frataxin gene (*FXN*) restored hypermethylation of *FXN* to WT levels, rescued the expression of *FXN* mRNA and FXN protein, and restored axonal projections. For FXS, we demonstrated *FMR1* promoter demethylation, *FMR1* reactivation, and FMRP restoration in PE-corrected iPSCs and iPSC-derived cortical neurons harboring the *FMR1* with repeat expansion. Together, these data demonstrate that iPSC-derived neuronal models can be a powerful tool for studying Prime Editing correction of REDs *in vitro.*

Disclosures: N. Haider: A. Employment/Salary (full or part-time):; Prime Medicine. **S. Kyrychenko:** A. Employment/Salary (full or part-time):; Prime Medicine. **S. Hernandez:** A. Employment/Salary (full or part-time):; Prime Medicine. **V. Chou:** A. Employment/Salary (full or part-time):; Prime Medicine. **H. Hartley:** A. Employment/Salary (full or part-time):; Prime Medicine. **J. Reichert:** A. Employment/Salary (full or part-time):; Prime Medicine. **H. Getachew:** A. Employment/Salary (full or part-time):; Prime Medicine. **H. Yang:** A. Employment/Salary (full or part-time):; Prime Medicine. **L. Fasching:** A. Employment/Salary (full or part-time):; Prime Medicine. **Y. Zhou:** A. Employment/Salary (full or part-time):; Prime Medicine. **C. Sun:** A. Employment/Salary (full or part-time):; Prime Medicine. **E. Zheng:** A. Employment/Salary (full or part-time):; Prime Medicine. **J. Tay:** A. Employment/Salary (full or part-time):; Prime Medicine. **J. Duffield:** A. Employment/Salary (full or part-time):; Prime Medicine.

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.20/A20

Topic: A.03. Stem Cells and Reprogramming

Support: 5U01DA054170-03

Title: Modeling cortical brain development using marmoset pluripotent stem cell-derived brain organoids

Authors: *E. DIAZ GUERRA[1,2,3], Y. NUNEZ[1,2], A. FERNANDEZ[1,2], G. MARTINEZ[1,2], C. NAVARA[1,2,3], J. HSIEH[1,2];
[1]Neuroscience, Developmental and Regenerative Biol., [2]Brain Hlth. Consortium, [3]Stem Cell Core, The Univ. of Texas at San Antonio, San Antonio, TX

Abstract: Non-human primate (NHP) models fill a critical gap in the translation of scientific discoveries from rodent models to humans. They offer a closer approximation to human brain anatomy, physiology, and behavior enabling the study of species-specific neurodevelopmental processes and associated disorders such as epilepsy, autism, and intellectual disability. In the last decade, the common marmoset (*Callithrix jacchus*), a small-bodied New World monkey native to South America, has emerged as a promising new NHP model to understand the primate brain. Their small size, short life span, rapid reproductive rate, reaching sexual maturity within a year and producing multiple offspring per litter, and amenability to genetic manipulation make them well-suited for laboratory housing and experimentation compared to larger primate species. However, the lack of a well-established *in vitro* system using marmoset pluripotent stem cells (PSCs) remains a challenge for molecular and cellular analyses in the marmoset brain. To bridge this gap, we are using marmoset PSCs to optimize the generation of marmoset brain organoids.

Specifically, we are generating cortical organoids (COs) and ganglionic eminence organoids (GEOs) to study the development of the cortex and the generation of the interneurons, respectively. Immunostaining of COs at different time points showed typical dorsal cortical features, such as cortical progenitor markers (FOXG1 and PAX6) and layer structure with deeper-layer (TBR1 and CTIP2), upper-layer neurons (SATB2, BRN2, and CUX1) and outer radial glial cells (HOPX). Furthermore, immunostaining of GEOs revealed ventral telencephalic markers (NKX2.1 and LHX6) and inhibitory neurons (GABA, SST, and Calretinin). To recapitulate the inhibitory neuron migration and cortical network activity, we will fuse COs and GEOs to generate cerebral assembloids (CAs) that represent a unique *in vitro* model for studying excitatory and inhibitory neuron interactions. Taken together, the marmoset brain organoid system will provide an *in vitro* platform for probing the impact of genetic mutations and environmental factors on brain development and facilitate translation into humans in neuroscience research and drug discovery.

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Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.21/A21

Topic: A.03. Stem Cells and Reprogramming

Title: Vta-nac assembloids revealed nad$^+$ deficiency in major depressive disorder

Authors: *M. TAO[1], Y. LIU[2];
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Abstract: VTA-NAc assembloids revealed NAD$^+$ deficiency in Major Depressive Disorder. Major depression is characterized by diverse debilitating symptoms, including hopelessness and anhedonia. The VTA-NAc circuit, comprising dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), is implicated in reward and motivation and has been reported to be dysregulated in depression. Despite numerous studies on the VTA-NAc circuit in Major Depressive Disorder (MDD), understanding its pathogenesis remains elusive, partly due to the absence of human models reflecting VTA-NAc circuit deficits for pharmacological investigations in MDD. Here, we developed 3D VTA-NAc assembloids by assembling VTA-like and NAc-like organoids. Synaptic connection with medium spiny neurons (MSNs) was established in NAc organoids and the MSNs showed the optically evoked inhibitory postsynaptic currents (oIPSCs) by labeling the VTA-like organoids with optogenetic virus. Compared to healthy controls, VTA-NAc projections, along with electrophysiological activity and dopamine transport, were significantly altered in sMDD-derived assembloids. Importantly,

by Chimeric organoids analysis, we characterized VTA derived from sMDD as the target region causing the VTA-NAc circuitry deficits. Also, transplantation of VTA-like organoids into the VTA region of mice approved the disrupted projection in sMDD and our data revealed that a decreased projection from VTA to NAc would lead to depressive-like behaviors. Due to ranscriptomic analysis revealed dysregulated NAD^+ related pathway, we then supplemented NAD^+ precursor and restored VTA-NAc circuits deficits, as well as depressive-like behaviors of mice. In summary, our findings highlight the pivotal role of NAD^+ in sMDD pathogenesis, and offer a human model for studying neural circuits and drug discoveries for sMDD.

Disclosures: M. tao: None. **Y. Liu:** None.

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.22/A22

Topic: A.03. Stem Cells and Reprogramming

Support: 1R01DA053372

Title: Is Klinefelter syndrome a synaptogenesis disease?

Authors: *H. ZHAO[1], D. ZHOU[2], G. G. HADDAD[3];
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Abstract: *Klinefelter syndrome (KS)* is the most common sex chromosome disorder and occurs in about 1 per every 650 newborn boys. KS patients are born with extra copies of the X chromosome, which adversely affects testicular growth and produces a reduced amount of testosterone (testosterone deficiency), exhibiting impaired neurocognition, language-based learning difficulties, and executive function. Due to limited studies so far focused on KS brains, our understanding of KS neuropathobiology at the molecular and cellular levels remains largely unknown. In the current study, we use iPSC-derived cortical organoids to model early neuronal development and explore the earliest cellular and molecular changes during KS fetal brain development, aiming to develop optimal therapeutic strategies for KS patients. We generated cortical organoids from KS and healthy iPSC lines and found that KS organoids were significantly smaller than control organoids even though the preparation started with the same number of cells. Further, we found that decreased organoid size was due to a decreased Ki67+ proliferation but not to an increased cell death. During neuronal differentiation, the expression of neural progenitor cells (Nestin), radial glial cells (Pax6), and mature neuronal markers (MAP2) were compared by western blot. Nestin expression significantly decreased in KS organoids and MAP2 expression significantly increased in KS organoids, suggesting a promoted neuronal differentiation and abnormal neurogenesis in KS organoids. RNA-seq showed 1555 differentially

expressed genes (DEGs) in KS organoids, including 630 up-regulated genes and 925 down-regulated genes. Within those genes, 80 genes were located on the X chromosome, including 58 up-regulated genes and 22 down-regulated genes. Gene ontology analysis showed that the significantly differentially expressed genes were enriched in biological processes such as nervous system development, neuron differentiation, and neurogenesis. IPA pathway analysis indicated that neuronal-related signaling pathways are significantly altered in KS organoids, especially in synaptogenesis, where SNARE, glutamate receptor, and CREB signal pathways. We conclude that KS organoids had altered neurogenesis and synaptogenesis as compared with control organoids. Our data provide cellular and molecular evidence for a neurobiological defect in synaptogenesis for the first time, explaining the cognitive deficits in KS patients. Further studies are ongoing to investigate the effect of testosterone on synaptogenesis and neurogenesis during early brain development between the two groups.

Disclosures: H. Zhao: None. **D. Zhou:** None. **G.G. Haddad:** None.

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.23/A23

Topic: A.03. Stem Cells and Reprogramming

Support: Promega Corporation
Pioneer Science/D'Or Institute for Research and Education (IDOR)

Title: Real-time evaluation of psychedelic effects on human neural stem cells using the nanoluc-halotag reporter system

Authors: T. HOANG[1], A. CUNHA[2], H. BORGES[3,4], C. PEDROSA[2], A. SHERWOOOD[5], ***S. REHEN**[2,4,6];
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Abstract: Psychedelics have demonstrated potential in modulating brain plasticity and neurogenesis, offering a novel pathway for therapies in psychiatry and neurology. In this study, we introduced a NanoLuc-HaloTag Reporter System, controlled by neuron-specific promoters, to monitor in real time the effects of specific psychedelic tryptamines on human neural stem cells. We used Adeno-Associated Virus (AAV) vectors to deliver these reporters and, after evaluating multiple AAV serotypes, identified AAV1 and AAV6 as the most effective for transducing human neuronal cells. We detected NanoLuc activity via luminescence assays as early as two days post-transduction. By the fifth day, fluorescence imaging had revealed HaloTag expression, facilitating precise spatial and temporal analyses. Luminescence from NanoLuc demonstrated

changes in synapsin expression, while fluorescence imaging detailed the localization and altered subcellular distribution of FOXG1. These results indicate that psychedelics can affect the differentiation of human neural stem cells in vitro. Our reporter system introduces a novel approach for real-time monitoring of human brain cells as they mature and integrate into neural networks, potentially aiding the screening of therapeutic compounds for neurological and psychiatric disorders.

Disclosures: T. Hoang: A. Employment/Salary (full or part-time):; Promega Corporation. **A. Cunha:** None. **H. Borges:** A. Employment/Salary (full or part-time):; Promega Corporation. **C. Pedrosa:** None. **A. Sherwoood:** A. Employment/Salary (full or part-time):; Usona Institute. **S. Rehen:** A. Employment/Salary (full or part-time):; Promega Corporation. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Usona Corporation.

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.24/A24

Topic: A.03. Stem Cells and Reprogramming

Support: Utah Genome Projects
NS123849

Title: Unveiling neurodevelopmental deficits in patients with MAST1 mutations using human stem cell-derived neurons and brain organoids

Authors: *H. ULLAH[1], K. NAPAN[2], M. SERRANO[8], E. B. TAYLOR[3], A. RICCIARDULLI[4], Y. KIM[5], Z. JIN[6], W. SHEN[9], C. MAGUIRE[10], R. MAO[11], L. BOTTO[7], J. CAREY[4], H. YOST[4], O. SHCHEGLOVITOV[5];
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Abstract: Neurodevelopmental disorders associated with autism, intellectual disability, and cortical malformations are frequently caused by rare genetic abnormalities. Rare *de-novo* mutations in the microtubule-associated serine-threonine kinase 1 (MAST1) have been found in multiple patients with severe intellectual disability and cortical malformations. However, the cellular and molecular mechanisms that are disrupted by MAST1 mutations remain largely unknown. In this study, we identified three patients with rare *de-novo* mutations in *MAST1 and* generated induced pluripotent stem cell (iPSC) derived neurons and brain organoids from

patients, parents, and isogenic CRISPR/Cas9-engineered control stem cells with a patient-specific mutation to study the cellular and molecular deficits associated with MAST1 deficiency. We found that MAST1-deficient organoids were significantly smaller in size as compared to control organoids and exhibited reduced expression of neuron-specific beta tubulin (TUJ1), as well as an elevated expression of an apoptotic marker, cleaved-Caspase-3. We also detected neurite outgrowth deficits in patient iPSC-derived neurons. We investigated the disrupted molecular mechanisms and demonstrated that expression of WT-MAST1 in patient MAST1-deficient neurons rescued the neurite outgrowth deficits. Overall, this study shows that MAST1 is an important regulator of neuronal anatomical development and survival and that the genetic restoration of MAST1 expression is a promising approach for future therapy development.

Disclosures: H. Ullah: None. **K. Napan:** None. **M. Serrano:** None. **E.B. Taylor:** None. **A. Ricciardulli:** None. **Y. Kim:** None. **Z. Jin:** None. **W. Shen:** None. **C. Maguire:** None. **R. Mao:** None. **L. Botto:** None. **J. Carey:** None. **H. Yost:** None. **O. Shcheglovitov:** None.

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.25/A25

Topic: A.03. Stem Cells and Reprogramming

Title: Insights into GLP1R mutation-induced epileptic encephalopathy using patient-specific organoid models.

Authors: *F. BEN-RACHED[1], H. ALMUTAIRI[3], N. STEINER[5], **H. FIUMELLI**[4], P. J. MAGISTRETTI[2];
[1]King Abdullah Univ. of Sci. and Technol., Thuwal, Saudi Arabia; [2]King Abdullah Univ. of Sci. and Technol., Thuwal, ; [4]Biol. and Envrn. Sci. & Engin. Div., [3]KAUST, Thuwal, Saudi Arabia; [5]Dept. of Physiol., Lausanne.

Abstract: This study investigates a rare homozygous splice site mutation in the *GLP1R* gene found in a ten-year-old Saudi patient with epileptic encephalopathy, which results in a profound cognitive delay and recurrent epileptic episodes. The mutation leads to an aberrant, truncated transcript missing exon 4, which causes a reading frame shift and introduces a premature stop codon. The link between this specific variant and the underlying physiopathology is unknown. To address this, we have developed an *in vitro* 3D brain model using induced pluripotent stem cells (iPSCs) derived from the patient for disease modeling. By day 28 of culture, brain organoids originating from these iPSCs exhibited spontaneous formation of external cysts filled with a large volume of cerebrospinal fluid (CSF)-like liquid. Mass spectrometry analysis of the fluid confirmed the production of CSF-specific proteins. Initial cell population analyses showed an enrichment of choroid plexus cells implicated in CSF production, as demonstrated by

quantitative real-time PCR, immunofluorescence assay, and electron microscopy. In parallel, we are generating cortical organoids to examine their electrical activity compared to isogenic controls to decipher the mechanisms at the basis of the patient's epileptic manifestations. Further research is ongoing to provide a comprehensive analysis and characterization of these organoids, focusing on the molecular and cellular mechanisms responsible for the observed pathology.

Disclosures: F. Ben-Rached: None. **H. Almutairi:** None. **H. Fiumelli:** None.

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.26/A26

Topic: A.03. Stem Cells and Reprogramming

Support: NIH R01 NS111986
Eagles Autism Foundation

Title: Elucidating the role of non-imprinted genes in Dup15q syndrome neuronal phenotypes

Authors: *D. ANJAN KUMAR, T. ROBINSON, E. S. LEVINE;
Univ. of Connecticut Hlth. Ctr., Farmington, CT

Abstract: Elucidating the role of non-imprinted genes in Dup15q syndrome neuronal phenotypes Deepa Anjan Kumar, Tiwanna M. Robinson, Eric S. LevineDup15q syndrome is a neurodevelopmental disorder caused by maternal duplication or triplication of chromosome 15q11-q13 region and is characterized by developmental delay, seizures, and autism. Of the duplicated genes in this region, *UBE3A*, which encodes a ubiquitin ligase, is only expressed from the maternal allele in neurons and is thought to be the main contributor for Dup15q syndrome phenotypes. However, overexpressing *UBE3A* alone in mouse models fails to accurately recapitulate behavioral phenotypes, indicating a role for other duplicated genes in the region. By using patient specific neurons derived from induced pluripotent stem cell lines and isogenic CRISPR-corrected control lines, we can gain a better understanding of the pathophysiology underlying this syndrome. We have shown that human Dup15q neurons exhibit a hyperexcitability phenotype characterized by increased action potential firing frequency and altered spontaneous excitatory and inhibitory synaptic activity. Overexpression of *UBE3A* alone fails to mimic all cellular phenotypes in Dup15q, indicating a role for other duplicated genes in this region. Non-imprinted genes in this region include a cluster of $GABA_A$ receptor subunit genes (*GABRB3, GARBA5,* and *GABRG3),* and *HERC2*, another ubiquitin ligase, all of which are associated with neurodevelopmental disorders. To evaluate the roles of these genes, we have normalized the expression of *GABRB3* and *HERC2* in Dup15q neurons using antisense oligonucleotides (ASO) and performed electrophysiological recordings at various developmental time points. Preliminary results indicate a role for *GABRB3* in altered synaptic transmission,

since normalizing the expression of this gene early in neuronal development using ASOs prevented this phenotype. Identifying the roles played by non-imprinted genes in this region is important for developing more effective therapies and for generating improved mouse models of Dup15q syndrome.

Disclosures: D. Anjan Kumar: None. **T. Robinson:** None. **E.S. Levine:** None.

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.27/A27

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant R21EY030727

Title: Emergence of desynchronized electrical activity in xenografted human cortical organoids

Authors: *K. E. HERREMA[1], E. A. MARTIN[2], M. WILSON[3], F. PUPPO[4], T. M. O'SHEA[1], A. MUOTRI[5], D. KUZUM[3], A. DEVOR[1], E. ZELDICH[6], M. THUNEMANN[1];
[1]Biomed. Engin., Boston Univ., Boston, MA; [2]Biol., Boston Univ., Boston, MA; [3]Electrical and Computer Engin., UC San Diego, La Jolla, CA; [4]UC San Diego, La Jolla, CA; [5]Pediatrics/Cellular Mol. Med., UC San Diego, La Jolla, CA; [6]Anat. & Neurobio., Boston Univ., Boston, MA

Abstract: Patient-specific tissue models have the potential to dramatically improve our understanding of neurodevelopmental diseases, providing new insights into biomolecular mechanisms, and giving rise to better therapies. Human cell-derived cortical organoids (hCOs), three-dimensional neural cell aggregates, which resemble the developing human cortex, have emerged as an avenue to model human development and disease. Unlike animal models, hCOs capture human-specific developmental features and can account for complex polygenic contributions to disease phenotypes. Previous work by us and others demonstrated that implanting hCOs into rodent brain leads to xenograft vascularization and facilitates maturation, synaptic connectivity, and integration of human neurons into host neuronal networks. Here, we labeled human, iPSC-derived neurons with GCaMP6s and transplanted healthy hCOs into mouse retrosplenial cortex. We then used a combination of 2-photon imaging and transparent surface graphene microelectrode arrays to perform longitudinal recordings of human neuronal activity in the xenograft. We observed a gradual shift in neuronal activity from low frequency, synchronous surges characteristic of an early fetal developmental stage to higher frequency, desynchronized events, consistent with neuronal maturation and network formation. We also observed that human neurons responded to visual stimulation of the contralateral eye of the mouse. Postmortem immunostaining revealed structural integration of the xenograft, with glial cell migration into host structures and human axonal projections extending to distant regions of the

host brain. Taken together, these results illustrate the feasibility of this longitudinal, multimodal monitoring approach for the investigation of human neurodevelopment in health and disease. Our ongoing study focuses on extending this approach to models of neurodevelopmental disorders, such as Down Syndrome, caused by the triplication of human chromosome 21 (trisomy 21).

Disclosures: K.E. Herrema: None. **E.A. Martin:** None. **M. Wilson:** None. **F. Puppo:** None. **T.M. O'Shea:** None. **A. Muotri:** None. **D. Kuzum:** None. **A. Devor:** None. **E. Zeldich:** None. **M. Thunemann:** None.

Poster

PSTR377

Pluripotent Stem Cells: Developmental Mechanisms and Disease Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.28/A28

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant R15NS131921

Title: Characterization of the CS79iBRCAn2 BRCA1 mutated patient derived stem cell line using an iPSC-BEC model

Authors: *N. ALEXANDER, K. BUCHANAN, B. J. KIM;
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Abstract: BRCA genes are considered tumor suppressor genes and help repair damaged DNA. Pathogenic germline mutations of BRCA genes are the most common hereditary cause of breast cancer and ovarian cancer. It has been established that BRCA mutations increase the risk of brain metastasis compared to the BRCA wildtype, and once metastasis occurs to the brain the disease is considered uncurable. The blood brain barrier (BBB) is essential for maintaining and regulating homeostasis of the central nervous system and is composed of highly specialized brain endothelial cells. Using an induced pluripotent stem cell (iPSC) based model, we characterized an iPSC line, CS79iBRCA-n2, from an invasive cancer patient harboring a BRCA1 mutation. This patient-derived iPSC line can be utilized to study BBB properties as our results show after differentiation into brain-like endothelial cells (BECs), BECs derived from this line express BBB markers such as tight junction proteins, and functional efflux transporters. Future application of patient-derived stem cell models could provide a platform to discover genetic predispositions to BBB disruption in individuals with BRCA1 mutations, as well as the potential molecular mechanisms contributing to brain metastasis.

Disclosures: N. Alexander: None. **K. Buchanan:** None. **B.J. Kim:** None.

Poster

PSTR378

Mechanisms of Dendritic Development and Synaptic Integration in Neurons

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.01/A29

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS084111
NIH Grant NS114914
NIH Grant NS119512

Title: Neurons rely on local PIP_2/ PIP_3homeostasis at ER-PM contact sites to sustain proper dendrite development

Authors: *C.-T. CHIEN, A. CHOI, M. SHELLY;
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Abstract: Phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) is a key phosphoinositide at the plasma membrane (PM) that mediates various downstream signaling which are essential during early neuronal development and specifically, dendrite maturation and arborization. These include the activation of phosphatidylinositol 3,4,5-triphosphate (PIP_3)-dependent Akt signaling, which has been known to play a critical role in dendrite development. In the developing brain, neurons are perpetually exposed to diffusible extracellular cues, including neurotrophic growth factors that trigger the use of $PI(4,5)P_2$, resulting in its rapid hydrolysis and depletion. Currently, little is known about how neurons regulate local $PI(4,5)P_2$ homeostasis to enable sustained $PI(4,5)P_2$/ PIP_3 signaling at subcellular compartments, such as dendrite branching points and dendritic tips, during early developmental stages. Recent studies conducted primarily in cell-lines demonstrated that the lipid transport protein, Nir2, is a key regulator of PM $PI(4,5)P_2$ replenishment and Akt signaling upon intense external cue stimulation by rapidly transporting specific lipid intermediates at ER-PM contact sites to accommodate the phosphatidylinositol (PI) cycle. We thus propose that local lipid transport mediated by Nir2 at ER-PM junctions is critical for maintaining PM $PI(4,5)P_2$ homeostasis and PIP_3-dependent Akt signaling, ultimately regulating dendrite growth and maturation during early neuronal development. Using short hairpin RNA (shRNA) to knockdown Nir2 expression through *in utero* electroporation, we observe severe defects in dendrite development, elongation and branching. Combining the genetically encoded PIP_3 biosensor, Akt-PH, with total internal reflection fluorescent (TIRF) microscopy, our data show that PM PIP_3 availability upon stimulation with the neurotrophin brain-derived neurotrophic factor (BDNF) is reduced following Nir2 knockdown (KD) in cultured cortical neurons, implicating shrinkage in the $PI(4,5)P_2$ pool. We further observed robust Nir2 recruitment to the ER-PM junctions as distinct puncta upon BDNF stimulation, indicating that Nir2 responds to PM $PI(4,5)P_2$ depletion and specifically translocates to ER-PM contact sites to facilitate local lipid exchange. Together, our study demonstrates that Nir2 regulates dendrite development via local $PI(4,5)P_2$ replenishment and subsequent PIP_3-Akt signaling in developing neurons.

Disclosures: C. Chien: None. **A. Choi:** None. **M. Shelly:** None.

Poster

PSTR378

Mechanisms of Dendritic Development and Synaptic Integration in Neurons

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.02/A30

Topic: A.05. Axon and Dendrite Development

Title: Developmental changes of synaptic inputs to Layer 5 pyramidal tract neurons in the mouse barrel cortex

Authors: *T. BABA[1], S. FUJIMOTO[1], M.-T. KE[2], T. IMAI[1];
[1]Kyushu Univ., Fukuoka, Japan; [2]Riken Ctr. For Developmental Biol., Hyogo, Japan

Abstract: Higher brain functions are acquired during adolescence. However, it remains unclear which specific circuits develop during adolescence. It has been generally believed that synapse elimination is dominant during this period. However, we found a dramatic increase in spine density in the apical dendrites of Layer 5 pyramidal tract (L5PT) neurons in the barrel cortex, suggesting that spine formation, rather than elimination, is more critical for cortical development at this stage.In this study, we investigated the sources of synaptic inputs to the newly formed spines during adolescence at the apical dendrites of L5PT neurons. L5PT neurons receive inputs from both cortical and thalamic neurons. We first investigated developmental changes of these inputs using antibodies against vesicular glutamate transporter (VGluT) 1 and 2: VGluT1 and VGluT2 antibodies label pre-synaptic terminals of cortical and thalamic axons, respectively. We found that the ratio of cortical to thalamic inputs (~1:3) does not change significantly during adolescence.Next, we considered two candidates for the increased presynaptic inputs: i) a specific layer within the barrel cortex, and ii) a long-range projection from specific brain areas. To investigate inputs from other layers of the barrel cortex, we introduced a presynaptic marker, synaptophysin-mRuby3, into Layer 2/3 using *in utero* electroporation. Synapse formation to L5PT neurons was evaluated, which was confirmed by colocalization of synaptophysin-mRuby3 and anti-Homer1 in EYFP-expressing L5PT neurons. We found a significant increase in inputs from Layer 2/3 neurons during adolescence.To identify candidate long-range inputs to L5PT neurons in the barrel cortex, we injected AAV-retro expressing tdTomato into the barrel cortex and analyzed labeled neurons comprehensively. We also referred to the Allen Mouse Brain Connectivity Atlas. AAV-retro labeled 19 cortical regions, some of which are not listed in the Allen Atlas. So far, we have identified some areas whose long-range synaptic inputs to L5PT apical dendrites increase during adolescence. For example, projections from the primary motor cortex and the posteromedial complex (POm) of the thalamus were found to be 5-10 times greater than what would be expected based on random synaptic connectivity probabilities. Thus, these brain regions may play important roles in establishing higher cognitive functions involving

the somatosensory cortex. Our approach focusing on the barrel cortex helps to understand adolescent cortical development at the circuit level.

Disclosures: T. Baba: None. **S. Fujimoto:** None. **M. Ke:** None. **T. Imai:** None.

Poster

PSTR378

Mechanisms of Dendritic Development and Synaptic Integration in Neurons

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.03/A31

Topic: A.05. Axon and Dendrite Development

Support: NIH R01NS086082

Title: Mirnome analyses reveal cell-type-specific requirements of micrornas in dendritic patterning and behavior in Drosophila somatosensory neurons

Authors: E. KAUFMAN, S. SAKHALKAR, S. BHATTACHARJEE, A. A. PATEL, F. CIGER, T. N. TRAN, M. N. CENTER, A. SAKURAI, *D. N. COX;
Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: The maintenance of proper synaptic connections by neurons is essential for the appropriate functioning of cognition and behavior. microRNAs (miRNAs) are a class of small noncoding RNAs that together with transcription factors, chaperonins, and ribosomal proteins regulate the neuronal cytoskeleton. The study of multi-dendritic (md) neurons of the *Drosophila melanogaster* peripheral nervous system has been insightful in providing essential information about the mechanisms regulating cell-type-specific cytoskeletal organization across different neuronal subtypes giving rise to distinct dendritic arborization architectures. Cell-type-specific miRNA expression profiling revealed differential expression of miRNAs across these md neuron subtypes. These differentially expressed miRNAs were then functionally validated through a neurogenetic phenotypic screen by overexpressing individual miRNAs in neuronal subtypes. Our analysis identified several miRNAs with previously unknown functions in dendritic development, providing novel insights into the molecular differences underlying neuronal type-specific dendritic arborization. An in-depth analysis of select miRNAs such as *miR-316* and *miR-1000* revealed that these miRNAs function in restricting dendritic arbor complexity. Overexpression of these miRNAs in each neuronal subtype led to dendritic hypertrophy, whereas overexpression of *miR-12* had a more sub-type-specific effect on dendritic arborization, leading to an increase in complexity in CIII neurons while restricting complexity in CIV neurons. We developed an integrative miRNA target prediction tool, IntramiRExploreR, to identify targets of intragenic miRNAs and identified widerborst (wdb), a subunit of PP2A enzyme as a target of *miR-12*. We explored structure-function relationships between dendritic architecture and sensory behavior which revealed that miRNA-mediated dendritic hypertrophy does not necessarily correlate with heightened behavioral sensitivity as measured by calcium imaging,

electrophysiology, and behavioral analyses. These results demonstrate the complex roles played by miRNAs in the regulation of dendritic architecture and behavior, further highlighting the need to study these molecules and determine how dysregulation in the network could lead to neurological disorders.

Disclosures: E. Kaufman: None. **S. Sakhalkar:** None. **S. Bhattacharjee:** None. **A.A. Patel:** None. **F. Ciger:** None. **T.N. Tran:** None. **M.N. Center:** None. **A. Sakurai:** None. **D.N. Cox:** None.

Poster

PSTR378

Mechanisms of Dendritic Development and Synaptic Integration in Neurons

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.04/A32

Topic: A.05. Axon and Dendrite Development

Support: DP1-MH119428

Title: Noncanonical Intercellular Communication in the Brain via Nanotubular Bridge Network

Authors: *M. CHANG[1,2], S. KRUESSEL[2], L. K. PARAJULI[4], J. KIM[3], S. OKABE[4], H. KWON[2];
[2]Neurosci., [3]Psychiatry and Behavioral Sci., [1]Johns Hopkins Univ., Baltimore, MD; [4]Cell. Neurobio., Univ. of Tokyo, Tokyo, Japan

Abstract: Recent investigations have shed light on the unique biology of filopodia, forming a bridge-like structure known as a nanotubular bridge (NB). These nanotubes create an additional layer of interconnected networks that facilitate intercellular material exchange. However, the intricate network remains largely unexplored in neurons due to technical challenges in discerning its infinitesimal anatomy within the brain's canonical neuropil. We developed dSRRF, a super-resolution microscopy technique capable of accurately delineating the morphology of dendritic protrusions. Employing this method, we successfully characterized atypical filopodial contacts, distinguishing them from neurites based on their molecular composition and plasticity in dissociated neurons. Experimentally induced increases in Ca^{2+} concentration in a single neuron propagate to distant neurons over several tens of micrometers non-synaptically, suggesting an NB-mediated calcium propagation mechanism. Utilizing imaging and machine-learning-based analysis, we confirmed the *in situ* presence of NBs connecting dendrites to other dendrites between cortical layer 5/6 pyramidal neurons in mice brains, distinguishing them from synaptic spines. Moreover, experiments involving the Infusion of human amyloid-beta (Aβ) into a single neuron via whole-cell patch-clamping demonstrated specific active transport of the peptide to neighboring cells along dendrites and NBs, implicating the NB network in Alzheimer's disease (AD) pathology. Intriguingly, we observed elevated NBs prior to the onset of amyloid plaque deposits in the medial prefrontal cortex (mPFC) of 3-month-old APP/PS1 mice. However, in the

6-month-old brain, numerous neurons with high Aβ accumulation exhibited impaired NB formation. Computational simulation based on NB network modeling elucidated how its over-activation leads to cellular amyloidosis, providing insight into the neuronal mechanisms underlying neurodegeneration of this newly discovered nanotubular connection.

Disclosures: M. Chang: None. **S. Kruessel:** None. **L.K. Parajuli:** None. **J. Kim:** None. **S. Okabe:** None. **H. Kwon:** None.

Poster

PSTR378

Mechanisms of Dendritic Development and Synaptic Integration in Neurons

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.05/A33

Topic: A.05. Axon and Dendrite Development

Support: FAPESP 2023-01789-9
Cnpq Capes 2021/06009-6

Title: Oubain effects on mitochondrial function and dendrite development in primary cortical neurons

Authors: *V. ARAUJO[1], *V. ARAUJO[2], A. SIENA[3], C. SCAVONE[3];
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Abstract: Mitochondria has a central role during neurodevelopment once it is responsible for ATP synthesis, intracellular calcium levels, and the management of reactive oxygen. In that context, its inhibition is capable of damaging neuronal development and is associated with neural disorders phenotypes, such as schizophrenia. With that in mind, this work intends to understand how Ouabain (Oua), a steroid related to the activation of neural protective signalling pathways, can interact with mitochondrial activity. To achieve that, we treated the primary cortical neuronal culture with Oua (10uM) for 2 hours. Then, we analysed the cellular viability by MTT assay (n=7); the oxygen consumption rate by Seahorse equipment (n=6); and the dendritic arborization by immunofluorescence labelling MAP2 (n=2). We saw that the Oua exposure did not change the neuronal viability (p = 0,613). However, we found a reduction in the basal respiration (p = 0.0053; 43.20 11.08%), the maximal respiration (p = 0.0338; 58.73 11.90%), the spare capacity (p = 0.0002; 38,73 6.911%) and ATP-linked respiration (p < 0.0001; 28.65 8.644%) when compared to the vehicle group (100%). Also, the Oua treatment was responsible for reducing the number of dendrites per neuron in 6.50.84 (p = 0.0002) and reducing the dendrites's size in 51.7 7.156m (p = 0.0038). With that data, we can conclude that Ouabain can affect mitochondrial function negatively and directly influence the development of dendrites in cortical neuronal cells. Study supported by FAPESP 2023-01789-9; 2021/06009-6.

Disclosures: V. araujo: None. **V. Araujo:** None. **A. Siena:** None. **C. Scavone:** None.

Poster

PSTR378

Mechanisms of Dendritic Development and Synaptic Integration in Neurons

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.06/A34

Topic: A.05. Axon and Dendrite Development

Support: NIH R01NS086082
2CI Neurogenomics Fellowship
GSU Brains & Behavior Seed Grant

Title: Ras gain-of-function mutation leads to dendritic hypertrophy in Drosophila melanogaster larval sensory neurons

Authors: *F. CIGER, R. A. M. IBRAHIM, T. RIOS, D. N. COX;
Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: Ras signaling pathway is a major regulatory pathway in cells that is involved in various processes such as cell proliferation, cell growth, gene expression regulation, cell-cycle arrest, and apoptosis. Ras' role in proliferation, cell-cycle arrest, and apoptosis have been widely studied as mutations in Ras pathway genes are reported in cancer patients. Mutations in this signaling pathway are also implicated in Rasopathies which are rare developmental disorders that lead to intellectual disabilities. Thus, it is important to understand how Ras affects cells of the nervous system. Although the role of Ras in spine development and neurite extension are well studied, a consensus is lacking on how it affects dendritic development. Ras is a small GTPase that is active in its GTP-bound state, and inactive in the GDP-bound state. Ras mutations linked to Rasopathies change the conformation of Ras and keep it in a constitutively active (CA) GTP-bound state, causing hyperactivation of Ras signaling. Drosophila melanogaster (Dmel) larval multi-dendritic (md) sensory neurons provide a powerful model for elucidating role(s) of Ras in dendritic development allow various genetic manipulations and can be imaged in vivo using confocal microscopy. These neurons are divided into four classes and have varying degrees of dendritic complexities with Class I (CI) neurons being the least complex, and Class IV (CIV) neurons being the most complex. *Dmel* larvae have two paralogs of Ras which are Ras85D and Ras64B. Expressing CA versions of Ras85D leads to increases in total dendritic length and number of branches in CI and CIV neurons. These increases in dendritic complexity are observed throughout the dendritic field in CIV neurons, however it is more distal to the soma in CI neurons. Expressing CA Ras64B leads to similar changes, although, the branch density is higher in CIV neurons expressing CA Ras64B compared to both control and CA Ras85D, whereas all the other measured dendritic parameters show higher increases in complexity for CA Ras85D in both classes of neurons. Additionally, CA Ras85D expressing CIV neurons show

defects in dendritic tiling, with dendritic branches crossing over to the adjacent neuron of the same class. This tiling defect is not as highly observed in CA Ras64B expressing neurons. Lastly, CA Ras85D leads to an increase in microtubule levels throughout dendrites of CIV neurons. Collectively, Ras is an important regulator of dendritic development that affects branching in two different classes of neurons. Future studies will reveal the mechanisms and downstream effectors through which Ras exert its effects on the dendrites.

Disclosures: F. Ciger: None. **R.A.M. Ibrahim:** None. **T. Rios:** None. **D.N. Cox:** None.

Poster

PSTR378

Mechanisms of Dendritic Development and Synaptic Integration in Neurons

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.07/A35

Topic: A.05. Axon and Dendrite Development

Support: NIH R01NS086082
GSU Brains & Behavior Seed Grant

Title: Roles of ribosome heterogeneity and local protein translation in regulating cell-type-specific dendritic architecture

Authors: *S. BHATTACHARJEE, F. CIGER, M. CENTER, E. N. LOTTES, D. N. COX; Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: Proper functioning of cells requires temporal and spatial control and maintenance of the proteome, achieved through a large consortium of cellular processes that form part of the proteostasis network (PN). The translation machinery includes the ribosome and associated proteins that form an integral part of the PN. Until the 1990s, ribosomes were thought to be homogeneous protein synthesis organelles that were not selective in which proteins they translated. However, emerging evidence from several organisms, including *Drosophila*, has challenged this dogma giving rise to the concept of specialized ribosomes which posits that ribosomes are, in fact, heterogeneous in composition and this heterogeneity regulates which mRNAs are actively translated. While the significance of local protein translation in neurons has primarily focused on their physiological roles in memory formation and synaptic plasticity, there remains a critical gap in our understanding of the role of local translation in regulating diverse neuronal architectures. Immunohistochemical (IHC) analyses show differential composition of the ribosome both within a cell-type as well as between neuronal subtypes. To better ascertain the requirement of ribosomal proteins in regulating dendritic morphology, we conducted a neurogenetic RNAi knockdown (KD) screen of a subset of 30 RPs in CI and CIV multi-dendritic (md) neurons subtypes in *Drosophila*. While most RP knockdowns exhibited dendritic defects in both CI and CIV neurons, select RPs showed cell-type-specific dendritic requirements. Further, loss of RPs that affected dendritic morphology, also led to defects in ribosome trafficking and

localization. Previous studies have shown that stoichiometric differences in ribosomal RPs can determine which pool of mRNAs are preferentially translated. We previously characterized the role of the cytoskeletal modulator Formin 3 (Form3) in regulating dendritic morphology in CIV md neurons. Loss of *form3* leads to severe reduction in dendritic morphology similar to that observed for the knockdown of *RpL7* and *RpL36A*. IHC analysis revealed that compared to control, *RpL7* KD leads to a significant reduction in Form3 protein levels while *RpL36A* KD did not. Collectively, these data provide evidence of differential RP requirements in regulating dendritic morphological diversity, as well as distinct roles of RPs in preferentially translating certain mRNAs compared to others (*e.g.* Form3), and in regulating ribosomal trafficking and global protein translation.

Disclosures: S. Bhattacharjee: None. **F. Ciger:** None. **M. Center:** None. **E.N. Lottes:** None. **D.N. Cox:** None.

Poster

PSTR378

Mechanisms of Dendritic Development and Synaptic Integration in Neurons

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.08/A36

Topic: A.05. Axon and Dendrite Development

Support: NIH grant GM118331
Royal Society Grant RG16955

Title: Loss of the ubiquitin ligase CUL9 leads to neuronal arborization defects and aberrant dopaminergic signalling

Authors: *E. HOLLVILLE[1,2], J. OBERHAUSER[3,2], S.-H. LEE[4], Y.-Y. I. SHIH[4], J. SIMON[5,2], S. S. MOY[6], M. P. DESHMUKH[7];
[1]Univ. of Aberdeen, Aberdeen, United Kingdom; [2]Neuroscience Center, University of North Carolina at Chapel Hill, Chapel Hill, NC; [3]Univ. of California San Francisco Neurosci. Grad. Program, San Francisco, CA; [4]Dept. of Neurol., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [5]Harvard T.H. Chan Sch. of Publ. Hlth., Boston, MA; [6]Psychiatry, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [7]Neurosci. Ctr., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Cullin ubiquitin ligases are emerging risk factors for neurodevelopmental and neuropsychiatric disorders. In the latest genome-wide association study, *CUL9* was reported as a high confidence risk gene for schizophrenia. The function of CUL9 is however largely under-characterized, particularly in the brain. We therefore sought to determine whether CUL9 influences neuronal development and function. Mice carrying a deletion of exons 2-7 of the *Cul9* gene (*Cul9* KO, C57BL/6 background) and their littermate controls (wildtype - WT) were used to investigate the effect of *Cul9* loss on neuronal morphology, behaviour, molecular signalling

and resting-state functional connectivity. To investigate whether CUL9 affects dendritic architecture, we performed morphological reconstruction of somatosensory L5 pyramidal neurons (Thy1-YFP, H x WT vs *Cul9* KO, males aged 2 months, n = 19/20) and observed that *Cul9* deficiency results in reduced dendritic length and arbor complexity. To examine the importance of CUL9 for brain function, we analysed the behaviour of WT and *Cul9* KO adult mice (aged 3-7 months, males n = 9/10, females, n = 9/10) and found that the effect of *Cul9* loss was particularly exacerbated in males, leading to an increase in motor activity and anxiety, and a reduction in social interactions. To uncover the molecular function of CUL9 in the brain, protein extracts from cortices of WT and *Cul9* KO mice (males aged 3-5 months, n = 6) were analysed by quantitative mass spectrometry (LC-MS). We observed an enrichment in dopaminergic signalling-associated proteins downregulated in the absence of *Cul9*. Consistently, *Cul9* KO males (2-5 months) displayed a blunted response to amphetamine-mediated locomotor sensitization (3.0 mg/kg, n = 6/10). To further investigate the essential neural networks affected by *Cul9* loss, a cohort of WT and *Cul9* KO males (aged 2.5-4.5 months, n = 8) were subjected to resting-state fMRI. Pair-wise and network analysis revealed that *Cul9* KO animals display increase resting-state functional connectivity within a subnetwork particularly associated with dopaminergic signalling: the amygdala - basal ganglia subnetwork. Altogether, our results indicate that CUL9 is required for normal neuronal morphogenesis and for normal dopaminergic signalling, connectivity and associated behaviours. Our results have implications for understanding the dopaminergic dysfunction observed in schizophrenia patients and raise the question of the molecular networks controlled by CUL9-mediated ubiquitination in neurons.

Disclosures: E. Hollville: None. **J. Oberhauser:** None. **S. Lee:** None. **Y.I. Shih:** None. **J. Simon:** None. **S.S. Moy:** None. **M.P. Deshmukh:** None.

Poster

PSTR378

Mechanisms of Dendritic Development and Synaptic Integration in Neurons

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.09/A37

Topic: A.05. Axon and Dendrite Development

Title: How come neurons come in such different sizes? Because they can, while constrained by their different levels of activity

Authors: L. XU[1], ***S. HERCULANO-HOUZEL**[2];
[1]Psychological Sci., Vanderbilt Univ., Nashville, TN; [2]Vanderbilt Univ., Nashville, TN

Abstract: Neurons in a single brain come in an enormous range of cell sizes. Our current working model to account for such enormous size variation in neurons considers that differentiating neurons have different levels of excitability, which leads to the prediction that, under supply-limiting circumstances, the more excitable a differentiating neuron is, the less it

will be able to grow. Conversely, less excitable differentiating neurons should afford to grow to larger sizes, and become larger neurons simply because they could, growing to exhibit low firing activity despite having less competition for blood supply. In this study, we explore two datasets of matching electrophysiological and morphological data from single excitatory and inhibitory neurons in the human cerebral cortex, curated with similar methods, to test the hypothesis that larger neurons have progressively lower firing rates as predicted. We discover that larger neurons indeed have progressively lower firing rates in ways that compensates for their elevated membrane capacitance, resulting in activity at a size-invariant power. Importantly, we find that excitatory and inhibitory neurons occupy two separate, non-overlapping spaces along a continuum of variation in several morphological and electrophysiological properties that scale uniformly across these cells. Human excitatory and inhibitory cortical neurons have similar total neurite length and surface area within the grey matter, which is however distributed differently between the two cell types: the same total neuritic length and surface area is distributed between dendrites and axon in an 80/20 proportion in excitatory neurons, but nearly 1/3 to 2/3 proportion in inhibitory neurons. Electrophysiologically, the one feature that qualitatively distinguishes the two cell types is the relationship between membrane capacitance and resistance, even though the product of these two features, which is the time constant, remains similar between neurons of the two types. The calculated energy cost per spike is universally predicted by dendrite surface area, agnostic of cell type, while the power for recharging the membrane (firing rate times energy per spike) does not vary with any morphological feature including dendrite surface area, suggesting that larger neurons have lower firing rate compensatory to their higher energy per spike, resulting in constant power. Our results support the proposition that neuronal cell size is self-regulated through the trade-off between excitability and cell growth, in the context of a limiting blood supply.

Disclosures: L. Xu: None. **S. Herculano-Houzel:** None.

Poster

PSTR378

Mechanisms of Dendritic Development and Synaptic Integration in Neurons

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.10/A38

Topic: A.05. Axon and Dendrite Development

Support: R01AA029114

Title: Nmda receptor-dependent nuclear calcium elevations during the terminal phase of radial migration and the initiation of the apical dendrite.

Authors: *J. ENCK[1], E. C. OLSON[2];
[1]SUNY Upstate MU, Syracuse, NY; [2]Neurosci. and Physiol., SUNY Upstate Med. Univ., Syracuse, NY

Abstract: The apical dendrite of cortical projection neurons (CPNs) arises from the direct transformation of the leading process of migrating neurons during the final stages of neuronal migration. Branched dendrite growth occurs within the cortical marginal zone (MZ), in close proximity to Cajal-Retzius neurons (CRNs) and their axonal projections. While CRNs are recognized for secreting Reelin, a glycoprotein required for dendritogenesis and cell positioning, we have found that neurotransmitters, likely released from CRNs, stimulate cytoplasmic calcium elevations in CPNs during this period of dendritic initiation and growth. Using nuclear-localized calcium indicators (H2B-GCaMP6s and H2B-CaMPARI2) we now show that nuclear calcium levels also rise during dendritic initiation. Importantly, chemical stimulation of CRNs by veratridine induces sustained cytoplasmic calcium elevations in CPNs, that are blocked by a GluN2B-specific NMDA receptor antagonist and partially attenuated by the glycine receptor antagonist strychnine. Multiphoton imaging reveals an increase in surface expression of NMDA receptors (super-ecliptic pHluorin-Grin1) during the terminal stage of migration. These newly inserted surface receptors are found in the perisomatic region and the forming apical dendrite. These observations outline a model wherein CRN activity causes the release of glutamate and glycine that both stimulates other CRNs and promotes nuclear calcium elevations in the arriving migratory CPNs. These calcium elevations may regulate transcriptional events that underlie the transition from migratory to post-migratory neuronal differentiation. The identification of non-synaptic CRN to CPN signaling during early development provides an additional role for CRNs during early cortical development and may help explain the early expression of autism-associated genes that encode synaptic proteins but are expressed in the developing cortex before morphological synapse formation.

Disclosures: J. Enck: None. **E.C. Olson:** None.

Poster

PSTR378

Mechanisms of Dendritic Development and Synaptic Integration in Neurons

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.11/A39

Topic: A.05. Axon and Dendrite Development

Support: March of Dimes Foundation
Whitehall Foundation Research Award
National Science Foundation
National Institute of General Medical Sciences of the National Institutes of Health

Title: The kpc-1 3'UTR facilitates dendritic transport and translation efficiency of mRNAs for dendrite arborization of a mechanosensory neuron important for male courtship

Authors: *M. SHIH[1], C.-F. CHUANG[1], C. CHANG[2];
[1]Univ. of Illinois at Chicago, Chicago, IL; [2]Biol. Sci., Univ. of Illinois at Chicago, Chicago, IL

Abstract: A recently reported Schizophrenia-associated genetic variant in the 3' UTR of the human furin gene, a homolog of C. elegans kpc-1, highlights an important role of the furin 3' UTR in neuronal development. We isolate three kpc-1 mutants that display abnormal dendrite arborization in PVD neurons and defective male mating behaviors. We show that the kpc-1 3′ UTR participates in dendrite branching and self-avoidance. The kpc-1 3′UTR facilitates mRNA localization to branching points and contact points between sibling dendrites and promotes local protein translation. We predict a secondary structural motif in the kpc-1 3' UTR required for dendrite self-avoidance. DMA-1 is a PVD dendrite branching receptor. Animals with dma-1 receptor over-expression exhibit similar dendrite branching and self-avoidance defects that are suppressed with kpc-1 over-expression. Our results support a model in which KPC-1 proteins are synthesized at branching points and contact points to locally down-regulate DMA-1 receptors to promote dendrite branching and self-avoidance of a mechanosensory neuron important for male courtship.

Disclosures: M. Shih: None. **C. Chuang:** None. **C. Chang:** None.

Poster

PSTR379

Genetic Models for Autism

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.01/A40

Topic: A.07. Developmental Disorders

Support: R01NS123163

Title: Proteomic and transcriptomic analysis towards the understanding of ASD: lessons from the PACS1 syndrome model

Authors: *X. GOMEZ MAQUEO[1], L. E. RYLAARSDAM[2], A. D. GUEMEZ-GAMBOA[1];
[1]Neurosci., Northwestern Univ., Chicago, IL; [2]Mol. and Med. Genet., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Modeling human brain development and autism spectrum disorder (ASD) remains a challenging task due to limited accessibility to embryonic and fetal tissue. To overcome these limitations, the use of human induced pluripotent stem cells (iPSCs) which can be differentiated into cortical brain organoids, offers an opportunity to model ASDs on a temporal scale. We used proteomic and transcriptomic approaches to characterize the regulatory landscape observed over maturation in brain organoids. We assessed the level of concordance across techniques to understand how well they recapitulate early stages of human brain development. Moreover, we determined how the regulatory landscape is dysregulated on a rare ASD-related disorder caused

by a recurrent missense *de novo* mutation in the PACS1 gene (PACS1 p.R203W). Proteomic data was obtained using tandem mass tag mass-spectrometry from brain organoids. Previously obtained single cell RNA transcriptomics and publicly available human fetal brain proteome and transcriptomes were used as references to assess the degree of concordance across protein and RNA to developmental time. Time series analysis showed that brain organoids displayed a dynamic proteome landscape which better represented the dynamics observed in earlier developmental stages of the fetal brain. At the transcriptomic level, similar biological functions as observed at the proteome level were regulated in organoids and fetal samples. However, there was low overlap between gene identities reported by both approaches, indicating that multiple regulation mechanisms occur at the protein and RNA level for the same biological processes. Accordingly, PACS1 p.R203W variant showed transcriptomic and proteomic alterations in related biological processes, but affecting different genes, 86 of which had been previously associated to ASD. At the protein level, the variant had a higher impact over axogenesis, membrane trafficking, and cell cycle, whereas pseudo-bulk transcriptomics indicated that the transition from early to later stages affected axon development, regulation of neurogenesis, and synapse organization. Our data highlights the need of applying multiple 'omics' over developmental time due to the multiplicity of regulation levels at which ASD-risk genes might be affecting brain development.

Disclosures: X. Gomez Maqueo: None. **L.E. Rylaarsdam:** None. **A.D. Guemez-Gamboa:** None.

Poster

PSTR379

Genetic Models for Autism

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.02/A41

Topic: A.07. Developmental Disorders

Support: HOPE for Harvey Foundation

Title: Development of an iPSC-based drug screening platform for DLG4-Related Synaptopathy

Authors: A. DUONG[1], P. ZHOU[1], M. NICHOLSON[1], *W. W. POON[1], A. C. PFALZER[2], D. V. LESSARD[1], J. SKINNER-FOSTER[3];
[1]NeuCyte, Inc., Mountain View, CA; [2]COMBINEDBrain, Brentwood, TN; [3]HOPE for Harvey Fndn., Austin, TX

Abstract: *DLG4*-related synaptopathy (DLG4-RS) is a rare neurodevelopmental disorder characterized by clinical symptoms including developmental delay, intellectual disability, and autism spectrum disorder. Approximately 50% of individuals diagnosed with DLG4-RS experience epilepsy, while approximately 40% experience regression in motor and language skills, highlighting the significant impact on this patient community. Currently, there is no cure

for this condition. Our study focuses on accelerating drug discovery efforts for *DLG4*-RS. Here, we describe the generation of cryopreserved iPSC-derived *NGN2*-glutamatergic neurons from both control and from isogenic *DLG4*-RS gene-edited iPSCs with the T654I mutation. In parallel, *NGN2*-glutamatergic neurons were also produced from a patient iPSC line with the T654I *de novo* mutation. The availability of these neurons enabled examination of neuronal electrophysiological properties by microelectrode array analysis, facilitating the identification of *DLG4*-related phenotypes for the development of a drug screening platform, including high-throughput screen (HTS) assays. RNA-seq analysis of cultured NGN2 neurons revealed altered pathways that could serve as potential therapeutic targets. Taken together, the development of patient-derived neurons will be an invaluable resource for identifying therapies for *DLG4*-related synaptopathies.

Disclosures: A. Duong: A. Employment/Salary (full or part-time):; NeuCyte, Inc. **P. Zhou:** A. Employment/Salary (full or part-time):; NeuCyte, Inc. **M. Nicholson:** A. Employment/Salary (full or part-time):; NeuCyte, Inc. **W.W. Poon:** A. Employment/Salary (full or part-time):; NeuCyte, Inc., UC Irvine. **A.C. Pfalzer:** A. Employment/Salary (full or part-time):; COMBINEDBrain. **D.V. Lessard:** A. Employment/Salary (full or part-time):; NeuCyte, Inc.. **J. Skinner-Foster:** None.

Poster

PSTR379

Genetic Models for Autism

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.03/A42

Topic: A.07. Developmental Disorders

Support: 21M149–AARE20–260
MCST R&I–2017–029T

Title: Identification of a novel Kv7.3 mutation associated with autism and schizophrenia inducing abnormal excitability in reprogrammed neurons

Authors: M. D`ADAMO[1], C. A. BROWNSTEIN[2], N. C. ANDERSON[3], S. M. HASAN[4], F. M. HAIDAR[5], E. BUTTERMORE[6], M. SAHIN[7], ***M. PESSIA**[8], J. GONZALEZ-HEYDRICH[9]; [1]Med., LUM Univ., Casamassima, Italy; [2]Genet. and Genomics, Div. of Genet. and Genomics, Manton Ctr. for Orphan Dis. Res., Boston Children's Hosp., Harvard Med. Sch., Boston, MA 02115, USA., Boston, MA; [3]Neurol., Boston Childrens Hosp., Framingham, MA; [4]Physiol. Dept., Kuwait Univ., Safat, Kuwait; [5]Physiol., United Arab Emirates Univ., Al-Ain, United Arab Emirates; [6]Rosamund Stone Zander Translational Neurosci. Ctr., Boston Children's Hosp., Harvard Med. Sch., Boston, MA 02115, USA, Boston, MA; [7]Dept of Neurol., Boston Children's Hosp., Needham, MA; [8]United Arab Emirates Univ., Al Ain, United Arab Emirates; [9]Dept. of

Psychiatry, Tommy Fuss Ctr. for Neuropsychiatry Dis. Res., Boston Children's Hosp., Harvard Med. Sch., Boston, MA 02115, USA, Brookline, MA

Abstract: Voltage-gated M-type K^+ channels are heterotetramers, commonly composed of two Kv7.2 (encoded by *KCNQ2*) and two Kv7.3 (encoded by *KCNQ3*) subunits. Kv7.3/7.2 channels are widely expressed in the brain where they regulate the resting membrane potential of neurons thereby determining excitability and firing pattern. A number of *loss-of-function* mutations have been identified in *KCNQ3*(Kv7.3) and associated with *benign familial neonatal epilepsy*. Most patients with this condition develop normally. In this study, we report a new *KCNQ3*(Kv7.3) frameshift mutation in a unique clinical case characterized by high-functioning autism diagnosed at age 3 years, as well as extreme noncompliance and aggressivity. Despite persistent oppositionality, this male child still had advanced language skills with an IQ of 114 at age 8. Around age 8 1/2, his functioning declined dramatically. His speech deteriorated rapidly, his thinking became highly disordered, and he developed paranoid delusions and obsessive-compulsive disorder (OCD). In early adolescence, he also experienced intermittent epilepsy, which subsequently abated. By late adolescence, after many rounds of treatment with antipsychotics, he remained highly thought-disordered, delusional, aggressive, and intellectually disabled (IQ= 60). MRIs were normal and a lumbar puncture unrevealing. Whole-exome sequencing demonstrated that the proband carried a mutation inherited from his mother resulting in an early stop codon. Electrophysiological investigations in homologous and heterologous systems revealed a significant *loss-of-function* of both homomeric Kv7.3 and heteromeric Kv7.3/7.2 channels. Blood cells were collected from the proband and his father, who did not carry the variant (control), and subsequently reprogrammed into cortical neurons which expressed Kv7.3 channels. Western blot analysis of the proband batches showed a significant decrease in KCNQ3 levels compared to control batches. Patch-clamp recordings demonstrated that the neurons differentiated from the proband had increased input resistance (R*in*), more depolarized membrane potentials (E*m*), and reduced M-type current amplitudes and densities compared with control cells. Firing pattern analysis unraveled a remarkably smaller percentage of neurons carrying the mutation capable of discharging multiple action potentials compared with controls. In summary, we report on a novel mutation disrupting Kv7.3 channel function that is associated with a unique phenotype characterized by high functioning autism early in life that later on evolved into OCD, schizophrenia, and intellectual disability.

Disclosures: M. D`Adamo: None. **C.A. Brownstein:** None. **N.C. Anderson:** None. **S.M. Hasan:** None. **F.M. Haidar:** None. **E. Buttermore:** None. **M. Sahin:** None. **M. Pessia:** None. **J. Gonzalez-Heydrich:** None.

Poster

PSTR379

Genetic Models for Autism

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.04/A43

Topic: A.07. Developmental Disorders

Support: COBRE Grant P20 GM148302

Title: Champ1 directs the maturation of synaptic function and firing activity in ipsc-derived excitatory neurons

Authors: *D. NETTLES, C. STANTON, L. L. MCMAHON, S. BERTO;
Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: Mutations in CHAMP1 are a major genetic risk factor for neurodevelopmental disorders (NDDs), as patients with CHAMP1 disorder are characterized by intellectual disability, autism spectrum disorder (ASD)-like behaviors, microcephaly, hypotonia, and dysmorphic features. CHAMP1 encodes for a zinc-finger protein involved in the maintenance of kinetochore-microtubule attachment and chromosomal segregation during mitosis, and in DNA repair mechanisms. However, the function of CHAMP1 in neurodevelopment remains relatively unexplored. We hypothesize that CHAMP1 orchestrates neuronal proliferation by modulating mitosis during prenatal development, ultimately promoting the maturation of synaptic and firing properties of excitatory neurons and synchronicity of local cortical circuits. CHAMP1 loss of function (LoF) could delay the maturation of both individual neurons and complex circuits.
To test our hypothesis, we induced neural progenitor cells (NPCs) and mature neurons using a dual-SMAD inhibition approach with one neurotypical iPSC line (Ctrl1), two patient donor lines (CHAMP1(c.1489c>T), CHAMP1(c.2094delT)), and one isogenic knockout line (iCHAMP1$^{-/-}$). After mitotic arrest using nocodazole treatment, CHAMP1 LoF significantly reduced the number of dividing NPCs. This data confirms that CHAMP1 regulates mitosis in neural lineage cells. To quantify the development of functional properties in maturing neurons, we used whole-cell patch-clamp electrophysiology at one and two months *in vitro*. CHAMP1(c.1489c>T), CHAMP1(c.2094delT), and iCHAMP1$^{-/-}$ neurons exhibited altered membrane properties and reduced action potential amplitude and frequency compared to Ctrl1 neurons. Additionally, CHAMP1 LoF reduced the amplitude and frequency of spontaneous excitatory postsynaptic currents at 1 month *in vitro.* More deficits were observed in CHAMP1(c.1489c>T) neurons compared to CHAMP1(c.2094delT), which correlates with the severity of CHAMP1 syndrome present in these patients.
Together these results suggest that CHAMP1 directs the maturation of excitatory synaptic and firing activity. To further explore the dose-dependent function of CHAMP1 in neurodevelopment, we will conduct western blot analysis and bulk-RNA sequencing. Future experiments will also quantify synchronous population firing using 2-photon calcium imaging with GFP-labeled excitatory neurons in both 2D neuronal culture and 3D brain organoids. These combined approaches will help reveal the function on CHAMP1 in human neurodevelopment.

Disclosures: D. Nettles: None. **C. stanton:** None. **L.L. McMahon:** None. **S. Berto:** None.

Poster

PSTR379

Genetic Models for Autism

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.05/A44

Topic: A.07. Developmental Disorders

Support: Showalter Research Trust
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Title: Deciphering autism-associated SCN2A deficiency with advanced human brain assembloids model

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Abstract: Autism spectrum disorder (ASD), affecting 1 in 36 children (cdc.gov) in the U.S., is characterized by deficits in social communication and interaction. Genetic research has identified the *SCN2A* gene, which encodes for the voltage-gated sodium channel $Na_V1.2$, as a key contributor to a monogenetic form of severe ASD. Most *SCN2A* mutations identified in ASD are loss-of-function, however, current treatments do not effectively treat patients with profound autism carrying *SCN2A* loss-of-function mutations. To advance our understanding of the role of *SCN2A* in ASD, here, we focus on the *SCN2A-C959X* mutation, a nonsense mutation that results in a *de novo* protein-truncating variant, which is found in children with profound autism. We firstly developed a 3D organoid model derived from hiPSCs to closely recapitulate the human brain for investigating how $Na_V1.2$ deficiency affects neuronal activity. Furthermore, recognizing the limitations in existing single brain region organoid models for studying human brain circuitry, we construct advanced human 'assembloids' which are composed of cortical pyramidal neurons and striatal medium spiny neurons (MSNs) to partially recapitulate the key circuitry implicated in ASD. This will allow us to delve into the specific contributions of *SCN2A* deficiency to neural circuit impairments with the ultimate goal of mitigating these *SCN2A*-associated impairments. By using assembloids that form circuity between these organoids, we are conducting molecular, imaging, and electrophysiological studies to elucidate how $Na_V1.2$ deficiency renders disease phenotypes. We found that *SCN2A-C959X* organoids exhibit reduced axonal projections from the cortex to the striatum and lower spine density in projected striatal

neurons. Mechanistically, RNA sequencing revealed downregulation of spine and axon-related gene pathways in these mutant human cortical-striatal assembloids. In summary, with this current study serving as an example by focusing on a typical *SCN2A* protein-truncating mutation, our innovative approach employing assembloids composed of 3D human brain organoids offers a promising avenue to explore the impact of *SCN2A* loss of function mutations on neuronal function and circuit impairments. We aspire to unravel the underlying mechanisms of $Na_V1.2$ deficiency-related diseases to help develop novel therapeutic strategies.

Disclosures: X. Chen: None. **J. Zhang:** None. **M. Wang:** None. **K. Wettschurack:** None. **M. Halurkar:** None. **M.I. Olivero acosta:** None. **M. Eaton:** None. **Z. Que:** None. **B. Deming:** None. **J. Wu:** None. **Y. Yoo:** None. **E. Creager:** None. **Y. Yang:** None.

Poster

PSTR379

Genetic Models for Autism

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.06/A45

Topic: A.07. Developmental Disorders

Support: NIH grant GR5272030

Title: The Histone Methyltransferase ASH1L Orchestrates Neuronal Structure and Function through Transcriptional Regulation in Human Neurons

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Abstract: Autism spectrum disorder (ASD) affects 1 in 36 individuals, presenting challenges in communication, social interaction, and repetitive behaviors. Chromatin regulators, particularly histone methyl transferases, are prevalent among high-risk variants associated with ASD. ASH1L encodes a histone methyltransferase that dimethylates lysine 36 on histone H3 (H3K36me2) and the trimethylation of lysine 4 on histone H3 (H3K4me3) and counteracts the activity of the Polycomb Repressive Complex 2 activity. We previously showed that ASH1L modulates neuronal structure, yet its impact on neuronal activity and the molecular signatures in human neurons constitutes a gap in knowledge. We used genome editing to generate induced pluripotent stem cells (iPSCs) with ASD-associated variants in ASH1L. We incorporated nonsense mutations in the chromatin binding domain (R2426*) and the catalytic domain (E2143*) of *ASH1L* using iPSCs from a neurotypical male individual. We find that glutamatergic excitatory neurons have defects in neuronal morphology and activity that are independent of the pathogenic variant. Moreover, we find that the changes in neuronal structure and function in both

mutants correlate with widespread dysregulation of various transcriptional programs related to chromatin biology, cell adhesion, synaptic function, and neuronal morphogenesis that are relevant to ASD pathobiology. Similarly, using a modified chromatin immunoprecipitation and sequencing technology - CUT&Tag, we determined the influence of distinct ASH1L variants on the histone modification landscape for H3K36me2, H3K4me3, and H3K27me3 on a genome wide scale. We find an overrepresentation of genomic regions important for neuronal structure and function differentially affected in neurons with either variant. Integrating the transcriptomic and epigenomic datasets is poised to allow us to uncover the molecular underpinnings associated with ASH1L dysfunction in human neurons. Finally, we find that small molecules targeting different epigenetic mechanisms show promise in ameliorating the ASH1L-mediated deficits in neuronal structure and function. in neuronal structure and activity. In summary, this study offers crucial insights into ASD pathogenesis, shedding light on the intricate relationship between ASH1L-mediated transcriptional and epigenetic programs that modulate human neuronal structure and function.

Disclosures: M. Jhanji: None. **C. Leung:** None. **J. Ward:** None. **F.D. Ritchie:** None. **C.L. Krall:** None. **B. Yoon:** None. **V. Corces:** None. **S. Berto:** None. **J.S. Liu:** None. **S. Lizarraga:** None.

Poster

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Topic: A.07. Developmental Disorders

Support: NIH grant 5R01NS120667-04

Title: Disrupted neurogenesis in the developmental gene DDX3X

Authors: *C. YANG[1], E. H. SHERR[2];
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Abstract: Disrupted neurogenesis in the developmental gene DDX3X
Objective:DDX3X encodes an RNA helicase of the DEAD-box helicase family and plays an important role in neurogenesis. Patients carrying DDX3X mutations exhibit a range of brain anatomy changes indicative of disrupted development. The objective of this study is to examine how DDX3X mutations disrupt the balance in the number of neural progenitor subtypes during early neurogenesis.Methods:PBMCs from patients with the DDX3X mutations T532M, R376C, and I415M and PBMCs from their healthy mothers are used to generate pluripotent stem cells (PSCs). Mutations T532M and I415M were previously found to have severe loss of helicase activity, and R376C was found to have a moderate loss of helicase activity. With dorsomorphin

and SB431542, PSCs are differentiated into dorsal neural progenitor cells. The number of radial glial cells (RGCs) and intermediate progenitors (IPs) are measured with the marker gene sets PAX6+/SOX2+/KI67+ and TBR2+/KI67+/TUJ1- respectively with ICC-IF and qPCR from differentiation day 0 to 40. Results:Mutations T532M and I415M cause an increase in the number of IPs while mutation R376C causes a decrease in the number of IPs. Consistent with this finding, BRN2, a transcriptional regulator of TBR2 that suppresses TBR2 expression, is decreased in neural progenitor cells with the T532M mutation. There was no significant change in the number of RGCs by DDX3X mutations.Conclusions:DDX3X disrupts early neurogenesis through the overproduction of IPs in severe mutations and the underproduction of IPs in moderate mutations.

Disclosures: C. Yang: 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 funding. **E.H. Sherr:** 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 funding.

Poster

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Genetic Models for Autism

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Topic: A.07. Developmental Disorders

Support: National Institutes of Health Convergent Neuroscience Initiative grant 1U01MH115747-01A1
National Institutes of Health grant U01MH116487
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Coalition to Cure CHD2
The SYNGAP1 Research Fund
The Sorensen Foundation Fellowship in Child & Adolescent Psychiatry

Title: Convergence of Autism Genes at the Cilium

Authors: *E. KOSTYANOVSKAYA, M. LASSER, B. WANG, J. SCHMIDT, E. BADER, K. MCCLUSKEY, J. ARBELAEZ, O. CASTILLO, D. B. KASTNER, M. STATE, H. WILLSEY; Univ. of California San Francisco, San Francisco, CA

Abstract: Hundreds of high-confidence autism genes have been identified, yet the relevant molecular underpinnings are unclear. Autism commonly co-occurs with cilia-related disorders, including congenital heart disease, hydrocephalus, and blindness, but the role of autism genes at the cilium has not been systematically investigated. Cilia are membrane-bound organelles critical

for neurogenesis, brain patterning, and neuronal activity, all processes strongly implicated in autism. Here we show that autism proteins converge in expression, localization, and function at cilia, and patients with pathogenic variants in these genes have cilia-related coincident medical conditions and disrupted biomarkers of ciliary function. This unprecedented degree of convergence provides strong evidence that cilia are relevant to autism biology and should be explored for therapeutic potential for treating impairing co-occurring medical conditions.

Disclosures: E. Kostyanovskaya: None. **M. Lasser:** None. **B. Wang:** None. **J. Schmidt:** None. **E. Bader:** None. **K. McCluskey:** None. **J. Arbelaez:** None. **O. Castillo:** None. **D.B. Kastner:** None. **M. State:** None. **H. Willsey:** None.

Poster

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Topic: A.07. Developmental Disorders

Support: BioNexus KC

Title: miRNA markers of neurodevelopmentally salient stress effect in pregnancy in disadvantaged communities

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Abstract: While progress has been made towards the understanding of genetic factors in neurodevelopmental disorders, environmental factors are less understood. One important environmental factor during pregnancy is stress, which is emerging as a critical factor in disadvantaged communities. Our lab has previously found epigenetic markers in mouse brains that were associated with prenatal stress and genetic stress susceptibility. A number of microRNA (miRNA) were also detected in both the brains of the prenatally stressed mouse offspring and in the blood of mothers of children with Autism Spectrum Disorder (ASD) who experienced prenatal stress exposure in a clinical study. With knowledge of which maternal miRNA changes are present during a pregnancy with prenatal stress, we moved on to examining whether this could potentially be a biomarker for maternal exposure to stress that could predict outcomes. This is of particular importance in disadvantaged communities where disparity is prominent and there is a high incidence of developmental disabilities. For the current study, buccal swabs were used to collect saliva from 83 pregnant African American women ages 18-40 during their 20 week ultrasound appointments. 2 surveys were also given to assess how much stress each participant had experienced during their pregnancy thus far. The saliva samples from

the 10 women who reported the highest amount of stress (high stress group) and the 10 who reported least amount or no stress at all (low stress group) in their surveys were chosen for miRNA analysis. We hypothesized that women in the high stress group would have miRNAs that were significantly differentially expressed when compared to those of women in the low stress group. Out of 6,631 total miRNAs analyzed, 34 reached or exceeded the threshold for significant differential expression. Of these 34, 5 were upregulated and 29 were down regulated in the high stress group compared to the low stress group. The next step for this study will be to run a gene ontology (GO) analysis to examine which genes the differentially expressed miRNAs regulate and what their role is in development. We also plan to examine the neurodevelopmental outcomes of these pregnancies, helping further determine predictive salience of these miRNAs. The identification of these miRNAs is a crucial first step in understanding the relationship between miRNA expression and stress exposure in this population, and could eventually lead to the identification of biomarkers that predict high risk for neurodevelopmental disorders. Furthermore, if mechanistic biomarkers are found, this would allow future studies to work toward developing an intervention to mitigate this risk.

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Poster

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Genetic Models for Autism

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Support: University of Modena and Reggio-Emilia and Fondazione Modena, Intramural Competitive Research Grant 2021
University of Modena and Reggio-Emilia and Fondazione Modena, Intramural Competitive Departmental Research Grant 2023

Title: Immunogenetic association supports the involvement of IL-4 in Autism Spectrum Disorder

Authors: *A. M. PERSICO[1], F. CHEHBANI[1], I. S. PIRAS[2], V. NAPOLIONI[3], A. MAVILLONIO[1], R. SACCO[4], A. GRANDE[1];
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Abstract: Autism Spectrum Disorder (ASD) is characterized by deficits in social reciprocity and communication, repetitive behaviors, restricted interests, and abnormal sensory processing. ASD is often accompanied by immune dysregulation, encompassing neuroinflammation, systemic

immune activation, abnormal adaptive and/or innate cellular response, allergies, autoimmunity. It is unclear whether this immune dysregulation contributes to ASD pathogenesis or represents a collateral by-standing phenomenon. We performed a genetic study in three stages: (a) Thirty-four known functional SNPs located in 26 genes involved in 16 different immune pathways were genotyped in an experimental sample of 502 Italian ASD families. After quality control, genotypes at 28 SNPs were retained, encompassing 22 genes belonging to 15 different immune pathways. Transmission disequilibrium was contrasted in ASD vs unaffected sibs using the family-based association test (FBAT). A significant association was found with rs2243250 (IL4 gene, adj $P=2.0 \times 10^{-4)}$, as well as with CTLA4 rs231775 and LTA rs909253; (b) A significant association with IL4 rs2243250, but not with rs231775, was replicated in a validation data set including 1,031 families from the AGRE collection (FBAT P=0.027); (c) a definitive confirmation was obtained in a third independent sample encompassing 381 Italian ASD families (FBAT P=0.037). The functional T allele at rs2243250 was consistently overtransmitted to ASD offspring in all three patient samples. This allele is associated with reduced IL-4 baseline expression in all tissues and enhanced IL-4 expression in EBV-transformed cells. However, rs2243250 alleles are also associated with differential expression of the KIF3A, SLC22A5, SLC22A4, SEPT8 and HSPA4 genes (eQTL database). Plasma levels of IL-4 were found significantly higher in 171 ASD children compared to 55 unaffected sibs (P=0.008; P=0.046 in 58 intrafamilial ASD-SIB pairs). No significant association between rs2243250 alleles and IL-4 plasma levels was recorded in this ASD-SIB combined sample. In conclusion, the T allele at rs2243250 displays the strongest and most consistent association with ASD among functional SNPs located in immune genes. This association is likely mediated by differential IL-4 gene expression, although mediation by other genes cannot be excluded at this time. In addition to playing a pivotal role in T_H2 immunity, the IL-4 pathway directly influences brain function and may mechanistically link allergies to autism, since rs2243250 is also significantly associated with asthma and atopic dermatitis (PheWAS database).

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Poster

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Genetic Models for Autism

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Topic: A.07. Developmental Disorders

Support: RO1NS091220 from NINDS

Title: Arid1b haploinsufficiency leads to neuroinflammation in the mouse paraventricular nucleus

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[1]Biol. Sci., Kent State Univ., Ohio, OH; [2]Kent State Univ. - Biol. Sci., Kent, OH; [3]Biol. Sci., Kent State Univ., Kent, OH

Abstract: Title: Arid1b haploinsufficiency leads to neuroinflammation in the mouse paraventricularnucleusAT-Rich Interactive Domain 1B (ARID1B) is a scaffolding protein that plays a critical role inbrain development. ARID1B haploinsufficiency is known to cause autism spectrum disorder(ASD). Our laboratory generated an Arid1b haploinsufficient (heterozygous) mouse to modelASD. We found that this mouse model validated ASD behavioral phenotypes such as socialimpairments and stereotyped behavior. However, the cellular and molecular mechanismunderlying these phenotypes in Arid1b haploinsufficient mice is unclear. Recent evidencesuggests altered immune activities in the postmortem ASD brain. Thus, we investigated theneuroimmune response to Arid1b haploinsufficiency in the brain. Utilizing our Arid1bhaploinsufficient mouse model, we explored neuroinflammation in the paraventricular nucleus(PVN), the primary relay for oxytocin and social behavior in the brain. We measured the levelsof a pro-inflammatory cytokine interleukin 6 (IL-6) and anti-inflammatory cytokines interleukin4 (IL-4) and interleukin 10 (IL-10). We also assessed a dual role cytokine interferon-gamma(IFN- γ). Our quantitative PCR showed that the PVN had an increase in IL-6 and IL-4 mRNAexpression, and a decrease in IL-10 and IFN- γ expression in Arid1b haploinsufficient mice. Wealso found abnormal oxytocin levels in the Arid1b haploinsufficient PVN. It will be interestingto see whether there is an interaction between inflammatory modulation and neuroendocrineregulation in the social nucleus PVN under the Arid1b haploinsufficient condition. Our resultsmay serve as a steppingstone to reveal the potential role of neuroinflammation in Arid1bhaploinsufficiency- associated ASD.

Disclosures: A. Smith: None. **T. Ford:** None. **W. Kim:** None.

Poster

PSTR379

Genetic Models for Autism

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Topic: A.07. Developmental Disorders

Support: R01HL139712
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W81XWH2010199
Foglia and Hill families

Title: Perinatal hypoxia phenocopies loss of autism associated Mll3 gene in neocortical pyramidal neurons

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Abstract: Chromatin modifier genes encoding histone H3 lysine methyltransferase (HMT) enzymes have been strongly implicated in both congenital heart disease (CHD) and neurodevelopmental disorders. Perinatal hypoxia is a known risk factor for autism spectrum disorders (ASD), has been shown to influence histone H3 lysine methylation in vitro and is associated with complex CHD. Recent large scale sequencing studies have shown a striking overlap between CHD and ASD risk loci, pointing to a shared genetic etiology between these conditions. Here we investigated the role of the ASD and CHD-associated HMT gene *Mll3* during neocortical excitatory neuron maturation and in motor and social neurobehavioral tasks. To understand whether exposure to perinatal hypoxia independently impacts the same processes, we employed a chronic hypoxia exposure paradigm and assessed phenotypic overlaps. RNAScope combined with immunohistochemistry was used to characterize *Mll3* expression in excitatory glutamatergic (VGLUT1+) and inhibitory GABAergic (GAD67+) neocortical neurons. *Emx1cre*-driven recombination allowed for conditional inactivation of *Mll3* in excitatory neurons. Crosses to *Thy1-GFP(M)* mice, or in utero electroporation (at E14.5) of membrane-targeted GFP constructs allowed for assessments of neocortical pyramidal neuron arborization and dendritic spine morphometry in deep and superficial layers respectively. VGLUT1 and PSD95 immunoreactivity was used to characterize excitatory synaptic puncta co-localization. Perinatal hypoxia exposure from P3-P11 was used to mimic oxygen desaturation due to complex CHD. Conditional *Mll3* mutants and hypoxic mice were behaviorally assessed using rotarod and 3-chamber sociability assays. Multiomic single nuclei profiling of neocortical tissue was used to detect unique and shared transcriptional and chromatin signatures between *Mll3* loss of function and hypoxia. *Mll3* expression was detected throughout the neocortex at all postnatal stages investigated. Conditional inactivation of *Mll3* in neocortical excitatory neurons led to a striking reduction in layer V pyramidal neuron spine density, with an accompanying increase in spine volume. Behaviorally, *Mll3* mutants displayed deficits in motor learning and sociability. Chronic perinatal hypoxia mimicked these structural and behavioral deficits. These results suggest *Mll3* loss and hypoxia cause overlapping phenotypic outcomes, hinting at shared mechanisms between complex CHD-linked cerebral hypoxia and ASD-associated genotypes during brain development.

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Poster

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Genetic Models for Autism

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Topic: A.07. Developmental Disorders

Support: NIMH 1R01 MH118631-01

Title: Restoration of developmental social recognition by normalizing hippocampal CA2 perineuronal nets in two genetic mouse models for neurodevelopmental disorders

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Abstract: Autism Spectrum Disorder (ASD) is a heterogeneous set of neurodevelopmental conditions, with defining characteristics of restrictive interests/repetitive behaviors and social communication/interaction deficits. Individuals with ASD and related conditions like Phelan-McDermid syndrome and Fragile X syndrome also have difficulty recognizing familiar faces as well as recognizing emotional expressions (Guillory et al., 2020; Holsen et al., 2008). Since social dysfunction in these conditions arises during childhood, understanding how neural mechanisms supporting social behavior develop atypically may suggest therapies to improve quality of life for people with ASD and related conditions. Using genetic knockout (KO) mouse models for Phelan-McDermid syndrome (*Shank3B* KO mice) and Fragile X syndrome (*Fmr1* KO mice), we found that postnatal day (P) 14 pups of both models exhibit social memory impairments during development that persist into adulthood, or P60. The CA2 region of the hippocampus supports emerging social memory in healthy mice (Diethorn and Gould, 2023a), and neurons in this region are surrounded by perineuronal nets (PNNs), specialized extracellular matrix structures which appear around the developmental onset of social novelty preference (Laham et al., 2021; Diethorn and Gould, 2023b). We found that both *Shank3B* KO pups and *Fmr1* KO pups have atypical, but different, CA2 PNN concentrations with the former exhibiting an excess and the latter exhibiting a deficiency. Using local injections of the degradative enzyme chondroitinase ABC to decrease excess PNNs in the CA2 of *Shank3B* KO mice and the growth factor neuregulin-1 to increase diminished PNNs in the CA2 of *Fmr1* KO mice during the early postnatal period, on P10, emerging social memory abilities were restored by P14 and improvements persisted into adulthood. These data suggest the existence of a critical period for CA2 development, where PNN concentration in an optimal range is required for healthy social behavior. Using in vivo electrophysiology, CA2 network activity, including sharp wave ripples and theta-gamma coupling, was also assessed in adult *Shank3B* KO and *Fmr1* KO mice during social interactions to identify aberrations in these properties that may contribute to impaired encoding and/or retrieval of social memories. Taken together, these studies characterize the trajectory of social memory impairments in two genetic mouse models for ASD, identify CA2 PNNs as successful targets for intervention, and aim to assess CA2 network activity during social interactions in these models.

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Poster

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Topic: A.07. Developmental Disorders

Support: Loulou Foundation

Title: Exploring antisense oligonucleotide strategy for CDKL5 deficiency disorder

Authors: *E. KELLY[1], Y.-H. HUANG[2], P. N. AWAD[1], M. FAGIOLINI[3], H. OLSON[2], T. YU[4];
[1]FM Kirby Neurobio. Ctr., [3]Neurol., [4]Div. of Genet. and Genomics, [2]Boston Children's Hosp., Boston, MA

Abstract: Cyclin-dependent kinase like 5 (CDKL5) is an essential protein kinase for early brain development. Pathogenic variants in the *CDKL5* gene give rise to CDKL5 deficiency disorder (CDD), a severe developmental disorder. Our work is testing the feasibility of antisense oligonucleotide (ASO) treatment for CDD. We are exploring splice-modulating ASO strategies for CDD including: (1) a mutation-specific ASO therapy which we developed to rescue splice defects of a recurrent pathogenic variant. We utilized patient fibroblasts to develop a splice-switching ASO strategy capable of restoring exon inclusion and protein expression of this variant. (2) We are testing newly developed ASOs to induce de-silencing of CDKL5 expression in a CDD mouse model. This ASO strategy rescues exon trapping in a lox-stop-cassette-inserted CDD mouse model (CDKL5^{loxstop}). We tested ASOs in patient iPSC and iPSC-derived neurons and identified two ASOs capable of promoting exon 14 inclusion (Figure 1A). Transfection of lead ASO into *CDKL5* c.2152G>A fibroblasts boosted CDKL5 protein expression (Figure 1B). The next step for this project is to evaluate whether downstream targets are also rescued and whether such ASOs are safe *in vivo*. For our ASO strategy in the CDKL5^{loxstop} mouse, we screened 13 ASOs *in vitro* using mouse embryonic fibroblasts and primary neuron culture derived from CDKL5^{loxstop} mice and found three candidate ASOs to then be tested *in vivo* (Figure 1C, D). Combinations of two of the three candidate ASOs have been administered to neonatal pups by intracerebroventricular injection and we have quantified CDKL5 expression at ~p30 by western blot analysis (Figure 1E). Currently, we are evaluating the effect of these ASOs on mouse behavioral, physiological, and morphological phenotypes. Our results indicate that ASO strategy allows re-expression of CDKL5 *in vivo* in both animal and human samples. Furthermore, our study provides further understanding of the basic biology of CDD and the feasibility of therapeutic trials for CDD.

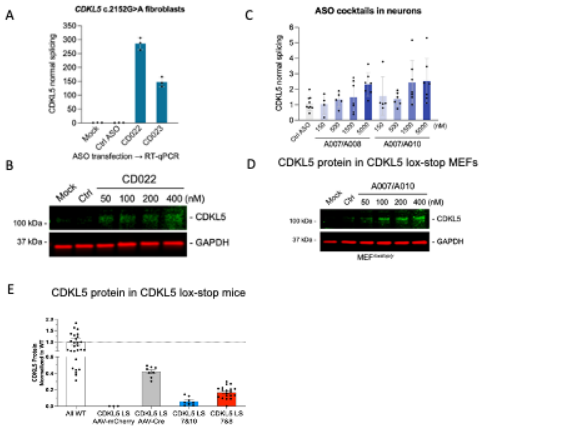

Figure 1: A: Levels of CDKL5 normal splicing quantified through RT-qPCR from patient fibroblasts following transfection with candidate ASOs. B: CDKL5 protein levels following ASO transfection show dose dependent increase. C: Levels of CDKL5 with normal splicing collected from primary neuron culture of CDKL5 lox-stop mutant mice following transfection with candidate ASOs. D: CDKL5 protein levels detected following ASO transfection in MEFs derived from CDKL5 lox-stop mice. Protein levels show a dose dependent increase following ASO treatment. E: CDKL5 protein levels detected following ASO transfection in MEFs derived from CDKL5 lox-stop mice. Protein levels show a dose dependent increase following ASO treatment. F: CDKL5 protein levels (from tissue collected at p30) resulting from intracerebroventricular injections of ASO cocktails or AAV-Cre-mCherry/AAV-mCherry virus in neonatal pups.

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Poster

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Topic: A.07. Developmental Disorders

Support: Orphan Disease Center 2022 Million dollar bike ride
The Hock E. Tan and K. Lisa Yang Center for Autism Research at Harvard University

Title: Choroid plexus disruptions in CDKL5 Deficiency Disorder

Authors: *P. N. AWAD[1], M. TRAPP[2], A. PATRIZI[2], M. FAGIOLINI[1];
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Abstract: The choroid plexus (ChP) is an intraventricular highly vascularized structure, which is primarily comprised of tightly interconnected, multi-ciliated and polarized epithelial cells that form the blood-cerebrospinal fluid (CSF) barrier (BCSFB). The ChP secretes CSF and controls

brain homeostasis by producing an abundant secretome that includes neuroactive substances, such as growth factors, hormone transporters and other signaling factors critical for brain development and physiological function. ChP disruptions affect normal brain development and homeostasis, and recent clinical studies have found enlarged ChP volumes in Schizophrenia and Autism Spectrum Disorder patients. However, the direct contribution of ChP dysfunction in neurodevelopmental disorders has not yet been established. Our project directly probes this relationship in CDKL5 Deficiency Disorder, a severe developmental and epileptic encephalopathy. Recent evidence has shown that the loss of CDKL5 leads to elongated motile cilia, which in turn affects the flow and movement of CSF, suggesting a role of CDKL5 in ciliated cells. Our results demonstrate that the loss of CDKL5 impacts ChP metabolic and lipidomic composition, causes structural alteration of ChP epithelial cells and functional changes in the BCSF barrier. These results suggest that CDKL5 plays a key role in ChP structure and function and opens the door of using its uniquely accessible structure as a potential therapeutic target of CDD.

Disclosures: P.N. Awad: None. **M. Trapp:** None. **A. Patrizi:** None. **M. Fagiolini:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.01/A55

Topic: A.08. Development of Neural Systems

Support: The Children's Glaucoma Foundation
Vision for Tomorrow
Fight for Sight

Title: Effect of Pax6 on corneal nerve fiber growth and patterning

Authors: *S. MOHAN[1], J. D. LAUDERDALE[2];
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Abstract: The Paired box 6 (PAX6) gene is best known for its role in eye development. It also plays key roles elsewhere in neuronal development including cell fate specification and axon guidance. This study aims to test the hypothesis that Pax6 plays a key role in innervation of cornea. The cornea is one of the most highly innervated tissues in the body. Cells in the cornea express various axon guidance molecules such as netrins, semaphorins and ephrin family members. Corneal nerve fibers play a critical role in maintaining the ocular surface. In humans, heterozygous loss-of-function mutations in Pax6 lead to aniridia, a congenital disorder affecting multiple tissues in the eye. To assess the role of Pax6 in the cornea, we evaluated changes in nerve fiber growth between $Pax6^{+/+}$ and $Pax6^{+/Sey\text{-}Neu}$ mice corneas. The $Pax6^{+/Sey\text{-}Neu}$ allele is a

spice junction mutation in the 3’ end of exon 10. Nerve fibers were visualized by immunofluorescent labeling using anti-β-tubulin III antibody and confocal microscopy. At birth, *Pax6*$^{+/+}$ and *Pax6*$^{+/Sey\text{-}Neu}$ mice both exhibit thick nerve fiber bundles in cornea. In wild type, by 2 weeks of age, these thick fibers are largely absent from the central cornea and numerous smaller fibers are observed. A stable pattern of innervation is observed by 8 weeks of age. In contrast, in *Pax6*$^{+/Sey\text{-}Neu}$ mice thick nerve fiber bundles are observed past 2 weeks. A significant difference in the nerve fiber pattern was observed at 4 weeks of age, thus suggesting difference in the maturation process of fibers in mutant corneas. In mutant mice, different patterns of nerve fibers correlated with transparent and opaque corneal regions suggesting nerve fiber reorganization correlating with changes in corneal microenvironment. For each time point, at least 3 biological replicates were tested. Given the functional relationship between corneal nerve fibers and ocular surface, we also examined the trigeminal ganglion. Trigeminal ganglion ophthalmic neurons innervate the cornea. In addition to the ocular surface, Pax6 is expressed in trigeminal ganglion neurons. This leads to the possibility that maintenance of the ocular surface requires normal levels of Pax6 in trigeminal ganglion neurons as well as in the corneal cells.

Disclosures: S. Mohan: None. **J.D. Lauderdale:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.02/A56

Topic: A.08. Development of Neural Systems

Support: HHMI
McKnight Foundation

Title: Investigation of Gene Expression Programs Regulating Vision-dependent Development of L2/3 Neuron-types in the Mouse Visual Cortex

Authors: *S. JAIN[1,2], J. YOO[3], S. BUTRUS[4], V. XU[5], F. XIE[5], K. SHEKHAR[4], S. L. ZIPURSKY[5];
[1]Georgia Inst. of Technol., Atlanta, GA; [2]Biological Chemistry, HHMI/ UCLA, Los Angeles, CA; [3]Biol. Chem., UCLA, Los Angeles, CA; [4]Chem. and Biomolecular Engin., UC Berkeley, Berkeley, CA; [5]Biol. Chem., HHMI/ UCLA, Los Angeles, CA

Abstract: Neuronal fate specification and incorporation of neurons into specific circuits requires precise spatial and temporal control of gene expression. Indeed, mis-regulation of gene expression in developing nervous systems is associated with disorders such as autism and schizophrenia. However, the mechanisms that allow thousands of cell-types to express specific sets of genes at appropriate times remain poorly understood. To address this problem, we collected single-nucleus ATAC and RNA-Seq data for all cells in the mouse visual cortex across

several time points during postnatal development. The chosen time points encompassed peak synaptogenesis and key periods of post-mitotic fate specification. Analysis of global patterns revealed that early gene expression programs (P6 - P8) consist of a common set of genes expressed broadly across cortical neuronal types. These programs are replaced by more cell type-specific programs at later time points. Interestingly, neurons express a highly type-specific program during peak synaptogenesis, which is enriched for genes encoding cell surface recognition molecules. Importantly, this feature is conserved across several animal species. Preliminary analysis also suggests that several signaling pathways play key roles in the regulation of these dynamic gene expression programs. We next focused on excitatory neuronal types present in the superficial cortical layers (L2/3), which are the last-born neurons and are critical for higher order processing via cortico-cortical connections. Our labs previously showed that L2/3 excitatory neurons form a continuum of transcriptomic identities bound by three archetypes (A, B and C), and that this continuum is disrupted by dark-rearing. While transcriptomic and phenotypic continuums have now been identified in several contexts, how they are generated during development remains largely unknown. We find that the L2/3 continuum forms gradually and continuously over development, with two of the three archetypes already present at P6. Preliminary analyses suggest that the rate of development of the continuum is controlled by vision. ATAC-Seq allowed us to identify several transcription factors that likely drive the formation of this continuum. These factors are now being tested via directed genetic perturbations.Taken together, our study identifies dynamic genetic programs that control cell-type specific gene expression during synaptogenesis and ones that drive the development of a complex transcriptomic continuum amongst L2/3 cortical neurons. We expect these programs to be critical for proper circuit formation and function of the mammalian visual cortex.

Disclosures: S. Jain: None. **J. Yoo:** None. **S. Butrus:** None. **V. Xu:** None. **F. Xie:** None. **K. Shekhar:** None. **S.L. Zipursky:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.03/A57

Topic: A.08. Development of Neural Systems

Support: National Institutes of Health

Title: Mechanistic studies of MOK-10 protein kinase function in stochastic left-right neuronal asymmetry

Authors: *J. YANG, P. SAHYOUNI, R. XIONG, C.-F. CHUANG;
Dept. of Biol. Sci., Univ. of Illinois Chicago, Chicago, IL

Abstract: Left-right asymmetry of the nervous system development is essential for brain function. In *C. elegans*, the AWC (amphid wing C) olfactory neuron pair differentiates asymmetrically into AWC^{ON} and AWC^{OFF} subtypes in a stochastic manner. The default AWC^{OFF} subtype is specified by voltage-activated Ca^{2+} channels (UNC-2/UNC-36 and EGL-19/UNC-36) and a downstream Ca^{2+}-regulated protein kinase cascade. Intercellular communication between the two AWC neurons and other neurons in an NSY-5 gap junction network activates SLO BK potassium channels to suppress the Ca^{2+} signaling pathway in the induced AWC^{ON} subtype. To identify the genes required for and/or regulating *slo-1* function in promoting the AWC^{ON} subtype, our lab performed a non-biased forward genetic screen to isolate *mok* (modifier of K^+ channel) mutants that suppressed the *slo-1(gf)* $2AWC^{ON}$ phenotype. One of the *mok* mutants, *mok-10(vy65)*, shows a $2AWC^{OFF}$ phenotype. We determined that *vy65* is a nonsense mutation in a gene encoding a protein kinase by one-step whole genome sequencing and single-nucleotide polymorphism mapping. Our genetic mosaic analysis with *mok-10* fosmid rescuing transgenes in *vy65* mutants suggests that *mok-10* acts cell autonomously and non-cell autonomously to promote the AWC^{ON} subtype. Our data from tissue-specific rescue experiments further support that *mok-10* acts in multiple tissues, including neurons, glial cells, and hypodermal cells, to promote the AWC^{ON} subtype. These results are consistent with the endogenous expression pattern of *mok-10* in these tissues, analyzed in our *ZF1::mNG::mok-10 knock-in* strain. To further validate the tissues required for *mok-10* function in AWC asymmetry, we will perform tissue-specific degradation of endogenous MOK-10 protein in wild type using the ZF1/ZIF-1 degradation system. Together, our study will provide insight into a novel non-cell-autonomous mechanism by which signals from non-neuronal cells regulate neuronal differentiation.

Disclosures: J. Yang: None. **P. Sahyouni:** None. **R. Xiong:** None. **C. Chuang:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.04/A58

Topic: A.08. Development of Neural Systems

Support: National Institutes of Health

Title: Investigating mechanisms of MOK-2 cell-cell junction protein in stochastic left-right neuronal asymmetry

Authors: *S. YUAN, J. YANG, C.-F. CHUANG;
Dept. of Biol. Sci., Univ. of Illinois Chicago, Chicago, IL

Abstract: The *C. elegans* AWC (amphid wing "C") olfactory neuron pair differentiates asymmetrically along the left-right axis into two different subtypes, AWC^{OFF} (default) and AWC^{ON} (induced). Asymmetric differentiation of AWC neurons is regulated by a stochastic

lateral signaling interaction and relative Ca^{2+} levels between two AWC cells. A calcium influx through voltage-gated calcium channels, UNC-2 (CaV2) and EGL-19 (CaV1), activates a Ca^{2+}-regulated kinase cascade to specify the default AWC^{OFF} subtype. Intercellular communication between the two AWC neurons and other neurons in a transient NSY-5 gap junction network represses this Ca^{2+}-mediated signaling to induce the AWC^{ON} subtype. In addition, our previous genetic study showed that SLO-1 BK K^+ channels act downstream of NSY-5 gap junctions to promote AWC^{ON}. However, the mechanism of how SLO K^+ channel function is regulated or mediated in AWC asymmetry remains to be determined. To explore the mechanism of the *slo-1* function in AWC asymmetry, we performed a non-biased forward genetic screen to identify *mok* (modifier of K^+ channel) mutants that suppressed the *slo-1(gf)* $2AWC^{ON}$ phenotype. From this screen, we identified a *mok-2(vy149)* mutant that showed a $2AWC^{OFF}$ phenotype, which suggests the role of *mok-2* in promoting AWC^{ON}. We identified a missense mutation in a gene encoding a cell-cell junction protein responsible for the *vy149* phenotype by one-step SNP mapping and whole genome sequencing. To investigate the mechanism of the *mok-2* function in promoting AWC^{ON}, we first determined the *mok-2* endogenous expression pattern by generating *ZF1::mNG::SEC::mok-2 knock-in* and *ZF1::mNG::mok-2 knock-in* using CRISPR-Cas9. We found that *mok-2* is expressed broadly in multiple head, body, and tail cells. In addition, ZF1::mNG::MOK-2 protein is localized at the cell-cell junction regions in these cells. To determine the cells required for *mok-2* function in AWC asymmetry, we will perform tissue-specific rescue experiments in *mok-2(vy149)* mutants and tissue-specific degradation of MOK-2 protein in wild type using the ZF1/ZIF-1 degradation system. The findings of these experiments will provide insight into the molecular and cellular mechanisms of *mok-2* function in regulating or mediating *slo-1* activity in AWC asymmetry.

Disclosures: S. Yuan: None. **J. Yang:** None. **C. Chuang:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.05/A59

Topic: A.08. Development of Neural Systems

Support: KAUST baseline research fund

Title: Investigating the development of layer 1 circuit in the visual cortex via longitudinal 2-photon imaging

Authors: *M. ALYAHYAY[1], M. SOHEIB[1], L. A. IBRAHIM[2];
[1]King Abdullah Univ. of Sci. and Technol., thuwal, Saudi Arabia; [2]Dept. of Neurobio., KAUST, Thuwal, Saudi Arabia

Abstract: Neonatal mammals are exposed to diverse sensory stimuli that interact with internally generated signals in the brain. Cortical layer (L) 1 in sensory cortices are modulated by 1) multi modal external stimuli, 2) state-dependent inputs, 3) combination of external and internal signals, suggesting that L1 circuits are directly involved in integrating external and internal representations. However, it remains unclear how these representations in L1 mature and function throughout developmental stages, nor how they transfer these integrated representations to L23 excitatory neurons. L1 act as a hub for integrating diverse inputs to cortical excitatory neurons via their apical dendrites. At the center of the hub are a subtype of inhibitory neurons, the neurogliaform (NGF) cells located in L1. Here we used longitudinal mesoscale 2-photon calcium imaging of the mouse visual cortex (VIS) from postnatal day (p) 10 (before eye opening) to adulthood. We have imaged calcium activity of hundreds of single cells simultaneously in L1 and L23 in a baseline period in which mice were presented with grey screen followed by representation of drifting grating in eight directions. This allowed us to record spontaneous activity and activity evoked by external stimuli. Longitudinal imaging provided us with the opportunity to investigate the development and function of L1 and L23 neurons at key developmental stages. Particularly, we inspected neurons prior to sensory experience, early stages of sensory exposure, visual critical period, and adulthood. We focused on the critical period and were able to track several neurons at p26, p28, p31, and p39. Our observations demonstrate that prior to eye opening, neuronal activity in L1 interneurons is marked by prolonged and synchronized events, which then desynchronize around the time of eye opening. During the second postnatal week, L23 neurons display spontaneous activity, which progressively diminished by the third postnatal week. Interestingly, both L1 and L23 neurons exhibited reliable responses to state dependent modulation prior to the critical period (modulated neurons prior to P28; L1: 303/319 neurons (94.9%), L23: 206/218 (94.5%)). The ratio of the neurons modulated during the critical period decreased in both L1 and L23 (modulated neurons after to P28; L1: 163/214 neurons (76%), L23: 139/295 (47%)), in line with more complex processing during the critical period. In conclusion, we follow the activity of single cells in VIS throughout different developmental stages, allowing us to investigate when and how different features such as state-dependence and sensory experience impact L1 and L23 neurons development and function.

Disclosures: M. Alyahyay: None. **M. Soheib:** None. **L.A. Ibrahim:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

Location: MCP Hall A

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Program #/Poster #: PSTR380.06/A60

Topic: A.08. Development of Neural Systems

Support: NIH Grant EY033874

Title: Defining the molecular mechanisms of DSCAM-mediated self-avoidance

Authors: *R. RANA, S. BHANDARI, A. M. GARRETT;
Wayne State Univ., Detroit, MI

Abstract: DSCAM (Down Syndrome Cell Adhesion Molecule) belongs to the Ig superfamily of cell adhesion molecules (CAMs), is associated with neurodevelopmental disorders including Down syndrome and autism, and plays a major role in neural development including neuronal self-avoidance. Self-avoidance encompasses iso-neuronal self-avoidance, where branches from the same neuron avoid each other, and homotypic self-avoidance, where neighboring neurons of the same type contact each other without becoming entangled. This phenomenon is exhibited by both dendrites and axons in vertebrates as well as invertebrates. In invertebrates like drosophila, extensive alternative splicing of Dscam1 generates more than 38,000 isoforms which provide molecular diversity to individual neurons. This enables neurons to distinguish between 'self' and 'non-self'. Although vertebrates DSCAM lacks such alternative splicing, it can promote self-avoidance by masking excessive adhesion mediated by other cell adhesion molecules. DSCAM's C-terminus is important for promoting self-avoidance in some but not in all cell types. The precise molecular mechanism behind DSCAM's self-avoidance is not clear. Our research aims to elucidate these molecular mechanisms in the mouse retina. Using a combination of in vivo and in vitro techniques, we are testing the hypothesis that homophilic DSCAM interactions mask adhesion by triggering changes in local CAMs, but that only a subset of CAMs requires DSCAM's C-terminus for masking. To test candidate mechanisms, we are focusing on the masking of cadherin-3 in an in vitro assay of adhesion. To test the role of DSCAM's C-terminus in masking diverse CAMs, we identified CAMs expressed in amacrine cell types with a differential dependence on these C-terminal interactions and are using retinal electroporation to ask if DSCAM's C-terminus is required for their masking. We aim to expand our understanding of intricate molecular machinery governing self-avoidance in vertebrate neural developmental processes and its role in the pathogenesis of different neurodevelopmental disorders.

Disclosures: R. Rana: None. **S. Bhandari:** None. **A.M. Garrett:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

Location: MCP Hall A

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Topic: A.08. Development of Neural Systems

Support: College of Liberal Arts and Sciences Undergraduate Research Initiative (LASURI) Scholarship
Howard L. Kaufman Scholarship from UIC

Title: Immobilization of C. elegans with different concentrations of an anesthetic for time-lapse imaging of dynamic protein trafficking in neurons

Authors: *C. A. SIETE, R. XIONG, A. KHALID, Y.-W. HSIEH, C.-F. CHUANG;
Dept. of Biol. Sci., Univ. of Illinois at Chicago, Chicago, IL

Abstract: To perform dynamic imaging of protein trafficking events or mitochondrial transport in *Caenorhabditis elegans*, worms are usually immobilized with anesthetics, microfluidics, polystyrene microbeads, or glue. Cholinergic agonists, such as tetramisole and levamisole that cause hypercontracted paralysis by mediating excitatory neurotransmission at the neuromuscular junctions, are commonly used for time-lapse imaging in *C. elegans*. As tetramisole and levamisole act on acetylcholine receptors, it has long been a concern that they may affect subcellular processes. Despite this, tetramisole and levamisole have still been used to image the axonal transport of proteins or mitochondria in *C. elegans* as seen in recent studies. Considering logically, lower concentrations of anesthetics should have less effect on subcellular processes, but higher concentrations should better immobilize animals. With this idea in mind, we wanted to determine whether different concentrations of tetramisole would affect the dynamic movement of the TIR-1 (Sarm1) scaffold protein that we have been studying in the asymmetric differentiation of the *C. elegans* AWC olfactory neuron pair. In this study, we compared the percentage and velocity of anterograde and retrograde trafficking events of TIR-1::GFP in worms immobilized with 0.5 mM, 1 mM, 2 mM tetramisole, or microbeads (without the use of anesthetics) against 7.5 mM tetramisole. Our results show that the percentage and average velocity of TIR-1::GFP moving events in the *C. elegans* AWC axons are not significantly different between worms immobilized with 7.5 mM tetramisole and other conditions. Our results support that using 7.5 mM tetramisole, compared to lower concentrations (0.5 mM, 1 mM, and 2 mM) of tetramisole, to immobilize worms for dynamic imaging has no significant effect on the axonal transport of TIR-1::GFP along the AWC axons.

Disclosures: C.A. Siete: None. **R. Xiong:** None. **A. Khalid:** None. **Y. Hsieh:** None. **C. Chuang:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.08/A62

Topic: A.08. Development of Neural Systems

Support: NINDS STTR NS120748

Title: Measurement of proprioception and discriminative touch in rats using SensiTrak, a largely automated forelimb stimulation and reward system

Authors: *D. YOO[1], A. RAMAMURTHY[3], C. SANCHEZ[4], A. M. SLOAN[5], J. B. CARMEL[2]; [2]Orthopedics, [1]Columbia Univ. Med. Ctr., New York, NY; [3]Columbia Univ., New York, NY; [4]Bioengineering, The Univ. of Texas At Dallas, Dallas, TX; [5]Vulintus Inc, Westminster, CO

Abstract: Current sensory assessments in rodents primarily assay pain (Von Frey filaments) or temperature (Hargreaves test), but not touch or proprioception. We developed a rodent behavior system that delivers texture and proprioceptive stimuli and measures the accuracy of the responses. The texture and proprioceptive modalities use a two-alternative forced-choice (2-AFC) paradigm that requires rats inside a reaching box to sample and identify a stimulus through an aperture. Rats are trained to indicate their choice with a left or right nose poke response for each stimulus type. Correct responses are rewarded with a food pellet. The setup is constrained so the rat can only sample stimuli with their right forepaw.
The texture discrimination task presents a random horizontal or vertical grating of raised ridges with peak-to-peak pitch distances ranging from 2.5-0.75mm. Healthy rats demonstrated a classic sigmoidal sensitivity function with a high response rate on coarser stimuli and near-chance performance on finely spaced stimuli. Measurements taken from this system can quantify deviations in response rates due to neurological injuries and trace recovery. To ensure rats rely on somatosensation of the forelimb, control experiments were conducted to test the use of auditory and visual cues. Rats were tested before and following injection with bupivacaine in the wrist, temporarily desensitizing the paw, and exhibited significant deviation in response rate, which was fully recovered after 24 hours. The same animals underwent a dorsal column lesion with more lasting effects, effectively impairing performance for ~4 days before returning to baseline levels.
The proprioceptive discrimination task consists of a similar setup in which the rats are trained to reach and hold a handle for 1.2 seconds while the handle moves left or right. The stimulus is dynamically controlled by software, which moves the handle randomly at a variable distance (0.5-3.0mm). Preliminary data show scaling of performance to the distance the handle moves. Control experiments were also done to validate that rats use forelimb sensation. Development of SensiTrak is ongoing, and future studies will test the sensitivity of the texture and proprioceptive discrimination tasks for measuring injury-associated impairment.

Disclosures: D. Yoo: 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.; Vulintus Inc. **A. Ramamurthy:** 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.; Vulintus Inc. **C. Sanchez:** None. **A.M. Sloan:** None. **J.B. Carmel:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

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Program #/Poster #: PSTR380.09/A63

Topic: A.08. Development of Neural Systems

Support: 1F31DC021366-01A1
DC017167

Title: Altered neurotrophin signaling increases excitability of neurons in the avian cochlear nucleus

Authors: *K. MCLELLAN[1], S. MOHAN[2], J. T. SANCHEZ[2];
[1]Northwestern Univ., Evanston, IL; [2]Dept. of Communication and Disorders, Northwestern Univ., Evanston, IL

Abstract: Neurotrophins are a class of signaling proteins that mediate the development of many neuronal structures. In the chicken auditory brainstem, the neurotrophin ligand-receptor pairs BDNF-TrkB and NT-3-TrkC regulate the development of nucleus magnocellularis (NM), the avian analog to the mammalian anteroventral cochlear nucleus. These neurotrophin receptors are differentially expressed across the tonotopic axis: TrkB is more highly expressed in high-frequency neurons, while TrkC regulates low-frequency neurons. Previous research shows that overexpressing TrkB throughout development causes morphological changes to NM neurons. However, it is unknown how altered BDNF-TrkB signaling affects the firing properties and ion channel composition of these cells. To study this, we used *in ovo* electroporation to genetically maintain the local expression of TrkB at a high level throughout the development and used whole cell patch clamp electrophysiology to record active, passive, and current properties of NM neurons. We found that genetically altering BDNF-TrkB expression significantly affects the firing patterns of NM neurons in response to various current injections. We also determined that the altered neurotrophin expression leads to reduced ionic currents from multiple types of voltage-gated channels. In sum, we suggest that the normal BDNF-TrkB signaling pattern regulates the expression of voltage-gated ion channels that underlie the controlled excitability and high temporal fidelity of NM neurons. Understanding the effects of neurotrophins on the development of the auditory brainstem is an essential step in investigating the mechanisms that may underly developmental and congenital hearing deficits.

Disclosures: K. McLellan: None. **S. Mohan:** None. **J.T. Sanchez:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

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Topic: A.08. Development of Neural Systems

Support: NIH Grant R01DC03292
NIH Grant R25GM061222
NIH Grant P50GM68762

Title: Annotation Enhancement Improves Analysis of Gene Expression during Xenopus Inner Ear Development

Authors: S. M. VIRK, J. VIRK, ***E. SERRANO**;
Biol., New Mexico State Univ., Las Cruces, NM

Abstract: The inner ear, a complex organ system crucial for vertebrate survival, requires precise gene expression for its development. Understanding gene expression changes during inner ear development is vital for treating the millions of people affected by hearing and balance disorders. Transcriptomic profiling with Affymetrix GeneChip™ *Xenopus laevis* Genome 2.0 microarrays has been used to evaluate gene expression profiles during inner ear development and the datasets are publicly accessible (GSE69546, GSE73828, GSE73829). Gene expression data comprise three *Xenopus* developmental stages, during which all eight end organs contain mechanosensory hair cells: larval stages 50 and 56, and the post-metamorphic juvenile stage. *Xenopus* is an established developmental model and is a useful system for inner ear research due to the organism's regenerative capacity. Moreover, investment in community resources such as Xenbase and the National *Xenopus* Resource have increased the feasibility of *Xenopus* for genetic studies of neurosensory systems. Here we present results of efforts to impart greater significance to the dataset by improving the annotation of Affymetrix Probe Set Identifiers (PSIDs) representing ~ 29,900 *X. laevis* transcripts. GCRMA was used for probe summarization and normalization. Differential expression analysis was completed using Analysis of Variance as implemented in JMP Genomics 5.0; pairwise comparisons were analyzed between all three developmental stages. Multiple test correction was made using a positive false discovery rate corresponding to q-value <= 0.01 and a minimum fold change set to 1.5. A minimum average intensity >=4 was required for a PSID to be considered upregulated. PostGreSQL was used to create a queriable database that included Affymetrix array Xl-PSIDs, Affymetrix annotation, Unigene annotation, Xenbase open access information (gene symbols and descriptions) and other microarray data. Analysis uncovered over 1500 candidate genes for differential gene expression in one or more pairwise comparisons of which over 200 Xl-PSIDs showed 3-fold change or greater between comparison groups, including those for *oncomodulin*, *sp8*, *wnt*, *pdlim*, and *neurod*. Notably ~1/3 of the Xl-PSIDs remained unannotated. Results presented here augment the potential of *Xenopus* as a model organism for studies of the genes essential for inner ear development and reveal candidate genes for functional analysis during inner ear development.

Disclosures: S.M. Virk: None. **J. Virk:** None. **E. Serrano:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

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Program #/Poster #: PSTR380.11/A65

Topic: A.08. Development of Neural Systems

Support: NSF Grant IOS #2029980

Title: Maintenance but not refinement of receptive field size in ferret primary visual cortex requires visual experience.

Authors: *P. F. FERNÁNDEZ[1,2], K. SUDANA[3], E. BEECH[3], V. SUÁREZ CASANOVA[4], S. V. GRISWOLD[4], S. D. VAN HOOSER[4], S. L. PALLAS[3];
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Abstract: The effect of visual experience on the development of receptive fields in visual cortex has been studied extensively in species with advanced vision, such as carnivores and primates. In those species, light exposure during development is necessary for receptive field refinement. More recently, studies have focused on rodent species with simpler visual systems. In crepuscular Syrian hamsters, receptive fields are fully refined before adulthood in both normal and dark reared conditions. However, in hamsters kept in the dark from birth, receptive fields re-enlarge in adulthood. Thus, in hamsters, visual experience plays a role in the maintenance, but not the refinement, of primary visual cortex (V1) receptive field sizes. In contrast, in nocturnal mice *(Mus musculus)*, although receptive fields gradually refine by puberty as in other species, refinement is maintained long into adulthood in both normal and dark reared mice (Fernández-Aburto et al., 2023). This suggests that in mice, unlike hamsters, visual experience is not necessary for either the refinement or the maintenance of V1 neuron receptive field size. In order to better understand how the development of receptive fields is influenced by ecological factors and phylogeny we studied the ferret (*Mustela furo*), a crepuscular carnivore species with a well-developed visual system. We hypothesized that ecological niche defines the need for visual experience in development of receptive fields more than phylogeny does, in order to match the need for visual experience with the degree of light exposure. We investigated the changes in receptive field (RF) size of V1 neurons (layer 2/3) in anesthetized ferrets at 45, 100, and 200 postnatal days (pnd). Our preliminary data show that V1 RF sizes are unrefined at 45 pnd in both normal (mean NR RF area = 93.8 ± 24.5 deg^2, n=21) and dark reared ferrets (mean DR RF area = 61.7±13.4 deg^2, *n*= 25), with a trend to show refined RFs at 100 pnd in both normal (mean NR RF area = 11.1 ± 1.64 deg^2, n=6) and dark reared ferrets ((mean DR RF area = 39.8 ± 4.52 deg^2, *n*=93). At 200 pnd, RFs in dark reared ferrets were larger than in normally reared ferrets (mean NR RF area = 75.8 ± 7.83 deg^2, n=40; mean DR RF area = 65.9 ± 10.5 deg^2, *n*=32). Overall, the role of visual experience in developmental refinement and maintenance of ferret V1 RFs in layer 2/3 resembles what we observed in hamsters but differs from our mouse data, suggesting that light exposure during the development is crucial for crepuscular species, regardless of their phylogenetic positions. This study is a significant step in understanding the role of visual experience in the development of visual processing in commonly studied species.

Disclosures: P.F. Fernández: None. **K. Sudana:** None. **E. Beech:** None. **V. Suárez Casanova:** None. **S.V. Griswold:** None. **S.D. Van Hooser:** None. **S.L. Pallas:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.12/A66

Topic: A.08. Development of Neural Systems

Support: OCENW.KLEIN.535

Title: Spontaneous activity in the visual cortex of neonatal mice is modulated by basal forebrain cholinergic activity

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Abstract: Development of sensory cortices depends on patterns of spontaneous activity, even before sensory inputs arrive, as in the mammalian visual cortex before eye opening. During these early stages of life, neuromodulators such as acetylcholine also play a crucial role in synaptogenesis and the development of neural circuits. Later in life, acetylcholine mediates attention and arousal, enhancing visual responses in the adult primary visual cortex. However, the functional role of acetylcholine in modulating early patterns of neuronal activity remains unclear. Here, we characterized the relationship between acetylcholine dynamics and neuronal activity in the developing mouse visual cortex using genetically encoded sensors and in vivo widefield and two-photon imaging. We found that increased levels of acetylcholine were correlated with periods of decreased and less synchronous activity in neurons of the primary visual cortex. At the network level, high levels of acetylcholine were also associated with changes in the size and propagation of neuronal activity patterns between the primary visual cortex and higher visual areas. By monitoring the body and facial movements of the mice, we found that the periods of high acetylcholine corresponded with active behavioral states as well as twitches and facial movements during putative sleep states. Pharmacological manipulation of the cholinergic signal showed that blocking of muscarinic receptors with atropine in the visual cortex increased the size and synchronicity of spontaneous events. Finally, we modulated both acetylcholine levels and patterns of spontaneous activity in the visual cortex by expressing excitatory and inhibitory DREADDs in cholinergic neurons of the basal forebrain. Our results demonstrate that acetylcholine plays a functional role in the visual cortex even before eye opening by shaping spontaneous activity patterns that are necessary for the proper development of visual cortical areas.

Disclosures: D. Cabrera Garcia: None. **J. Abeje:** None. **C. Lohmann:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

Location: MCP Hall A

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Topic: A.08. Development of Neural Systems

Support: Clarendon Fund
Royal Society
MRC

Title: Differential circuit and molecular profile of somatostatin interneurons in postnatal mouse neocortex across development

Authors: *I. LAZARTE[1], E. NTAOUKA[1], L. GEYER[2,3], J. HJERLING LEFFLER[4], S. J. B. BUTT[1];
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Abstract: The intricate architecture of the mammalian cortex is characterized by the remarkable diversity of neuronal populations that underlie its function. Understanding the processes governing the development and function of these neural circuits has been a longstanding pursuit in neuroscience research. Recently, our group has made significant efforts in elucidating the physiological and molecular underpinnings of cortical interneuron development, specifically focusing on the differences between two critical regions of the cortex: primary visual cortex (V1) and primary somatosensory cortex (S1). Based on our combined electrophysiology and RNA sequencing of single neurons experiments with Patch-seq, we found evidence suggesting that V1 Layer 5 (L5) somatostatin-expressing (Sst) interneurons exhibit distinct developmental trajectories compared to their counterparts in S1 L5 Sst neurons. These findings challenged previous assumptions and highlighted the nuanced nature of cortical interneuron development, particularly in the context of region-specific molecular markers and gene expression patterns. Crucially, one of the most prominent molecular markers identified in this context is EphA4, a member of the Eph receptor family known for its role in axon guidance and neuronal circuit formation. To delve deeper into EphA4's role, we knocked out EphA4 in Sst interneurons and characterized their physiology, local inhibitory inputs, and thalamic inputs. We also examined the population dynamics of cortical neurons across development. Our results offer valuable insights into the molecular mechanisms underpinning cortical interneuron development, with broader implications for understanding neurodevelopmental disorders and neural circuit plasticity.

Disclosures: I. Lazarte: None. **E. Ntaouka:** None. **L. Geyer:** None. **J. Hjerling Leffler:** None. **S.J.B. Butt:** None.

Poster

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Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

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Topic: A.08. Development of Neural Systems

Support: R01 EY030893-05
NIH Training Grant (LK) 5T32EY025187-07

Title: Stereotyped Patterns of Sequential Activity in Neonatal Visual Cortex

Authors: *L. KETTLEWELL[1], A. J. SEDERBERG[2], G. B. SMITH[3];
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Abstract: Visual perception relies on precise network organization in the visual cortex. Very early in development, even prior to eye-opening, activity patterns in the ferret visual cortex exhibit modular structure and long-range spatial organization. The spatial structure of these early long-range networks may serve as a precursor for mature network organization. However, the temporal features of these early networks, which may contribute to their continued plasticity and development, remain largely uncharacterized. To address this, we imaged ongoing spontaneous network activity in the developing ferret visual cortex utilizing a fast calcium indicator (GCaMP8m) and widefield imaging at high temporal resolution (50Hz). Using this approach, we captured hours of spontaneous activity consisting of thousands of identified spatio-temporal sequences (mean=1352 sequences, n=6 animals). The spatial structure of this activity was highly modular, exhibiting spatially segregated active domains consistent with prior work, and highly complex temporal dynamics. We found that the majority of activity sequences showed a clear dynamic component in which modules activate sequentially across the field of view (mean=91.4%, CI=81.5%-95.7%, n=6 animals), and we only rarely observed instances of large modular events emerging as a single instantaneous pattern. Most dynamic activity sequences exhibited complex and non-linear propagation, with only a minority well-fit with a linear traveling wave (mean=35.6%, CI=24.0%-51.5%, n=6 animals). Notably, we found that spatiotemporal sequences occur in repeated and stereotyped motifs, reoccurring across hours of imaging (median=90.0%, IQR=81.6-96.4%, n=30 clusters from 5 animals). In addition, spontaneous activity appeared to progress through highly similar spatio-temporal trajectories across multiple events. By identifying the most frequently occurring single-frame spatial activity patterns, we found that we could successfully predict the spatiotemporal activity both preceding and following these conserved states (-300ms to 1000ms, n=5 animals). Intriguingly, the trajectories within groups of sequences sharing a common single-frame activity pattern were more coherent following a conserved activity state than preceding it, suggesting that activity trajectories may proceed along a subset of possible states. Together, our results demonstrate that

spontaneous activity in the early developing cortex exhibits a rich spatiotemporal structure, providing important constraints for models of cortical development and plasticity.

Disclosures: L. Kettlewell: None. **A.J. Sederberg:** None. **G.B. Smith:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: A.08. Development of Neural Systems

Support: NIH Grant R01DC019814
NIH Grant R01DC004199

Title: GABA co-release at glycinergic synapses supports the development of a specialized functional synaptic architecture

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Abstract: Gamma-aminobutyric acid (GABA) and glycine are the prevalent inhibitory neurotransmitters in the brain and spinal cord and are usually released from distinct neuronal populations. During development, however, neurons in many brain areas release both GABA and glycine, but the developmental function of this co-release has remained obscure. To shed light on this question, we investigated the maturation and refinement of the auditory brainstem pathway from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO). MNTB-LSO synapses transiently co-release GABA and glycine before hearing onset, when the pathway undergoes refinement and synaptic maturation. To disrupt GABA co-release, we used knockout mice of either sex in which MNTB neurons lack both GABA-synthesizing enzymes, GAD67 and GAD65 (dKO mice). Analysis of MNTB-evoked synaptic responses in LSO neurons recorded in slices revealed that loss of GABA co-release had no effect on the normal developmental elimination or strengthening of MNTB fibers. However, the loss of GABA co-release led to striking changes in the functional architecture of MNTB-LSO synapses. dKO mice showed a 67% increase in miniature event amplitudes due to a 43% increase in postsynaptic glycine receptors, a 27% decrease in the number of release sites, and a 42% decrease in readily releasable vesicle pool (control: 13-22 neurons, dKO: 13-22 neurons). While these changes had no effect on synaptic transmission at low-frequency stimulation, they significantly degraded the strength, fidelity, and temporal accuracy of synaptic transmission under physiologically realistic high-frequency stimulations (50 Hz) (control: 18 neurons, dKO: 21-22 neurons). These results demonstrate the crucial role of GABA co-release in the maturation of the functional architecture

of glycinergic MNTB-LSO connections, which enables reliable synaptic transmission at high and ongoing activity levels that typically occur *in vivo*.

Disclosures: J. Lee: None. **K. Kandler:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

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Program #/Poster #: PSTR380.16/A70

Topic: A.08. Development of Neural Systems

Support: NIH Grant EYEY034310-01A1

Title: Investigating the role of GABAergic interneurons in the maturation of neocortical state dependent activity and cortical activity modulation

Authors: *J. BARNETT[1], R. BATISTA-BRITO[2];
[1]Albert Einstein Col. of Med., Bronx, NY; [2]Neurosci., Albert Einstein Col. of Med., New York, NY

Abstract: The intricate development of neocortical circuits in humans spans over two decades, from the perinatal settling of newly born neurons to the refinement of synapses that extends into early adulthood. A remarkable aspect of this protracted developmental process is the dynamic interplay between emerging neuronal subtypes and their activity, a process crucial for shaping mature brain function. Among these neuronal populations, Vasoactive Intestinal Peptide (VIP) neurons, a type of GABAergic interneuron, hold particular significance, as they have been implicated in the regulation of locomotion and behavioral arousal in adult mammals. Similarly to most neuron subtypes, the activity patterns of VIP interneurons in mice undergo profound changes between the postnatal stage to adulthood.
Within the cortical sensory areas, a critical developmental time period occurs during the transition from preparatory stages for sensory information processing to the integration of external stimuli during active sensing. For instance, in mouse pups, this transition surrounding eye opening at postnatal day 14 is critical. In humans, the last trimester of pregnancy corresponds to the first postnatal weeks in mice and is a critical time for cortical circuit assembly, and a highly sensitive period for neurodevelopmental disorders. This transition from innate neural predispositions to sensory-driven responses highlights the dynamic nature of cortical circuit development in early postnatal life.
Emerging evidence suggests that cholinergic input plays a crucial role in modulating the activity of VIP and other GABAergic interneuron populations. Specifically, cholinergic signaling has been shown to preferentially project to GABAergic interneurons, including VIP interneurons, in various cortical regions, especially during intense periods of neuronal development. We hypothesize that cholinergic input serves as a key regulatory signal in the maturation and

function of VIP interneurons during cortical development, impacting behavioral modulation in the visual cortex at eye-opening in mice.
We will use a variety of novel, *in-vivo* tools such as dual color two-photon calcium imaging and electrophysiology to investigate the interplay of cholinergic and VIP interneuron activity. This study can help elucidate the role of cholinergic signaling in the maturation of cortical circuits via GABAergic interneuron activity.

Disclosures: J. Barnett: None. **R. Batista-Brito:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

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Topic: A.08. Development of Neural Systems

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UNAMPAPIIT-IN210823.
CONAHCyT. Ciencia de Frontera, Paradigmas y Controversias de la Ciencia 2022. 319880.

Title: Ontogeny of the thyrod hormone signaling in the retina of zebrafish: effects of the thyroidal status on retinal morphology and color preference.

Authors: *I. LAZCANO[1], S. PECH-POOL[1], A. OLVERA VIDAL[1], V. DARRAS[2], A. OROZCO[1];
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Abstract: Thyroid hormones (THs) regulate several processes of development including that of the retina. Experimental evidence has shown that disruption of the thyroidal status affects retina development from fish to mammals, but the mechanisms involved in this disarray are far from being elucidated. In the present study, we investigated the expression profile of genes involved in TH signaling during early retinal development in the zebrafish *(Danio rerio)*. We further analyzed the impact of thyroidal status on retinal morphology and color preference in the zebrafish larvae. To conduct ontogeny analyses, we microdissected zebrafish retinas at 3, 4 and 5 days post-fertilization (dpf) and analyzed the expression of TH receptors *thraa, thrab, thrb*, TH metabolizing deiodinases *dio2 and dio3b,* and the transmembrane transporter *mct8* using qRT-PCR. For thyroid status experiments, we used 5 µM of iopanoic acid (IOP) and 0.025 µM T3 to reduce or increase TH bioavailability, respectively during the embryonic to larval stages (0 to 5 dpf). At the end of the treatments, larvae were submitted to a color preference paradigm and/or euthanized for retinal histological analysis. In general, the elements of thyroid signaling tended to increase their expression from 3 to 5 dpf, however *dio3b* expression showed the most dramatic

changes, increasing 5-fold at 4 dpf and up to 15-fold at 5 dpf, when compared to 3 dpf. These results show that all TH signaling components are upregulated during retina development. IOP and T3 selectively affected the development of retinal cell layers. T3 induced an increase in thickness of the ganglion cell layer and a decrease of the outer nuclear layer; while IOP treatment slightly decreased the thickness of the outer nuclear layer. Compared to the control group, T3 significantly decreased red color preference by 34% while IOP increased green and yellow color preference by 78% and 48% respectively, suggesting that a TH-signaling alteration could result in a visual impairment. Together, these results highlight the critical role of thyroid hormone signaling during retinal development in zebrafish.
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Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: A.08. Development of Neural Systems

Support: NSF Grant 2212591

Title: Direction selective tectal and midbrain tegmental neurons in the Xenopus tadpole

Authors: *K. ZHENG, U. G. UDOH, K. G. PRATT;
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Abstract: There are two direct projections from the tadpole retina to the midbrain: the retinotectal projection and the retinotegmental projection. The well-studied retinotectal projection is considered homologous to the mammalian superior colliculus (SC). The retinotegmental projection is most likely the accessory optic system (AOS), a highly conserved retinofugal projection which has been described across a wide range of vertebrates, from adult frogs to humans. The tegmental neurons of the AOS are known to be direction selective. They are activated optimally by slow velocity motion and, in turn, elicit compensatory optokinetic and optomotor reflexes - eye and head/body movements, respectively, that stabilize the visual world as the organism moves through space. If the tegmental neurons of the tadpole midbrain are part of the AOS, they should be direction selective. Hence, in this study, we measured direction selectivity expressed by tadpole tegmental neurons and, for comparison, tectal neurons. For this, slow-moving bars of light moving in four directions (up, down, left, right) were projected onto one eye of the tadpole, and resulting synaptic responses recorded (whole-cell voltage-clamp) from individual neurons in the contralateral tectum and tegmentum. Recordings were carried out

between developmental stage 42 (approximately 5 days postfertilization, when the retinal ganglion cell axons have just begun to innervate their midbrain targets), to developmental stage 48/49 (10-18 days postfertilization). A direction selectivity index (DSI) for each orientation was calculated for each neuron. A DSI > 0.15 was considered direction selective. Based on this threshold, approximately 45% of both tectal and tegmental neurons recorded were determined to be direction selective. This percentage appeared consistent across the developmental stages studied. The majority of tectal neurons were observed to be direction selective across the horizontal (left-right) orientation, while the majority of tegmental neurons were observed to be direction selective across the vertical (up-down) orientation. The finding of direction selective tegmental neurons is consistent with them being part of the AOS. We also identified direction selective neurons in the optic tectum, which may be homologous to the direction selective neurons of the mammalian SC that are known to be associated with saccades. Also, our finding that direction selectivity is present as soon as the retinal ganglion cell axons innervate their respective targets (stage 42) in both the tectum and tegmentum suggests that direction selectivity is achieved by molecular cues instead of activities.

Disclosures: K. Zheng: None. **U.G. Udoh:** None. **K.G. Pratt:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

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Program #/Poster #: PSTR380.19/A73

Topic: A.08. Development of Neural Systems

Support: NIH 1 R03 MH127401-01

Title: Developmental trajectory of astrocytes and parvalbumin cells in Shank3 mouse model of autism

Authors: *S. DILLON[1,2], N. HARDING[3], G. QUINTANA[3], K. SOH[3], M. LEHAR[3], T. DEEMYAD[3];
[1]Johns Hopkins Univ. Otolaryngology Res., Baltimore, MD; [2]Otolaryngology-HNS, Johns Hopkins University, Baltimore, MD; [3]Otolaryngology-HNS, Johns Hopkins Univ., Baltimore, MD

Abstract: Experience-dependent plasticity shapes primary senses in the brain through changes in neural cells, including excitatory pyramidal cells, inhibitory interneurons, and astrocytes. This process is vital for understanding neurological conditions like autism spectrum disorder (ASD). Cortical astrocytes, for instance, boost neuron efficiency, enhancing sensory processing, while GABAergic interneurons like PV interneurons are crucial for network precision and functions such as working memory and social interactions. Shank3, a key gene in autism, influences these cells, with reports of reduced PV cells and increased astrocytes upon mutation. Despite this, the

effects of the Shank3b isoform on the morphological maturation of astrocytes and PV cells in cortical circuits are not well-defined. Using PALE labeling, electron microscopy and immunohistological assays, we examined these cells' development in the auditory and somatosensory cortices of Shank3b knockout mice, revealing significant temporal and spatial patterns. Our preliminary findings from Shank3b KO mice show a significant reduction in the number of PV cells and an increase in astrocyte cell body density, particularly in layers 4 and 5 of the auditory and somatosensory cortices, observable as early as P16. Additionally, Sholl analysis of astrocytes indicates a reduction in their distal processes, in proximity of patches of reduced PV cells. This suggests that the structural complexity of astrocytes and their perisynaptic territories is substantially altered without the Shank3b isoform. Collectively, these results imply that the absence of the Shank3b isoform may affect the maturation and possibly the interaction of PV cells and astrocytes within the cortical circuit of these specific cortices.

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Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.20/A74

Topic: A.08. Development of Neural Systems

Title: Ontogeny of structure and neuronal diversity of the spinal cord dorsal horn

Authors: *R. B. ROOME[1], L. FLORES[2], D. NARDINI[4], R. R. WACLAW[5], J. E. JOHNSON[3], A. LEVINE[1];
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Abstract: The nervous system is arguably the most complex organ system in the body by the sheer diversity of molecularly distinct neuron types. The spinal cord’s relative experimental tractability has placed it at the front of studies attempting to understand neuronal diversification, which occurs in the spinal cord by specification of progenitor domains within the neural tube (a consequence of opposing morphogen gradients), and by progenitors varying the neurons they produce over time, ultimately forming the dorsal and ventral horns. While this model explains the ontogeny of the ventral horn, it has yet to explain dorsal horn ontogeny: rather than remaining throughout neurogenesis, the five dorsal-most progenitor domains transform into one large domain (dIL; dorsal interneuron-late) which produces almost the entirety of the dorsal horn, comprised of roughly two-thirds of spinal cord neurons. To begin forming a theory of dorsal horn ontogeny, we comprehensively birthdated spinal neurons using EdU at 12-hour

intervals throughout neurogenesis in separate cohorts of mouse embryos. This showed that the deepest excitatory neurons were born earliest and the most superficial excitatory neurons were born latest, while inhibitory neuron birth order followed a discontinuous pattern of laminar formation. We hypothesized therefore that the structure of the laminae is dependent on the orderly production of excitatory dIL neurons. Indeed, embryos absent of excitatory neurons (null for Gsx1 and Gsx2), but not inhibitory (null for Ptf1a) or sensory neurons (Pax3:Cre; Isl2:DTA), showed disorganized lamination. We next developed a single-cell RNA-sequencing database of 95000 embryonic spinal neurons, showing six progressively-born families of excitatory and inhibitory dIL neurons respectively. These families could be separated into neuron subtypes which varied in their expression of Zic family transcription factors. We hypothesized that Zic genes pattern dIL progenitors and found that embryos null for Zic1/4 are deficient in Zic-high neuron variants and abundant in Zic-low variants. Comparing these data with adult single-cell RNA-seq atlases, we can reconcile all spinal neuronal diversity with developmental processes, producing a comprehensive theory of spinal cord neuron diversification.

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Poster

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Topic: A.08. Development of Neural Systems

Support: NIH Grant R90DA060338
UChicago Neuroscience Honors Program

Title: The Role of Hox Genes in the Differentiation of Serially Homologous Mechanosensory Circuits in Drosophila Larvae

Authors: *G. HU[1], E. HECKSCHER[2], D. VASUDEVAN[1];
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Abstract: Neural stem cells differentiate into neural subtypes to create unique circuits along the anterior-posterior axis. The diversity of these neural circuits is critical to enabling organisms to react to their environments. Yet, the basis for how this variety of neural circuits emerges from repetitive stem cells is a relatively unknown area. This project uses Drosophila larvae to understand the role Hox genes play in determining unique sensory encoding along the A-P axis. The Drosophila ventral nerve cord is a segmented spinal cord equivalent. Each segment contains differentiated neurons arising from one of 30 neural stem cells. This project will be focusing on interneurons which are the progeny of one neural stem cell, neuroblast 3-3. These interneurons

are Even-skipped(+) and are known as Even-skipped lateral interneurons, or ELs in Drosophila. Even-skipped interneurons are sensory processing and are conserved across flies, frogs, and mice. Despite originating from the same stem cell, ELs in abdominal segments A1 and A2 respond uniquely to mechanosensory stimuli. Segment A1 responds to low and high frequencies while segment A2 only responds to low frequencies, indicating differences in sensory encoding along the AP axis. Hox genes are a potential regulator of this A-P diversity due to their role in A-P patterning. Different Hox genes are expressed in A1 (Ubx) and A2 (AbdA). To test their role, we aim to overexpress and knockdown AbdA expression in ELs using the UAS-Gal4 system. Using Calcium imaging at different frequencies of vibrational stimuli, we will assay EL activity. We expect that overexpression of AbdA will result in segment A1 showing similar responses to segment A2. On the other hand, the knockdown of AbdA could either shift the Ubx domain or the AbdB domain. This could result in segment A2 responding to high-frequency stimulus similarly to A1 or a neuronal response pattern that reflects the activity of terminus ELs regulated by AbdB. Differences in activity between AbdA mutants and wildtype Drosophila will inform us about the role Hox gene expression plays in the differential A-P sensory encoding observed in ELs along the ventral nerve cord. This has implications for how different sensory stimuli are encoded differently in various regions of the VNC.

Disclosures: G. Hu: None. **E. Heckscher:** None. **D. Vasudevan:** None.

Poster

PSTR380

Molecular Mechanisms and Circuit Development in Sensory and Neural Systems

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Topic: A.08. Development of Neural Systems

Support: Moorfields Eye Charity
Research to Prevent Blindness
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Title: Developmental expression of Vax2 and Cyp26a1/c1 in the zebrafish retina

Authors: *C. OLMOS[1], T. YOSHIMATSU[2];
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Abstract: The central vision originates from a high-acuity area (HAA) of the retina or the fovea in humans. Despite the importance of the HAA for our vision, developmental mechanisms underlying the formation of the HAA remain unclear. To investigate the roles of retinal axis formation in specifying the location of the HAA, we evaluated the expression of region-specific genes in the presumptive HAA location during development in zebrafish. We performed in-situ hybridization (ISH) in the retina of larval zebrafish at 2.5, 3 and 5 days post fertilization (dpf)

and in the wholemount retina from adult zebrafish to evaluate the distribution pattern of Vax2, Cyp26a1 genes. Vax2 is a transcription factor responsible for the formation of the ventral region, whereas Cyp26a1 is specifically expressed in the middle along dorsal-ventral axis, forming a horizontal stripe. We found that, at 2.5 dpf, the expression of the vax2 and cyp26a1 are mutually exclusive, forming a sharp boundary between vax2 and cyp26a1 positive domains. However, we found that this boundary disappears by 3 dpf. Vax2 positive domain becomes smaller and shifts temporally while cyp26a1 domain expands, resulting in the formation of vax2 and cyp26a1 double positive region in the presumptive HAA. To test the necessity of vax2 in regulating the location of the cyp26a1 positive domain, we generated vax2 mutant zebrafish using CRISPR/Cas9. We aim to investigate the cyp26a1 expression patterns in vax mutants. As Cyp26a1 regulates an aspect of the HAA specialization, specifically suppression of rod photoreceptor genesis in the HAA, we will further examine whether the location of the HAA changes in vax2 mutants.

Disclosures: C. Olmos: None. **T. Yoshimatsu:** None.

Poster

PSTR381

Comparative Neuroanatomy and Neurodevelopment Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.01/A77

Topic: A.10. Development and Evolution

Title: Establishing the Pig as a Translational Animal Model for Neurodevelopment

Authors: *L. T. SUTKUS[1], Z. LI[2], R. N. DILGER[3];
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Abstract: Across the field of neuroscience, translational animal models are widely utilized to investigate neurodevelopmental processes. One remaining challenge in comparative species studies is establishing a direct translational timeline for each developmental event from conception through birth and adulthood. In 2001, Clancy and colleagues proposed the "Translating Time" model that included 9 mammalian species and estimated the timing of 95 empirically derived neural events, providing a comprehensive formula for direct translation of developmental markers across species. However, a notable omission from this widely accepted model was the domestic pig. For decades, the domestic pig has been an important preclinical animal model for studying neurodevelopment, and yet the direct translation between humans and pigs remains ambiguous. Therefore, there is a need to include the domestic pig into this model to further establish the pig as a translational animal model for neurodevelopment. Following the methods described by Clancy and colleagues (2001), an extensive literature review was conducted to identify the post-conception date (PCD) of as many neurodevelopmental events as

possible in the domestic pig. A total of 28 events were identified and the corresponding PCD was utilized to calculate a species-specific constant, resulting in a range of values from 1.5-3.2 for the domestic pig. Given this range, we optimized the pig's species constant through minimizing the error sum of squares (SSE) between predicted PCDs and actual PCDs using grid search and gradient descent approaches. Across both methods, the same species score of 2.151 was derived with an SSE of 2,565.72. This species score places the domestic pig between the cat (1.808) and the macaque (2.255), thereby reinforcing the translational power of the pig comparable to non-human primates. Furthermore, we modeled the domestic pig's PCDs to align with those of other established mammalian species, allowing direct temporal comparisons. This study further expanded the translating time model and solidified the domestic pig as a prominent translational animal.

Disclosures: L.T. Sutkus: None. **Z. Li:** None. **R.N. Dilger:** None.

Poster

PSTR381

Comparative Neuroanatomy and Neurodevelopment Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.02/A78

Topic: A.10. Development and Evolution

Support: Brains and Behavior Seed Grant (Georgia State University)
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Title: Patterns of cortical connectivity in primates and carnivores

Authors: *A. MURPHY[1], K. A. PHILLIPS[3], S. BROSNAN[4], L. A. PARR[5], A. V. KUKEKOVA[6], J. PARGETER[7], D. STOUT[8], C. SHERWOOD[10], T. M. PREUSS[9], S. BARTON[1], E. E. HECHT[2];
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Res. Ctr., [8]Emory Univ., Atlanta, GA; [10]Dept. of Anthrop. and Ctr. for the Advanced Study of Human Paleobiology, George Washington Univ., Washington, DC

Abstract: Many aspects of brain structure appear to be constrained by developmental processes. Our study analyzes how structural connections within the cerebral cortex differ across mammals in the context of two developmentally grounded hypotheses about brain connectivity: the rostrocaudal gradient hypothesis and the association network hypothesis. The rostrocaudal gradient hypothesis suggests that long distance connections should increase towards the rostral end of the brain. The association network hypothesis suggests that long distance connections should increase with greater distance from primary sensory regions of the brain. Here, we examine species differences in broad patterns in corticocortical connectivity by measuring spatial changes in connectivity across a variety of mammalian brains. Our dataset includes both primates, as well as carnivores, representing two clades which have independently evolved large and complex brains. DTI scans of capuchins, macaques, chimpanzees and humans were analyzed using probabilistic tractography. Voxelwise analysis was performed using white matter as a seed mask and cortical grey matter as a target mask. The resulting connectivity matrix was used to produce measures of streamline count and average path length from each voxel in cortex to the rest of cortex. These variables were measured along the rostrocaudal axis of the brain as well as with increasing distance from primary sensory regions (V1, S1, A1). We found that as brain size increased brains were increasingly characterized by association network patterns of connectivity. Comparisons with carnivore brains, including the silver fox and domesticated dogs, suggest that there may be several clade-specific characteristics of connectivity.

Disclosures: A. Murphy: None. **K.A. Phillips:** None. **S. Brosnan:** None. **L.A. Parr:** None. **A.V. Kukekova:** None. **J. Pargeter:** None. **D. Stout:** None. **C. Sherwood:** None. **T.M. Preuss:** None. **S. Barton:** None. **E.E. Hecht:** None.

Poster

PSTR381

Comparative Neuroanatomy and Neurodevelopment Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.03/A79

Topic: A.10. Development and Evolution

Support: Fred B. Snite Foundation

Title: Systematic review and meta-analysis of sex differences in the human fetal brain

Authors: M. A. PAULUS[1], J. E. SCHUMAN[1], ***L. ELIOT**[2];
[1]Chicago Med. Sch., Rosalind Franklin Univ. of Med. & Sci., North Chicago, IL; [2]Rosalind Franklin Univ. of Med. & Sci., North Chicago, IL

Abstract: Background: Sex differences in child neurobehavioral health suggest that male and female brains may differ early in development. We took advantage of recent advances for in-utero magnetic resonance imaging (MRI) to conduct a systematic literature review and meta-analysis of brain sex differences in human fetuses.
Methods: This study was pre-registered in the PROSPERO database of prospectively registered systematic reviews. A literature search in PubMed, yielded 4,054 studies published between 2002 and 2023. The studies were screened by two independent reviewers per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standards. Studies were included if either structural or functional MRI was used to image brains of healthy human fetuses in utero and at least some results were reported stratified by sex. After title and abstract screening, 457 studies remained for full-text screening, which resulted in 35 total studies meeting inclusion criteria. We focused our analysis on 24 of these that reported sex-disaggregated data on the same measure across three or more studies or independent samples.
Results: Gestational age ranged from 18 to 39 weeks. Pooled effect sizes (Hedges' g) revealed significantly larger male brains based on global volumes (total intracranial, total brain, and lateral ventricle; 8 studies, 11 independent samples) and linear measures (fronto-occipital, biparietal, and cerebellar transverse diameters; 4 studies) but inconsistent sex differences in cerebellar and hippocampal volumes and corpus callosum length. Among 12 studies reporting brain growth trajectories, 5 reported faster male growth, 5 found no sex differences, and 2 reported mixed results. Among 10 studies measuring resting fetal brain activity with fMRI, 5 reported no significant sex differences and 5 reported at least some significant sex differences, but there was little replication of the specific networks identified to differ between males and females.
Conclusion: This study found evidence for larger brain size and faster brain growth in human males compared to females before birth. However, existing studies have not identified reliable sex differences in regional brain structures or functional connectivity. These findings provide a reference for future investigations into sex differences in fetal brain development and underscore the need for larger and higher-resolution prenatal MRI studies.

Disclosures: M.A. Paulus: None. **J.E. Schuman:** None. **L. Eliot:** None.

Poster

PSTR381

Comparative Neuroanatomy and Neurodevelopment Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.04/B1

Topic: A.10. Development and Evolution

Support: Harvard Brain Science Bipolar Disorder Seed Program

Title: Left-right asymmetry of neuroanatomy in the mouse brain

Authors: *A. SILBERFELD[1], J. ROE[3,4], J. ELLEGOOD[5], J. P. LERCH[6], L. QIU[5], Y. KIM[7], J. LEE[1], W. D. HOPKINS[8], J. GRANDJEAN[9], Y. OU[10], O. POURQUIE[2];
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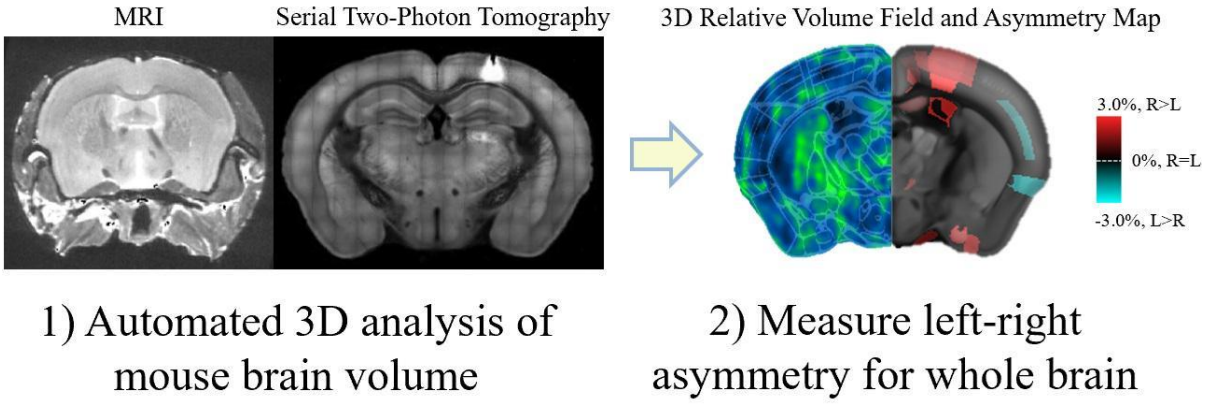
Abstract: Left-right asymmetry of the human brain is widespread through its anatomy and function. However, limited microscopic understanding of it exists, particularly for anatomical asymmetry where there are few well-established animal models. In humans, the brain shows a subtle, brain-wide series of regional asymmetries in cortical thickness or surface area, in addition to a macro-scale twisting in which the right hemisphere is shifted anteriorly relative to the left - the *cerebral petalia*. Here, we ask whether neuroanatomical asymmetries can be observed in mice, leveraging 7 different neuroimaging cohorts of animals from 6 different research groups (~3,500 animals). We found an anterior-posterior pattern of volume asymmetry in mice, where anterior regions are larger on the right while posterior regions are larger on the left. This pattern appears driven by a similar trend in surface area asymmetry and is supported by a concordant pattern of positional asymmetries. The cerebral hemisphere as a whole appears shifted anteriorly on the right while the brainstem and cerebellum appears shifted anteriorly on the left. These results together indicate a small torsion of the brain is present in mice, similar to the *cerebral petalia* in humans. Notably, these results are true across 2 fundamentally distinct image modalities: MRI and Serial Two-Photon tomography. To validate our analysis pipeline, we recapitulated well-established regional sex differences in the mouse brain. Additionally, we demonstrate that left-right flipping of the images prior to analysis indeed results in a flipping of the asymmetry pattern. By establishing a signature of anatomical asymmetry in the mouse brain, we aim to provide a foundation for future studies to probe the mechanistic underpinnings of anatomical brain asymmetry seen in humans - a feature of the brain with extremely limited understanding.



Disclosures: A. silberfeld: None.

Poster

PSTR381

Comparative Neuroanatomy and Neurodevelopment Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.05/B2

Topic: A.10. Development and Evolution

Title: Dense trunk tip muscle reconstruction in african and asian elephants revealed by automatic segmentation

Authors: *L. EIGEN[1], P. LADENBURGER[1], B. BRENCE[3], A. SHUBITIDZE[1], D. BAUM[3], T. HILDEBRANDT[4], M. BRECHT[1,2];
[1]BCCN Berlin, [2]NeuroCure Cluster of Excellence, Humboldt Univ. of Berlin, Berlin, Germany; [3]Zuse Inst. Berlin, Berlin, Germany; [4]Leibniz Inst. for Zoo and Wildlife Res., Berlin, Germany

Abstract: Elephants rely heavily on their dexterous trunks for daily life. This muscular hydrostat enables elephants to perform very intricate movements to achieve a diverse range of tasks, such as socializing, foraging, and moving objects. The trunk tip differs between African (*Loxodonta africana)* and Asian (*Elephas maximus)* elephants. While African elephants have one dorsal and one ventral finger, allowing them to pinch, Asian elephants have one dorsal finger and a ventral bulbous trunk tip lip, which they use to wrap around objects. We performed dense muscle fascicle reconstruction in three African and three Asian elephant hemi trunk tips with a combination of manual and automated segmentation on high-resolution microfocus tomography (microCT) scans to compare the muscle architecture in the trunk tips between these two elephant species. We found that the dexterous trunk tip in both species contains thousands of tiny muscle fascicles, which points to miniaturization in elephant dexterity, allowing very intricate and fine-tuned movements. In both species, muscle fascicles in the trunk tip are predominantly radial. The muscle architecture changes towards the distal trunk tip, becoming more radial and less longitudinal, and the trunk tip finger only contains radial muscle fascicles. Asian elephant trunk tips have more longitudinal muscle fascicles compared to African elephants and fewer transversal fascicles. We propose that these differences in muscular architecture can be attributed to the different strategies that are employed by these two species, namely wrapping versus pinching.

Disclosures: L. Eigen: None. **P. Ladenburger:** None. **B. Brence:** None. **A. Shubitidze:** None. **D. Baum:** None. **T. Hildebrandt:** None. **M. Brecht:** None.

Poster

PSTR381

Comparative Neuroanatomy and Neurodevelopment Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.06/B3

Topic: A.10. Development and Evolution

Support: DFG, German Research Foundation – EXC-2049 – 390688087
ERC Synergy Grant 'BrainPlay - the self-teaching brain' - 810580

Title: Elephant brain size and proportions

Authors: *M. SHAH[1], O. HEISE[2], P. BUSS[4], L.-M. DE KLERK-LORIST[5], S. HETZER[6,7], J.-D. HAYNES[9,8,10], T. HILDEBRANDT[11], M. BRECHT[3];
[1]Life Sci., Bernstein Ctr. for Computat. Neuroscience, Humboldt-Universität zu Berlin, Berlin, Germany; [2]BCCN, Berlin, [3]BCCN Berlin, Humboldt Univ. of Berlin, Berlin, Germany; [4]South African Dept. of Agriculture, Land Reform and Rural Develop. (DALRRD), Skukuza, South Africa; [5]Dept. of Agr., Land Reform and Rural Develop., Skukuza, South Africa; [6]Charite Univ. Medicine, Dept. Neurol., Berlin, Germany; [8]Berlin Ctr. for Advanced Neuroimaging, [7]Bernstein Ctr. for Computat. Neurosci., Berlin, Germany; [9]Charite - Univ. Med., Berlin, Germany; [10]Berlin Ctr. for Advanced Neuroimaging, Berlin, Germany; [11]Leibniz-Institut für Zoo- und Wildtierforschung, Berlin, Germany

Abstract: Elephants are the largest terrestrial animals, but our knowledge of their brains is limited. We studied brain size, proportions and development in Asian (*Elephas maximus*) and African Savanna (*Loxodonta africana*) elephants. Specifically, we weighed, photographed, and analyzed postmortem MR scans of elephant brains in addition to collecting elephant brain data from the literature. Despite their smaller body size adult Asian female elephants have substantially and significantly heavier brains (mean 5444 ± 939 g SD) than adult African savanna female elephants (mean 4253 ± 377 g SD). In line with their larger body size adult African savanna male elephants (mean 5764 ± 1274 g SD) have significantly heavier brains than of African female elephants; the brain weight of adult male Asian elephants remains unclear. Elephant brain weight increases approximately threefold postnatally. This postnatal increase that is similar to that of the human brain, but is larger than the postnatal growth seen in non-human primates. Asian elephants likely (but not certainly) have more cerebral cortical gray matter than African ones, whereas their cerebellum is relatively smaller (19.4% of total brain weight) than African elephants (22.9%). Our data add to the growing list of brain differences between Asian and African elephants and emphasize the need to collect more brain data from these animals.

Disclosures: M. Shah: None. **O. Heise:** None. **P. Buss:** None. **L. de Klerk-Lorist:** None. **S. Hetzer:** None. **J. Haynes:** None. **T. Hildebrandt:** None. **M. Brecht:** None.

Poster

PSTR381

Comparative Neuroanatomy and Neurodevelopment Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.07/B4

Topic: A.10. Development and Evolution

Support: NIH Grant R36MH135619-01

Title: Transcriptomics, development, and the parcellation of the human cerebral cortex

Authors: *L. KING[1], K. S. WEINER[2];
[1]Dept. of Neurosci., Univ. of California, Berkeley, Oakland, CA; [2]Psychology, Univ. of California, Berkeley, Berkeley, CA

Abstract: Introduction: Transcriptomic contributions to the functional and anatomical parcellation of the human cerebral cortex (HCC) has become a major interest in cognitive neuroscience. Prior research shows that active transcription of a small set of genes contributes to large-scale gradients and functional hierarchies in the HCC. Recent work also shows that different sets of genes contribute to anatomical and multimodal organization of regions throughout the HCC. While our work has largely explored the relationship between transcriptomics and parcellations of the adult HCC, here, we aim to examine the relationship among transcriptomics, development, and the parcellation of the HCC using the BrainSpan atlas (BSA). Methods: The BSA contains RNA sequencing samples from various brain regions across 31 developmental stages from 8 gestational weeks to 40 years of age. Using the top 200 genes identified in our previous work that have different molecular functions and biological processes contributing to the anatomical and multimodal parcellation of the HCC, we mapped the expression of these 200 genes in the developmental samples and applied distance metrics to examine how the expression of these 200 genes changes over time within and between regions. Results: Our preliminary data identify a potential cyclic pattern of expression change in which periods in late prenatal, late childhood, and late adolescent stages have the greatest difference in the expression of the 200 ‘adult’ genes compared to other stages. Furthermore, some functional regions seem to have similar developmental trajectories (such as M1 and S1) while other regions (such as V1) have unique developmental trajectories. Conclusions: Altogether, these preliminary findings suggest that the expression of important organizational genes in adulthood vary in their expression across development in a potential cyclic pattern that is either i) shared between a subset of regions or ii) unique to other regions.

Disclosures: L. King: None. **K.S. Weiner:** None.

Poster

PSTR381

Comparative Neuroanatomy and Neurodevelopment Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.08/B5

Topic: A.10. Development and Evolution

Support: R01DA054900
R25NS130965-02

Title: Single cell RNA sequencing reveals a largely conserved transcriptional landscape of the ventral pallidum across rodents and primates

Authors: *M. LYNCH[1,2], L. FANG[1], L. YANG[1], V. D. COSTA[3], V. K. SAMINENI[1], M. CREED[1];
[1]Washington Univ. in St. Louis, St. Louis, MO; [2]Neuroprep Postbaccalaureate Program, Washington University in St. Louis, St. Louis, MO; [3]Dept. of Behavioral Neurosci., Oregon Natl. Primate Res. Ctr., Beaverton, OR

Abstract: The ventral pallidum (VP) is a region within the basal ganglia that plays a critical role in regulating emotional processing and motivated behaviors. Neuronal subpopulations have been previously classified by their neurochemical and projection identities, but the molecular heterogeneity underlying the functional diversity of the VP has yet to be uncovered. Extensive behavioral pharmacology and circuit neuroscience studies in rodents have manipulated the VP and suggested that modulating this nucleus may be a viable target for disorders of reward processing (such as major depressive disorder or substance use disorders). However, whether the cell types of this nucleus are conserved in humans is unknown. Given their phylogenic similarity to humans, old-world monkeys are frequently used as models to study cognitive processes, including reward processing, relevant to humans. Here, we sought to bridge the gap between common rodent and primate model organisms with respect to VP cellular composition. We used snRNA-seq and multiplexed fluorescence in situ hybridization to define distinct cell type clusters in the VP in mice (Mus musculus), macaques (Macaca mulatta), and baboons (Papio anubis). We found a notable level of transcriptional similarity within molecularly defined neuronal cell types across all three species. Gabaminergic, glutamatergic, and cholinergic populations are represented in our cross-species conserved clusters which we validate using in situ hybridization. We have identified key neuropeptides, neurotransmitters, and neurotransmitter receptors within the VP that can be targeted in different neurochemically defined cells. We found no obvious topography to the identified cell types. Ultimately, our results provide a transcriptional, cross-species atlas for understanding the structure and function of the VP.

Disclosures: M. Lynch: None. **L. Fang:** None. **L. Yang:** None. **V.D. Costa:** None. **V.K. Samineni:** None. **M. Creed:** None.

Poster

PSTR381

Comparative Neuroanatomy and Neurodevelopment Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.09/B6

Topic: A.10. Development and Evolution

Support: Intramural Research Program of the National Institute of Mental Health

Title: Cytoarchitectonic characteristics of the amygdala complex of tarsiers (Tarsius)

Authors: *M. K. L. BALDWIN[1], A. C. CUMMINS[2], J. H. KAAS[3], E. A. MURRAY[4];
[1]Lab. of Neuropsychology, Natl. Inst. of Mental Hlth., Bethesda, MD; [2]LN NIMH NIH, Bethesda, MD; [3]Psychology Dept., Vanderbilt Univ., Nashville, TN; [4]Lab. of Neuropsychology, NIMH, NIH, BETHESDA, MD

Abstract: To better understand anatomical features that may contribute to functional differences of the amygdala across popular rodent and primate models in research, we are performing a comparative study to characterize the anatomy of amygdala nuclei across rodent and primate taxa. Tarsiers are key to this comparison because of their phylogenetic position; they diverged from the common ancestor of haplorrhine primates early in evolution. In addition, tarsiers have well-developed visual systems, features of which are shared with New and Old World monkeys. At the same time, however, tarsiers have retained many brain features present in strepsirrhines, including a small frontal cortex. Using tissue stained for Nissl, parvalbumin (Pv), and calbindin (Cb), we analyzed the lateral, basal, and accessory basal nuclei of the amygdala complex. These nuclei are easily identified across species, receive input from the visual system, and share connections with frontal and temporal cortex. The lateral nucleus contains densely packed small- to medium-sized Nissl-stained cells and is further distinguished from surrounding structures by dense Cb+ neuropil. Medium and large Pv+ cells are distributed throughout the lateral nucleus, whereas dense Pv+ stained neuropil is limited to the mediodorsal aspect of the nucleus. Nissl-stained sections reveal clear magnocellular (Bmc) and parvocellular (Bpc) divisions of the basal nucleus. The Bmc contains medium to large Pv+ cells and dense Pv+ neuropil, whereas the Bpc contains far fewer and smaller Pv+ cells with weak to no Pv+ neuropil staining. The accessory basal nucleus can be distinguished from the basal nucleus by darker Cb+ neuropil staining and dense medium to small Nissl-stained cells. Cb+ cells with large processes can be found throughout the basal and accessory basal nuclei. When comparing staining patterns in tarsiers with those observed in rats and macaque monkeys, clear differences are observed. First, similar to rats and unlike macaques, tarsiers lack a clear and large intermediate division of the basal nucleus. Second, unlike rats and similar to macaques, the density of Pv+ neurons within the basal nucleus of tarsiers is higher within the Bmc versus the Bpc. Overall, the highest number of Pv+ cells in tarsiers is in the lateral nucleus, followed by the Bmc; the Bpc and accessory basal nuclei have relatively fewer Pv+ cells. Differences in Pv+ cell distributions across taxa could reflect specializations of information processing associated with an expanded visual system and primate-specific interactions between the amygdala and temporal and/or frontal cortex.

Disclosures: M.K.L. Baldwin: None. **A.C. Cummins:** None. **J.H. Kaas:** None. **E.A. Murray:** None.

Poster

PSTR381

Comparative Neuroanatomy and Neurodevelopment Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.10/B7

Topic: A.10. Development and Evolution

Title: Comparative anatomy of perineuronal nets in fish and mouse

Authors: *E. GAHTAN, **A. ALYAN**;
Cal Poly Humboldt, Arcata, CA

Abstract: Perineuronal nets (PNNs) are extracellular matrix protein-saccharide structures encapsulating some neurons, notably parvalbumin (PV)-expressing GABAergic neurons, throughout the mammalian CNS. PNNs are thought to stabilize synapses, protecting them but limiting future plasticity, and they may be one mechanism of critical period plasticity, such as ocular dominance formation in visual neurons. Zebrafish are an important model organism with potential advantages for studying the role of PNNs in neural plasticity. However, we could not find published literature establishing the presence of PNNs in any fish, so we aimed to fill that gap. We labeled PNNs with Wisteria Floribunda Agglutinin (WFA), a standard method, in brain sections from adult mouse (as a positive control), zebrafish (of interest as a research model), and rainbow trout (to assess generalizability across fish), and co-labelled PV neurons using immunofluorescence to assess for colocalization. PNNs were identified based on WFA labeling intensity surrounding PV neurons or surrounding other, non-fluorescent neurons that were visible as gaps in the fluorescent extracellular matrix. As expected, in mouse cortex we observed PNNs around PV neurons. Of 4 mouse PV neurons documented, putative PNNs surrounded all 4 (100%). Zebrafish and trout brains showed similar levels of WFA-PV colocalization in sections from the pallium, tectum, and cerebellum. In zebrafish, 28 PV neurons were documented and all (100%) had putative PNNs. In trout, PNNs surrounded 20 out of 25 PV neurons (80%). Diffuse, net-like WFA labeling was found in mouse and fish brains, supporting the idea that PNNs form around diverse neuron types. Our results strengthen the hypothesis that PNNs exist in the brains of all vertebrates, but the key question is whether PNNs in fish have homologous functions in neural plasticity. This could be answered by adapting methods from rodent research and exploiting unique advantages of zebrafish methods.

Disclosures: E. Gahtan: None. **A. Alyan:** None.

Poster

PSTR381

Comparative Neuroanatomy and Neurodevelopment Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.11/B8

Topic: A.10. Development and Evolution

Support: DP1OD031273

Title: Comparative anatomy of the mammalian thalamus in rodents and marsupials

Authors: *S. PAL[1], N. GE[1], L. J. RICHARDS[2];
[1]Washington Univ. in St. Louis, St. Louis, MO; [2]Dept. of Neurosci., Washington Univ. in St. Louis, Saint Louis, MO

Abstract: The thalamus is the relay station of the brain, conveying sensory information to the neocortex. In contrast to the laminar patterning of the neocortex, thalamic neurons aggregate to form anatomically distinct groups of nuclei that differ from each other in function, connectivity, and molecular identity (Jones, 2003). Thalamic pre-patterning by a combinatorial expression of regulatory genes in the embryonic day (E) 12.5 mouse brain, sets up the framework for thalamic nuclei specification at E16.5 when expression patterns of these genes become refined and are retained postnatally (Nakagawa and O'Leary, 2001). Thalamocortical projections relay peripheral input to the neocortex in a sensory modality-specific manner. In addition, within each modality, the projections are topographically organized, thus generating area-specific maps in the postnatal cortex. These early thalamocortical interactions could be critical in shaping the proportions of area-specific circuits (Lopez-Bendito and Molnar, 2003). To understand how the basic anatomy of thalamocortical circuits might have evolved between different branches of mammals, we have studied a mouse-sized Australian marsupial, the fat-tailed dunnart (*S. crassicaudata*). Dunnarts are born at early stages equivalent to embryonic (E)day 10 in mice and seven weeks gestation in humans. Like other mammals, dunnarts have a six-layered cerebral cortex and undergo major stages of cortical development inside their mother's pouch (Suarez et. al., 2017). We examined the anatomy and gene expression of the thalamus of developing and adult dunnarts compared to mice. Using dunnart-specific riboprobes for known thalamic markers, we found that anatomically segregated groups of nuclei parcellate the dunnart thalamus, like the mouse. Combinatorial expression patterns of key genes enabled us to identify boundaries between first order (FO) and higher order (HO) thalamic nuclei, for each modality. A key point of difference between the dunnart and mouse was *Calb2* which is specific to HO thalamic nuclei in the embryonic and adult mouse. In dunnarts, *Calb2* labeled both FO and HO visual thalamic nuclei in the developing joeys, while in the adult, its expression was restricted to the HO visual nucleus. The fat-tailed dunnart therefore offers unprecedented advantages as a model to investigate how FO and HO nuclei differentiate during development and to identify changes in the evolution of sensory circuits.

Disclosures: S. Pal: None. **N. Ge:** None. **L.J. Richards:** None.

Poster

PSTR381

Comparative Neuroanatomy and Neurodevelopment Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.12/

Topic: A.10. Development and Evolution

Support: AU- CVM Animal health and disease research program
NIH R21HD101964

Title: Translating time: how old is a dog in human years

Authors: *E. J. MARSHALL[1], B. RIGBY DAMES[2], A. A. DE SOUSA[3], C. J. CHARVET[3];
[1]Auburn Univ., Auburn, AL; [2]Univ. of Bath, Bath, United Kingdom; [3]Col. of Vet. Med., Auburn Univ., Auburn, AL

Abstract: Aging is a complex process many species undergo. Dogs could be an excellent animal model for human disease because they live in similar environmental conditions to humans, have a shorter lifespan, and experience similar health-related diseases to humans. For example, dogs can develop cognitive dysfunction syndrome which is similar to dementia. Aligning dog age with those of humans could be crucial for advancing human research. We collected timepoints across pre- and postnatal ages to generate age alignments across humans and dogs. We draw from our long-term resource called Translating Time, which equates corresponding ages across humans and model systems. The dataset now includes more than 600 time points across mammals, including humans, non-human primates, rodents, and carnivores. From these data, we considered 119 timepoints collected from both various dog breeds and humans. These time points are collected across multiple tissue types, and include the age at which the organism transitions from 4-cell and 8-cell stages of embryonic development, age of tooth eruption, bone ossification, and onset of plaques and tangles. We fit a smooth spline through these data to predict human age based on dog age. According to this model, a 1-year-old dog is equivalent to a human in their early twenties, and a 5-year-old dog is equivalent to a human in their mid-thirties. Dogs develop very rapidly in comparison to humans, especially in their first year of life. Aligning ages between dogs and humans is vital to relate findings from humans to companion animals.

Disclosures: E.J. Marshall: None. **B. Rigby Dames:** None. **A.A. de Sousa:** None. **C.J. Charvet:** None.

Poster

PSTR381

Comparative Neuroanatomy and Neurodevelopment Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.13/B9

Topic: A.10. Development and Evolution

Title: Brn1/2 function in neocortical size determination and microcephaly

Authors: *S. BARAO[1], Y. XU[1], J. P. LLONGUERAS[1], R. VISTEIN[2], L. A. GOFF[1], K. J. NIELSEN[1], B.-I. BAE[3], R. S. SMITH[4], C. A. WALSH[5], G. L. STEIN-O'BRIEN[6], U. MULLER[1];
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Abstract: The neocortex varies in size and complexity among mammals due to the tremendous variability in the number and diversity of neuronal subtypes across species. The increased cellular diversity is paralleled by the expansion of the pool of neocortical progenitors and the emergence of indirect neurogenesis during brain evolution. The molecular pathways that control these biological processes and are disrupted in neurological and psychiatric disorders remain largely unknown. Here we show that the transcription factors BRN1 (POU3F3) and BRN2 (POU3F2) act as master regulators of the transcriptional programs in progenitors linked to neuronal specification and neocortex expansion. Using genetically modified lissencephalic and gyrencephalic animals, we found that BRN1/2 establish transcriptional programs in neocortical progenitors that control their proliferative capacity and the switch from direct to indirect neurogenesis. Functional studies in genetically modified mice and ferrets show that BRN1/2 act in concert with NOTCH and primary microcephaly genes to regulate progenitor behavior. Analysis of transcriptomics data from genetically modified macaques provides evidence that these molecular pathways are conserved in non-human primates. Our findings thus establish a mechanistic link between BRN1/2 and genes linked to microcephaly and demonstrate that BRN1/2 are central regulators of gene expression programs in neocortical progenitors critical to determine brain size during evolution.

Disclosures: S. Barao: None. **Y. Xu:** None. **J.P. Llongueras:** None. **R. Vistein:** None. **L.A. Goff:** None. **K.J. Nielsen:** None. **B. Bae:** None. **R.S. Smith:** None. **C.A. Walsh:** None. **G.L. Stein-O'Brien:** None. **U. Muller:** None.

Poster

PSTR381

Comparative Neuroanatomy and Neurodevelopment Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.14/Web Only

Topic: A.10. Development and Evolution

Title: Role of hnRNPs in neuroplasticity and neurological disorders: an evolutionary perspective

Authors: *S. JHA[1], N. M J[2];
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Abstract: The heterogeneous nuclear ribonucleoproteins (hnRNPs) are central regulators of several fundamental biological processes. Studies using model organisms, have implicated hnRNPs in transcriptional and post-transcriptional regulation, telomere maintenance, stem cell maintenance, among other processes. Our *In-silico* analysis suggested hnRNPs to be a regulator of basal and exercise-induced adult hippocampal neurogenesis, a form of neuroplasticity

implicated in normal brain function and several neurological disorders. Interestingly, further interactome studies identified hnRNPs as major hubs of protein interaction networks regulating psychiatric co-morbidities. Though hnRNPs are known to be conserved across eukaryotes, the evolutionary conservation/diversification of their functions across species is yet to be understood. Deciphering the evolutionary relationship between hnRNPs across species will help in understanding their roles in neuroplasticity and neurological disorders. To this end, we employed computational analyses to identify potential hnRNP orthologs in eighty eukaryotic species, and their interactors. A comprehensive analysis of the biological processes influenced by hnRNP interactomes is currently underway. Experimental studies in this regard would be of scientific and clinical importance, owing to the druggability of several human hnRNPs.

Disclosures: S. Jha: None. **N. M j:** None.

Poster

PSTR381

Comparative Neuroanatomy and Neurodevelopment Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.15/B10

Topic: I.03. Anatomical Methods

Title: Elucidating regional homologies of the hagfish brain by combining new tissue clearing and imaging methods with classic neuroanatomy techniques.

Authors: *M. RUDD[1], H. DONG[2];
[1]Neurobio., UCLA, Los Angeles, CA; [2]UCLA, Los Angeles, CA.

Abstract: Cyclostomes provide a unique window into the evolutionary history of vertebrate brains. Among the two extant classes, Myxini (Hagfish) and Hyperoartia (Lampreys), Lampreys have historically been used for comparative vertebrate studies. However, despite over a century of research on Hagfish, it is only with the recent sequencing of their genome that interest in their evolutionary significance has been reinvigorated. This resurgence is particularly pertinent to the study of vertebrate brain anatomy. By employing advanced fixation and tissue-clearing techniques alongside non-viral tracing, immunofluorescent staining, and Golgi-Cox staining, we are exploring the neuroanatomy of the Pacific Hagfish. Our approach allows for detailed examinations of regional connectivity, cell-type specific spatial molecular expression patterns, and neuron morphology. These insights enable us to draw new comparative conclusions regarding vertebrate neuroanatomy. Furthermore, the development of these combined methodologies demonstrates that tissue processing and imaging technologies have now advanced to the point where they can be effectively applied to non-genetically modified species to yield precise anatomical insights.

Disclosures: M. Rudd: None.

Poster

PSTR382

G-Protein Coupled Receptors

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.01/B11

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

Support: NIH R-01 DA032895

Title: Distinct populations of adrenergic receptors mediate norepinephrine induced PKA and PKC signaling in the nucleus and at the plasma membrane of mouse astrocytes

Authors: *J. MAGLASANG[1], P. J. GASSER[2];
[1]Marquette Univ., Milwaukee, WI; [2]Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: Location bias, the idea that GPCRs can signal from membranous organelles within cells to add another level of specificity and regulation, is an emerging phenomenon in G protein coupled receptor (GPCR) signal transduction biology. In astrocytes, norepinephrine (NE) exerts powerful influences on metabolic, neuroprotective, and immunoregulatory functions through activation of G-protein-coupled α- and β-adrenergic receptors. Until recently, NE-induced activation of adrenergic receptors has been assumed to occur exclusively at the plasma membrane (PM). However, we have recently identified functional β1-adrenergic receptors (β1-ARs) localized to the inner nuclear membrane in astrocytes and demonstrated that NE accesses and activates nuclear β1-ARs via transporter-mediated uptake. These findings suggest that the overall response of astrocytes to NE involves actions mediated by receptors at various subcellular locations. Here, we aim to determine the relative contributions of adrenergic receptor subtypes to NE-induced increases in protein kinase A (PKA) and protein kinase C (PKC) activity at the astrocyte nucleus and at the PM. To monitor kinase activity, cultured mouse astrocytes were transfected with ExRai-AKAR2 or ExRai-CKAR2, an excitation-ratiometric biosensor for PKA and PKC respectively, targeted to either the nucleus or to the PM. Transfected astrocytes were then treated with adrenergic agonists or antagonists while monitoring excitation ratio responses. NE induced rapid increases in PKA activity at both the nucleus and at the PM, whereas only rapid increases in PM PKC activity was observed. NE-mediated increases in nuclear PKA activity were completely abolished by preincubation with the β1-selective antagonist CGP 20712. NE mediated increases in PM PKA activity were not blocked by CGP20712, the β2-selective antagonist ICI 118, 551, the β3-selective antagonist L748, 337, or a combination of all three beta selective antagonists. Only a cocktail consisting of all three beta selective antagonists plus prazosin, an α1- adrenergic receptor antagonist, blocks the PM PKA response to NE. The α1- adrenergic receptor agonist phenylephrine and the α2- adrenergic receptor agonist dexmedetomidine were both equally able to induce rapid increases in PM PKC activity, similar to NE, whereas the β-adrenergic receptor agonist isoproterenol had no effect. These data suggest that NE induced signaling events at the astrocyte nucleus and PM are

initiated by distinct populations of adrenergic receptors, providing a powerful mechanism for location bias in adrenergic regulation of astrocyte biology.

Disclosures: J. Maglasang: None. **P.J. Gasser:** None.

Poster

PSTR382

G-Protein Coupled Receptors

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.02/B12

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

Support: NIH Grant R01- DA032895

Title: Intranuclear signaling pathways involved in norepinephrine-induced regulation of astrocyte gene expression

Authors: *B. FOSGITT[1], P. J. GASSER[2], J. MAGLASANG[2];
[1]Marquette Univ., Milwaukee, WI; [2]Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: Norepinephrine (NE) exerts powerful influences on metabolic, neuroprotective and immunoregulatory functions of astrocytes. These effects, mediated by G-protein-coupled α- and β-adrenergic receptors, include both rapid changes in enzyme activity and delayed changes in gene expression. Until recently, all effects of NE were believed to be mediated by receptors localized exclusively to the plasma membrane, but recent studies in cardiomyocytes have demonstrated that adrenergic receptors can also signal from intracellular membranes, including the nuclear membrane. We have identified functional β1-adrenergic receptors (β1-ARs) localized to the inner nuclear membrane in astrocytes and demonstrated that NE accesses and activates nuclear β1-ARs via transporter-mediated uptake leading to rapid increases in nuclear PKA activity. These findings raise the hypothesis that NE-induced regulation of astrocyte gene expression is mediated in part by nuclear β1-ARs. To begin to test this hypothesis, we examined the effects of NE on astrocyte expression of mRNA for PPP1R3C (Protein Targeting to Glycogen (PTG), a gene previously shown to be upregulated by NE. Preliminary data reveals that nuclear β1-ARs have roles in the regulation of this gene. In the present study, we tested the hypothesis that NE-induced increases in PTG mRNA expression are mediated by PKA. Primary mouse astrocytes were pretreated for 15 minutes with vehicle or one of two PKA inhibitors: KT5720, an inhibitor of the catalytic subunit, or Rp-cAMPs, a cAMP analog. After pretreatment, astrocytes were exposed to 1uM NE or vehicle for 15 min, followed by washing, incubation for two hours, and extraction of total RNA for mRNA quantification by qPCR. Astrocytes responded to a brief pulse of norepinephrine with robust increases in PTG mRNA which were not inhibited by pretreatment with either PKA inhibitor. We then tested the contributions of Epac (Exchange protein activated by cAMP) to NE-induced increases in PTG mRNA. Pre-treatment of astrocytes with the selective Epac inhibitor ESI-09 completely blocked NE-induced increases

in PTG mRNA. As other studies have shown that Epac can act by stimulating Akt activity, we examined the role of Akt in NE-induced gene expression. Pretreatment of astrocytes with MK2206, a potent and selective inhibitor of Akt, completely blocked NE-induced increases in PTG mRNA. These data suggest that NE increases PTG mRNA expression in astrocytes through activation of Akt via an Epac-dependent process.

Disclosures: B. Fosgitt: None. **P.J. Gasser:** None. **J. Maglasang:** None.

Poster

PSTR382

G-Protein Coupled Receptors

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.03/B13

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

Support: NIH Grant AG005214
DeArce-Koch Foundation

Title: Allosteric modulators of acetylcholine efficacy at M1 muscarinic receptors: novel mechanisms for therapeutic intervention

Authors: *W. S. MESSER, Jr.[1], S. VENTRESCA[2], C. WIDMAN[3], I. T. SCHIEFER[4], G. J. ELMSLIE[5], J. ELLIS[6];
[1]Pharmacol., Univ. of Toledo, Toledo, OH; [2]Univ. of Toledo, Toledo, OH; [3]Univ. of Toledo, Maumee, OH; [4]Medicinal and Biol. Chem., Univ. of Toledo, Toledo, OH; [5]Psychiatry and Behavioral Hlth., Pennsylvania State Univ. Col. of Med., Hershey, PA; [6]Psychiatry and Pharmacol., Penn State Univ., Hershey, PA

Abstract: Allosteric modulation of neurotransmitter activity at G protein-coupled receptors represents an attractive approach for therapeutic intervention. Most positive allosteric modulators (PAMs) increase neurotransmitter potency by enhancing the affinity of endogenous neurotransmitters. Allosteric modulation of neurotransmitter efficacy provides an alternative approach to increase receptor signaling, especially in neurological disorders associated with decreased receptor levels. We previously identified a novel series of compounds, including CW-6-65, that enhanced acetylcholine (ACh) efficacy at M_1 muscarinic receptors as measured by [^{3}H] arachidonic acid release. Well characterized M_1 PAMs such as BQCA did not enhance ACh efficacy. In the current studies, we used the TRUPATH system to assess whether CW-6-65 enhances ACh responses at the level of G protein signaling. Plasmids for human M_1 receptors were transiently co-transfected with plasmids for $G_{\alpha;q}$-RLuc8, $G_{\beta;3}$, and $G_{\gamma;9}$-GFP2 G protein subunits to generate a bioluminescence resonance energy transfer (BRET) signal in HEK 293T cells. Receptor activation by ACh was measured as a loss of BRET signal. At high levels of M_1 receptor expression, a robust BRET signal was measured in the absence of ACh that was reduced by ACh in a dose-dependent manner. BQCA and CW-6-65 (at 10 μM) did not alter the

magnitude of the ACh-stimulated loss of BRET signal. Co-transfection of G protein plasmids with a lower level of M_1 receptor plasmid (thereby reducing levels of receptor reserve) resulted in a maximal ACh response that was enhanced by 10 μM CW-6-65, but not by BQCA. Both CW-6-65 and BQCA exhibited intrinsic activity in the TRUPATH assay. The data suggest that the novel PAM of ACh efficacy CW-6-65 exerts its action, at least in part, through enhancement of G protein signaling. Allosteric modulation of ACh efficacy could provide a means for enhancing cholinergic activity in systems with impaired muscarinic receptor signaling, including Alzheimer's disease and schizophrenia.

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

Poster

PSTR382

G-Protein Coupled Receptors

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.04/B14

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

Support: NIH Grant AG005214

Title: Use of acetylcholine mustard to enrich the M_1 subtype of muscarinic receptors

Authors: *J. ELLIS[1], G. J. ELMSLIE[2];
[1]Penn State Univ., Hershey, PA; [2]Psychiatry and Behavioral Hlth., Pennsylvania State Univ. Col. of Med., Hershey, PA

Abstract: There are no brain regions that express a pure or nearly-pure population of the M_1 subtype of muscarinic receptors (mRs). However, the cortex and hippocampus are important brain regions that express a preponderance of the M_1 subtype (approx. 60%), along with a significant component of M_2 (<40%) and a minor component of M_4; M_3 and M_5 expression is negligible. The ability to reduce the M_2 and M_4 components in these regions, thereby enriching the M_1 component, could be useful in many studies. Mustard analogs of many receptor ligands have been used to irreversibly label receptors. In aqueous solution, mustard groups cyclize to form aziridinium ions that closely mimic quaternary amines. Such a reactive intermediate may therefore bind in the same orientation within the same binding pocket of the receptor that accommodates the related reversible ligand. Once oriented, it is capable of alkylating any of a number of nearby amino acid side chains. In principle, a mustard analog of an agonist might yield irreversible activation of the receptor; however, acetylcholine mustard (AChM) has been widely reported to possess agonist activity at mRs only until alkylation is achieved, at which point the receptor becomes inactivated. Contrary to these previous reports, we have found that

agonist activity persists after irreversible binding of AChM at all five subtypes of mRs. The Gq-linked subtypes (M_1, M_3, and M_5) were evaluated by measuring release of radiolabeled arachidonic acid from intact CHO cells. For the Gi-linked subtypes (M_2 and M_4), enhancement of the binding of the GTP analog GTPγ^{35}S was assayed in membranes of CHO cells. Notably, the M_2/M_4 PAM LY2119620 further enhanced these Gi responses in a dose-dependent manner. It is known that the Gi-linked mRs possess greater affinity for classical agonists, compared to Gq-linked mRs. Therefore, we investigated the relative potencies of AChM with LY2119620 at M_1 and M_2/M_4 mRs; as expected, the Gi-linked subtypes exhibited much greater sensitivity to these ligands in an assay measuring the inhibition of the binding of the non-selective antagonist [^{3}H]N-methylscopolamine. Furthermore, when we mixed membranes containing M_1 mRs with those containing M_2 (or M_4) mRs, then pretreated the membrane mixture with AChM/LY2119620 and washed the membranes by centrifugation, we dramatically enriched the proportion of the M_1 subtype in the mixture. Starting material that was 60% M_2 and 40% M_1 was restored to essentially 100% M_1; even material that was 75% M_2 and only 25% M_1 was restored to nearly 90% M_1. This approach could facilitate detailed investigations of the characteristics of M_1 mRs in clinically relevant conditions and brain regions.

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

Poster

PSTR382

G-Protein Coupled Receptors

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.05/B15

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

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

Title: Dopamine, acetylcholine, and adenosine evoke differential responsiveness across Medium Spiny Neurons

Authors: *A. KONOMI PILKATI[1], C.-F. BOWIN[3], M. Z. LEONARD[5], A. T. SÖRENSEN[2], J.-F. PERRIER[6], E. S. CALIPARI[5], K. L. MADSEN[7], U. GETHER[4];
[1]Copenhagen Univ., Kobenhavn NV, Denmark; [2]Ctr. for Neurosci., Copenhagen Univ., Kobenhavn N, Denmark; [3]Univ. of Copenhagen, Copenhagen, Denmark; [4]Univ. of Copenhagen, Copenhagen N, Denmark; [5]Vanderbilt Univ., Nashville, TN, ; [6]Univ. Copenhagen, Copenhagen, Denmark; [7]Univ. of Copenhagen, Fac. of Hlth. and Me, Copenhagen, Denmark

Abstract: The basal ganglia are a collection of subcortical nuclei that have been linked to movement, emotion, motivation, and learning. The striatum, which serves as the gateway to the basal ganglia circuit, receives dopaminergic neurons from the midbrain as well as other forms of input from various brain regions. The striatum is structurally and functionally segregated; the

dorsal striatum is involved in habit formation whereas the ventral striatum is in goal-directed actions. Its main cell type is the medium spiny neurons (MSN), further divided into dopamine D1-receptor (D1R-MSNs) and D2-receptor MSNs (D2R-MSNs). The D1R is considered to have less affinity to dopamine (DA) (responding at the µM range) as compared to the high-affinity D2R (nM range). It has accordingly been assumed that D1R-MSNs sense phasic release of DA while D2R-MSNs sense lower levels of tonic DA release. Here, we study D1R-MSNs sensitivity to DA in striatal primary cultures derived from D1-Cre mice pups or wild-type E19 rat embryos and in acute brain slices transduced and stereotactically injected with the genetically encoded protein kinase A (PKA) sensor ExRai-AKAR2 respectively. By using live imaging epifluorescence and 2-photon microscopy, we can track PKA activity in individual neurons with high temporal and spatial resolution. Strikingly, we observe that individual D1R-MSNs exhibit differential responsiveness to DA with a subpopulation of D1R-MSNs responding to nanomolar concentrations of DA while other D1R-MSNs require micromolar concentrations. The D1R-MSNs display a DA-response spectrum. This phenotype appears to be spatially segregated in the striatum and is seemingly characteristic of MSN when compared to other neuronal types. Surprisingly, the heterogenous responsiveness is not restricted to dopamine but is also present in other neurotransmitter stimulations such as acetylcholine and adenosine. In addition to revealing a so far unappreciated dynamic signaling heterogeneity of MSNs, the data challenge the classical assumption of low-affinity D1Rs and high-affinity D2Rs, and this could be potentially applied to other GPCRs. Further ongoing studies are targeted toward investigating the differential PKA dynamics in vivo through GRIN lens microendoscopic imaging and in vivo 2-photon imaging, as well as the putative role in DA-linked memory processes.

Disclosures: A. Konomi Pilkati: None. **C. Bowin:** None. **A.T. Sörensen:** None. **J. Perrier:** None. **K.L. Madsen:** None. **U. Gether:** None.

Poster

PSTR382

G-Protein Coupled Receptors

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.06/B16

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

Support: NIAAA R15AA027909 to ESO

Title: Behavioral and neuro-immune consequences of selective Cannabinoid CB2 receptor deletion from microglia and dopamine neurons in the effects of alcohol

Authors: *E. S. ONAIVI;
Biol., William Paterson Univ., Wayne, NJ

Abstract: There is mounting evidence supporting the involvement CB2 cannabinoid receptors (CB2Rs) in neuroinflammation associated with the effects of cannabinoids. The expanding

endocannabinoid system referred to as endocannabinoidome (eCBome) has been linked to the modulation of alcohol induced neuroinflammation. The discovery of CB2Rs in neurons and glia cells has raised questions regarding their role in regulating neuroinflammation and behavior. How CB2Rs modulate behavioral and neuroinflammation induced by alcohol was investigated in conditional knockout (cKO) mice with selective deletion of CB2Rs from dopamine neurons (*DAT-Cnr2*) and in separate group from microglia (*Cx3Cr1-Cnr2*) with C57BL/6J as wild type controls. Adult male and female mice from 3-5 months were used in the studies. Behavioral tests including motor function and alcohol preference tests were used to evaluate behavioral alterations induced by 8 and 16% alcohol. For preference testing, individually housed mice (N = 10 mice per group) were used following conditioning to two-bottle choice paradigm. The amount of alcohol consumed by each animal was recorded over five consecutive days between 10 and 11 AM. The ratio of alcohol to water consumed and the total fluid consumption, were calculated to obtain a preference ration. ELISA assay was used to investigate the level of pro-inflammatory cytokines, tumor necrosis factor- α (TNF-α), interleukin-6, (IL-6), interleukin-1α (IL-1α), and interleukin-1β, (IL-1β) in the striatum and prefrontal cortical regions of the animals. CB2R cKO mice with deletion of CB2Rs from dopamine neurons, DAT-*Cnr2* and those with deletion from microglia Cx3cr1-*Cnr2* displayed differential phenotypes. DAT-*Cnr2* cKO mice displayed hyper-psychomotor responses and were insensitive to the rewarding effects of alcohol but not to cocaine, whereas Cx3cr1-*Cnr2* cKO mice failed to display hyperactivity but were sensitive to the rewarding effects of alcohol and exhibited increased weight gain compared to the DAT-*Cnr2* and wild type (WT) controls. The results also showed that the cell-type specific deletion of CB2Rs and alcohol intake increased the proinflammatory cytokine in the striatum and prefrontal cortex. In summary the selective deletion of CB2Rs from either dopamine neurons or microglia differentially modifies behavioral effects with neuroinflammatory changes induced by alcohol. These findings suggest the involvement of CB2Rs in modulating the behavioral and neuroimmune alterations induced by alcohol may be potential therapeutic targets in CNS and alcohol use disorders associated with neuroinflammation.

Disclosures: E.S. Onaivi: None.

Poster

PSTR382

G-Protein Coupled Receptors

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.07/B17

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

Support: Fondecyt 1221030
Fondecyt 1161030
R01 DA038598

Title: A Heterodimer Complex between TAAR1 and 5HT1A receptors

Authors: *A. I. ROBLES[1], L. DINAMARCA[2], A. CACERES-QUEZADA[1], A. FIERRO[1], G. E. TORRES, Sr.[3];
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Abstract: The Trace Amine-Associated Receptor 1 (TAAR1) is a G Protein Coupled Receptor (GPCR) that displays potential as a therapeutic target for several psychiatric and neurological conditions. Previous studies have demonstrated that activation of TAAR1 can decrease the firing rate of dopaminergic or serotonergic neurons, leading to a reduction in extracellular monoamine levels. The modulation of firing rate in dopaminergic transmission occurs through the association of TAAR1 and D2 receptors, which form a heterodimeric complex. However, the molecular mechanism of firing rate modulation by TAAR1 in serotoninergic neurons remains unexplored. To investigate this question, we employed computational and biochemical methods to explore the potential dimer formation between TAAR1 and one of the main serotoninergic receptors expressed in raphe neurons, the 5HT1A receptor. Through computational approaches such as homology modeling, docking, and molecular dynamic simulations, we predicted a contact surface and an interaction network between both proteins with their respective ligands at the binding site. We found that both receptors form interactions between the transmembrane domains 5 and 6 of each receptor, which were stable during the simulations. Our predictions from computational studies were corroborated by co-localization studies and proximity ligation assay (PLA) in HEK-293 cells transfected with TAAR1 and 5HT1A. The description of this new interaction presents new opportunities to develop drugs targeting the TAAR1-5HT1A heterodimer to regulate not only the function of these neurons, but also mood disorders.

Disclosures: A.I. Robles: None. **L. Dinamarca:** None. **A. Caceres-Quezada:** None. **A. Fierro:** None. **G.E. Torres:** None.

Poster

PSTR382

G-Protein Coupled Receptors

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.08/B18

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

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

Title: Optogenetic endogenous serotonin transmission in mouse prefrontal cortex: acute impact and consequences of chronic perturbation

Authors: *S. POWER[1], D. SARGIN[3], E. K. LAMBE[2];
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Abstract: The medial prefrontal cortex is essential for cognition, executive function, and emotional behavior. Serotonergic afferents densely innervate the prefrontal cortex and pharmacological interventions targeting the serotonin system influence cognitive and emotional processing. While exogenous serotonin and related agonists regulate neuronal activity in this region, the neurophysiological consequences of synaptically-released endogenous serotonin have only begun to be characterized. Here, we use optogenetics and whole cell recording in brain slices from transgenic mice to probe the impact of endogenous serotonergic transmission in medial prefrontal cortex. First, we probe the frequency dependence of endogenous synaptic serotonin signaling in the major output neurons in prefrontal cortex, revealing strong frequency-dependent regulation. Next, we intervene pharmacologically to explore the interplay of serotonergic 5-HT_{1A} receptor-mediated inhibition and 5-HT_{2A} receptor-mediated excitation. Finally, we test the impact of acute and chronic SSRI treatment on signaling and the capacity for adaptation. Optogenetics gives a critical opportunity to test the acute impact of endogenous serotonin in prefrontal cortex and its response to chronic perturbation.

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

Poster

PSTR382

G-Protein Coupled Receptors

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.09/B19

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

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

Title: Bridging the Gap: Role of Serotonin on Cannabinoid Receptor Regulation

Authors: *G. CARRASCO;
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Abstract: The serotonin (5-HT) and endocannabinoid systems play crucial roles in regulating various aspects of neuronal function and behavior. 5-HT is involved in modulating mood, cognition, and several physiological processes. Cannabinoid receptors, particularly CB1R and CB2R, are part of the endocannabinoid system and mediate the physiological and psychoactive effects of marijuana-like compounds. Here, we studied the interplay between these two systems, which is paramount, as their dysregulation is implicated in various neurological and psychiatric disorders, such as drug abuse disorders. We investigated the effect of cocaine, which blocks the reuptake of monoamines including 5-HT into presynaptic terminals, on the expression of

cannabinoid receptors. We also sought to identify molecular mechanisms by which 5-HT_{2A} receptors may regulate cannabinoid receptor expression.
Rats were treated with either saline or cocaine (15mg/kg, bid,ip) for 7 days and sacrificed 48 h after the last cocaine administration. Using qRT-PCR, we found a statistically significant ($p<0.05$) cocaine-induced upregulation of CB2R mRNA in hypothalamic paraventricular nucleus (PVN), prefrontal cortex (PFCx), nucleus accumbens (NAcc) and central (CEA) and basolateral (BLA) amygdala of rats withdrawn from cocaine. CB1R was significantly ($p<0.05$) upregulated in CEA. Western blots were then used to study the effect of cocaine on CB1R or CB2R protein expression. We found statistically significant ($p<0.05$) increased levels of CB2R in NAcc, CEA and BLA of rats withdrawn from cocaine. CB1R protein levels were not significantly ($p<0.05$) modified in the studied tissues.
We then studied the effect of (+/-)DOI, a selective 5-$HT_{2A/2C}$ receptor agonist, on the expression of cannabinoid receptors. We found that in rat neuronal cells, CLU213 or A1A1v cells, (+/-)DOI induced a significant ($p<0.01$) upregulation of both CB1R and CB2R in both neuronal cell models. Whereas MDL100907 or ketanserin, selective 5-HT_{2A} receptor antagonists, fully inhibited ($p<0.01$) the (+/-)DOI-induced upregulation of CB1R in neuronal cells, the (+/-)DOI-induced CB2R upregulation was partially ($p<0.05$) inhibited. We also used either U73122, a phospholipase C Beta inhibitor, or PD198306, a MEK1/2 inhibitor, which are (+/-)DOI-activated signaling pathways in these cells. Preincubating cells with PD198306, but not U73122, prevented the DOI-induced CB1R upregulation in neuronal cells.
In summary, our data identified regionally-specific regulation of brain cannabinoid receptors by cocaine and suggest a new mechanism by which 5-HT_{2A} receptors would regulate the expression of cannabinoid receptors in neuronal cells.

Disclosures: G. Carrasco: None.

Poster

PSTR382

G-Protein Coupled Receptors

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.10/B20

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

Support: NIH Grant 1R01AG078129-01A1

Title: 5ht2c agonism as a treatment for age-related neural hypoexcitability and weakness

Authors: *N. R. KERR[1], F. B. DARVISHI[2], A. ROSHANI DASHTMIAN[2], S. AYYAGARI[2], P. MOORE[2], A. KETABFOROUSH[1], B. CLARK[3], W. ARNOLD[1];
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Abstract: Over 40% of older adults report limitations in performing tasks essential to daily living, with 15-30% unable to lift or carry 10 pounds. Weakness is the primary characteristic of sarcopenia, which is well known to be a major contributor to physical limitations, fall risk, hospitalization, frailty, and premature death. There is growing evidence supporting that neural hypoexcitability is a critical contributor to age-related weakness. For instance, prior work has shown that reductions in motor unit firing rate result in an estimated 25-30% reduction in muscle force output in weak older adults. Persistent inward currents (PICs) play a vital role in the repetitive firing of motor neurons, which are activated by serotonin signaling via the 5HT2c receptor. Importantly, PICs are capable of enhancing synaptic input up to five-fold, leading us to hypothesize 5HT2c agonism is a promising target for ameliorating age-related neural hypoexcitability and weakness. To explore this hypothesis, we began by evaluating the effect of a single dose of lorcaserin, a highly selective 5HT2c agonist, on neural excitability in 26-month-old male and female mice. We performed *in vivo* electrophysiological assessments by stimulating the spinal cord and measuring electrical activity in the gastrocnemius muscle. We found that a single dose of lorcaserin (1.5 mg/kg) was sufficient to increase motor evoked potential following cervical spinal cord stimulation (cMEP) and repetitive cMEP amplitude. Maintenance of H reflex amplitude across a train of repetitive nerve stimulation was observed, suggesting acute lorcaserin treatment increases neural excitability and activation. Next, we assessed muscle force in the gastrocnemius in response to single, 15, 30, 50 and 150 Hz stimuli in the spinal cord following lorcaserin treatment. Mean force output was significantly increased in lorcaserin treated mice. Finally, a single dose of lorcaserin significantly improved motor coordination (rotarod) and motor power performance (weighted cart pull) in aged mice. Importantly, the magnitude of motor function improvement following drug treatment correlated with the Single Motor Unit Potential amplitude, a measure of motor unit remodeling, suggesting that mice with greater motor unit deficits respond best to 5HT2c agonism. Overall, our data suggests that 5HT2c agonism is a promising therapeutic approach for treating age-related neural hypoexcitability and weakness. Importantly, 5HT2c agonism may be an effective strategy for treating weakness and physical frailty in the older adults, greatly improving quality of life and healthspan.

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Poster

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G-Protein Coupled Receptors

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.11/B21

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

Title: Serotonin-mediated ER-Ca^{2+}—cAMP pathway regulates the second-scale coincidence time window of learning

Authors: *X. LI, Y. WANG, Y. LI;
Peking Univ., Beijing, China

Abstract: In Pavlovian learning, the temporal coincidence of the neutral conditioned stimulus (CS) and unconditioned stimulus (US) is essential for the formation of associative learning. Our previous research in *Drosophila* revealed that this critical coincidence time window for olfactory associative learning is regulated bidirectionally by serotonin signaling from dorsal paired medial (DPM) neurons to Kenyon Cells (KCs) in the mushroom bodies through 5-HT1a receptor. Nevertheless, the specific intracellular biochemical downstream cascade of the 5-HT receptor that are responsible for the integration of CS and US remains unclear. Recognizing the essential role of Ca^{2+}-dependent adenylyl cyclase Rutabaga (Rut) and cAMP in olfactory learning within KCs, we utilized functional imaging using a novel intensiometric cAMP sensor, G-flamp1. Our finding shows that CS-US pairing triggers Rut-dependent cAMP facilitation (ΔcAMP). Notably, we found that the coincidence time window for inducing ΔcAMP is bidirectionally regulated by 5-HT via the 5-HT1a receptor. Through both *in vitro* and *in vivo* characterization of the 5-HT1a receptor, we identified its interaction with two distinct downstream G proteins, Go and Gq. These versatile signaling pathways act synergistically to regulate the coincidence time window of ΔcAMP. Specifically, 5-HT1a couples with Go to reduce the basal cAMP level, thereby enhances the signal-to-noise ratio of ΔcAMP. Simultaneously, 5-HT1a couples with Gq to cache the CS-related Ca^{2+} signal, activating Rut and promoting ΔcAMP. These findings underscore the *in vivo* functionality of promiscuous GPCR signaling and clarify the mechanism by which dual downstream signals orchestrate the temporal coding essential to Pavlovian learning.

Disclosures: X. Li: None. **Y. Wang:** None. **Y. Li:** None.

Poster

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G-Protein Coupled Receptors

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Program #/Poster #: PSTR382.12/B22

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

Support: SUPPORTED BY CINVESTAV

Title: The N217D mutation affects the intracellular signaling of the human histamine H_2receptor stably expressed in CHO-K1 cells

Authors: *M. CARRASCO-MEZA[1], J.-A. ARIAS-MONTANO[2];
[1]Physiology, Biophysics and Neurosci., CINVESTAV, Mexico City, Mexico; [2]Physiology, Biophysics and Neurosci., Cinvestav-IPN, Ciudad de Mexico, Mexico

Abstract: In addition to its function in the gastrointestinal and immune systems, the histamine H_2 receptor (H_2R) participates in the regulation of several functions of the central nervous system. The brain histaminergic system has been implicated in the pathophysiology of schizophrenia and the H_2R antagonist famotidine was shown to attenuate the negative symptoms of the disease. Furthermore, Orange et al. (Mol. Psychiatry 1: 466-469, 1996) reported a higher prevalence of the N217D mutation in patients with schizophrenia. However, the effects of this mutation on H_2R function have been scarcely investigated. To address this issue, the N217D mutation was reproduced in the human H_2R (hH_2R) and CHO-K1 cells were stably transfected with the native (hH_2R_{WT}) or the mutant (hH_2R_{N217D}) receptors. H_2Rs couple to Ga/s proteins and thus to the cAMP/PKA pathway, and cAMP accumulation assays show reduced constitutive activity and agonist-induced cAMP formation (efficacy and potency) for the hH_2R_{N217D}. These preliminary results indicate that the mutation impairs the pharmacological properties and signaling of the hH_2R, effects that could be linked to the pathophysiology of schizophrenia.

Disclosures: M. Carrasco-meza: None. **J. Arias-Montano:** None.

Poster

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G-Protein Coupled Receptors

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

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EXC 257 NeuroCure

Title: It's all in your head: layer 6b and the orexin-activated neurons of the human cortex

Authors: *T. A. ZOLNIK[1], A. MEENAKSHISUNDARAM[1], M. MUELLER[2], J. ONKEN[1], T. SAUVIGNY[3], U.-W. THOMALE[1], U. C. SCHNEIDER[1], R. N. SACHDEV[4], P. FIDZINSKI[1], M. HOLTKAMP[1], D. SCHMITZ[1], A. M. KAINDL[1], J. GEIGER[1], Z. MOLNÁR[2], B. J. EICKHOLT[1], M. E. LARKUM[4];
[1]Charité Univ. of Med. Berlin, Berlin, Germany; [2]Oxford Univ., Oxford, United Kingdom; [3]Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [4]Humboldt Univ. of Berlin, Berlin, Germany

Abstract: Does orexin - the powerful wake-promoting neuromodulator - activate neurons in our cortex? This question has become particularly important in light of recent findings in mice showing that orexin-activated cortical neurons (OCNs) - which reside mainly in the deepest cortical layer, layer 6b in rodents - powerfully drive attention-associated high gamma oscillations, strongly suppress sleep-associated slow waves, and engage the higher-order thalamocortical system, a key network for higher cognition. Moreover, a lack of orexin in humans causes the debilitating sleep-wake disorder narcolepsy and deficits in sustained attention, whose circuit basis remains unknown. Nevertheless, the human cortex does not have a layer 6b like rodents and it is not clear whether orexin-activated neurons reside in our cortex. If we have OCNs, then our cortex has a previously unrecognized network that may be important for our cognitive performance and wakefulness. Therefore, in living human cortical slices from clinically necessary surgical resection, we used electrophysiology and a new high-throughput drug screening method to determine whether human cortical neurons respond directly to orexin. We found that a subpopulation of our deepest cortical neurons - at the bottom of layer 6 and in white matter - are indeed powerfully activated by orexin. These human OCNs were also activated by dopamine, serotonin, and other wake-promoting neuromodulators, in addition to stimulant drugs. Although they are the deepest cortical neurons, human OCNs project to layer 1 at the top of the cortex. In mice, these analogous projections drive apical dendrites associated with sensory perception. Human OCNs include both pyramidal and multipolar morphology, which correspond to distinct projection neurons with unique influences on thalamocortical operations in mice. Finally, human OCNs were highly excitable and were driven by callosal axon stimulation. Interestingly, human and mouse OCNs were largely analogous, suggesting that OCNs are conserved across mammalian species and may be critical in humans (like in mice) to enhance and sustain cortical activity. This overlooked, small group of neurons could be an essential part of our wake-promoting circuitry, vital for understanding narcolepsy and our most important cognitive functions. OCNs should therefore be investigated as a target for therapeutic intervention.

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Poster

PSTR382

G-Protein Coupled Receptors

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.14/B24

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

Title: Quantitative 3D imaging of orexin and orexin receptor distribution in the intact mouse brain

Authors: *H. H. HANSEN, L. LYDOLPH LARSEN, T. TOPILKO, L. S. DALBØGE, J. HECKSHER-SØRENSEN, U. ROOSTALU;
Gubra, Horsholm, Denmark

Abstract: Background & Aim: Orexins (OX, also termed hypocretins) and their cognate receptors, OX1R and OX2R, play an important role in controlling several fundamental physiological functions such as sleep/wakefulness, appetite regulation, energy homeostasis, reward and reproduction. Hence, disturbances in the orexin system are implicated in several diseases, notably narcolepsy, for which there is a considerable interest in developing more effective treatments. To improve circuit-level understanding of the orexin receptor system, the present study aimed to generate a complete 3D map of the orexin system in the intact mouse brain at single-cell resolution using whole-brain immunohistochemistry (IHC) and in situ hybridization (ISH). Methods: Male C57BL/6J mice (8-9 weeks old, n=18) were maintained under a reversed light/dark cycle (lights off 3 AM, lights on 3 PM) and terminated 6hrs into the dark phase (9 AM). Brains were perfusion-fixed and processed for whole-brain IHC (orexin, n=6 mice) and ISH (OX1R and OX2R, n=6 mice per transcript). Upon clearing, whole-brains were scanned using light sheet fluorescence microscopy (LSFM) followed by AI-based, automated 3D quantitative image analysis.
Results: Orexin-expressing neurons were exclusively located in the hypothalamus, being particularly abundant in the lateral hypothalamic area with projections throughout the brain. In contrast, OX1R and OX2R showed brain-wide expression, however, with distinct differences in anatomical localization. Strong OX1R expression was detected in a subset of brain stem nuclei (nucleus raphe pallidus, parabrachial nuclei, locus coeruleus, laterodorsal tegmental nucleus, dorsal raphe nucleus). While high Ox1r expression was observed in the hypothalamus (ventromedial nucleus of the hypothalamus), striatal and septal expression was low. OX2R expression was particularly high in several motor brain stem circuits (tegmental reticular nucleus, pontine grey, lateral reticular nucleus, lateral paragigantocellular nucleus) as well as in the striatum. Whole-brain expression maps for orexin, OX1R and OX2R were consistent with public 2D IHC and ISH data.
Conclusions: We here provide complete 3D whole-brain maps of orexin, OX1R and OX2R expression in the mouse. These maps will be highly instrumental in preclinically characterizing the central effects and biodistribution of agonists and antagonists targeting the orexin receptor system.

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Poster

PSTR382

G-Protein Coupled Receptors

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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

Support: NIH grant UF1NS133763
NIH grant R21DA049569

Title: Optogenetic control of an intracellular pool of GPCRs

Authors: *C. BOWMAN[1], P. O'NEILL[2];
[1]UCLA Chapter, Westwood, CA; [2]Psychiatry and Biobehavioral Sci., Univ. of California Los Angeles, Los Angeles, CA

Abstract: G protein coupled receptors have well established roles in mediating communication between cells by sensing extracellular cues. However, many GPCRs have been reported to localize not only to the plasma membrane, but also various organelle membranes. The roles for GPCR signaling at organelle membranes are not well understood. An important experimental limitation has been the inability to selectively activate GPCRs inside of a cell without activating the cell surface receptors. We generated a genetically encoded "opto-ligand" that provides photoswitchable control over the B2R bradykinin receptor. B2R has a known nuclear localization sequence, and it has been reported to localize to both the nucleus and plasma membrane. Here we show that when the opto-ligand is expressed in the cytosol of HEK293T cells, it generates nuclear Ca2+ responses upon photoactivation. The responses only occur if the cells are transfected with the B2R receptor, and they are blocked by a small molecule inhibitor of Gq signaling. Our results support the conclusion that the opto-ligand provides optical control over an intracellular pool of B2R receptors. It can be used in future experiments to interrogate nuclear B2R signaling in various physiological processes, and may produce more general insights into the roles of GPCR signaling at organelle membranes.

Disclosures: C. Bowman: None. **P. O'Neill:** None.

Poster

PSTR382

G-Protein Coupled Receptors

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.16/B26

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

Support: HRSA-T99HP39202

Title: Enhanced locomotor activity in GPR4 knockout mice to cocaine administration

Authors: J. CASCONE, D. GLASER, R. AHSAN, E. HE, S. DING, E. CHAI, P. SHET, M. WACKER, *X. CHU;
Univ. of Missouri-Kansas City Sch. of Med., Kansas City, MO

Abstract: G-protein coupled receptor subtype 4 (GPR4) is a proton-sensing receptor implicated in various physiological and pathological processes, such as angiogenesis and inflammation. GPR4 knockout (KO) mice have been utilized to demonstrate the impacts of this receptor in various tissues or systems including the cardiovascular, renal, skeletal, and brain blood vessels. However, little is known about the effects of GPR4 with cocaine addiction. Cocaine inhibits the reuptake of neurotransmitters such as dopamine, serotonin, and norepinephrine in the brain and triggers serious maladaptation in the human body. In this study, we aim to provide preliminary evidence of how GPR4 KO mice react to acute and chronic cocaine administration. 3- to 4-month-old GPR4 KO and age-matched wild-type (WT) mice were tested in our study. Mice (WT and GPR4 KO) received either daily cocaine (20 mg/kg) or saline (20 mg/kg) injections for 5 consecutive days. Mouse locomotor activity was assessed before and after injection using an infrared photo-cell-based, automated Versamax animal activity monitoring system. 2 weeks after the last day of injections, the mice were subjected to a final challenge injection of cocaine (10 mg/kg) and locomotor activity was assessed. We found that GPR4 KO mice showed increased locomotor activity after each cocaine injection when compared to WT in both male and female mice. There was no significant difference in locomotor activity before cocaine injection. The difference in locomotor response between GPR4 KO and WT mice was more exaggerated in males as compared to females. Both WT and GPR4 KO mice showed behavior sensitization to challenge cocaine injection, with a relatively increased locomotor response in the GPR4 KO group. Our results demonstrate an increase in locomotor activity in response to cocaine in GPR4 KO mice, suggesting that GPR4 plays a critical role in the behavioral response to cocaine. This implies that GPR4 could have endogenous protective effects against cocaine administration. A GPR4 agonist, therefore, might reduce cocaine's neurological effects. However, further investigation is required to determine if targeting GPR4 could be used as a therapeutic strategy against cocaine addiction.

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Poster

PSTR382

G-Protein Coupled Receptors

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

Support: NIH Grant U18 DA052543
NIH Grant T32 DA007287

Title: Discovery of GPR52 G Protein-Biased Agonists With Reduced Receptor Desensitization

Authors: *J. A. ALLEN[1], R. E. MURPHY[1], P. WANG[1], S. ALI[1], D. E. FELSING[2], H. R. SMITH[1], H. CHEN[2], J. ZHOU[2];
[1]Univ. of Texas Med. Br., Galveston, TX; [2]Dept. of Pharmacol. and Toxicology, Univ. of Texas Med. Br., Galveston, TX

Abstract: Orphan GPR52 is emerging as a promising neurotherapeutic target. GPR52 is a brain specific orphan G protein-coupled receptor that activates the Gs/cAMP signaling pathway and is primarily expressed in the human striatum in indirect pathway medium spiny neurons. The unique brain and striatal expression profile of GPR52 has distinguished this orphan receptor as a promising drug target for neurological and psychiatric disorders including schizophrenia, Huntington's disease, and substance use disorders. We have pursued drug discovery to create novel GPR52 agonists integrating chemical synthesis and pharmacological evaluations, and here we describe a new series of 3-((4-Benzylpyridin-2-yl)amino)benzamides as GPR52 agonists with G protein biased activity. We employed an iterative drug design strategy and structural optimization of compound 4a, that led to the discovery of a series of potent GPR52 agonists. Compounds 15b, 15c, 15d, 24b, 24e, 24f, and 24k were found to be more potent for GPR52 Gs/olf-mediated cAMP signaling than both 4a and our previously optimized compound 4b. Our studies into GPR52 Beta-arrestin recruitment activity of this series revealed that the indoline carboxamide compound 4a was unexpectedly highly potent for GPR52 Beta-arrestin recruitment (EC50 = 22 nM). Opening the indoline ring system to create 10a profoundly reduced GPR52 Beta-arrestin recruitment potency and efficacy (EC50 = 483 nM, Emax = 70%). This finding provides a structural basis to create GPR52 G protein-biased agonists, and indicates the indoline ring system is important for imparting potent GPR52 Beta-arrestin recruitment. 10a and 24f, with greater bias for G protein/cAMP signaling, also induced significantly less receptor desensitization, indicating that reducing GPR52 Beta-arrestin activity with biased agonism results in sustained Gs/cAMP activation. Molecular docking studies of compounds 4a, 15b, and 24f with GPR52 revealed a conserved binding mode with three pairs of hydrogen bonds, pi-pi stacking, and hydrophobic interactions. Further exploration of compounds 15b and 24f indicated excellent target selectivity, but limited brain exposure warranting further optimization. These balanced and biased GPR52 agonists provide important pharmacological probes to determine the impact of biased agonism on GPR52 function. In addition, these compounds provide an innovative conceptual framework for evaluating reduced Beta-arrestin-mediated desensitization of GPR52, which may provide sustained agonist efficacy for neurotherapeutic applications.

Disclosures: J.A. Allen: A. Employment/Salary (full or part-time):; University of Teaxs Medical Branch. 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.; Maplight Therapeutics. **R.E. Murphy:** None. **P. Wang:** None. **S. Ali:** None. **D.E. Felsing:** None. **H.R. Smith:** None. **H. Chen:** None. **J. Zhou:** None.

Poster

PSTR382

G-Protein Coupled Receptors

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.18/B28

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

Title: Metabotropic glutamate receptor 3 differentially regulates excitability in human vs mouse L2/3 cortical pyramidal neurons

Authors: *A. GALAKHOVA[1], T. S. HEISTEK[1], S. IDEMA[2], N. VERBURG[2], H. D. MANSVELDER[1], N. A. GORIOUNOVA[1];
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Abstract: The excitability of cortical neurons is critical for cognitive function and is tightly regulated by modulatory receptors, such as the metabotropic glutamate receptor 3 (mGluR3). This receptor has recently been shown to improve working memory in humans, but also has general pro-cognitive effects. Moreover, mGluR3 has neuroprotective effects for neurodegenerative diseases, stroke, traumatic brain injury, epilepsy, schizophrenia, and certain types of pain. Thereby, mGluR3 might be a promising target for treatment of human cognitive disorders, but its mechanisms of action in human circuits of cognition are completely unknown. Here we investigated the role of this receptor in regulating neuronal excitability and distribution in human cortical circuits in tissue from neurosurgery. Using RNA-seq and Patch-Seq data, we found that mouse L2/3 pyramidal cell (PC) types abundantly express this receptor. In contrast, human L2 pyramidal neurons barely express this receptor, while human-specialised non-homologous PC types in L3 show strong expression. mGluR3 activation by the receptor-specific agonist N-acetyl-aspartyl-glutamate (NAAG) reduced cellular excitability in human L3 PC neurons, but did not affect excitatory synaptic transmission evoked by extracellular stimulation. NAAG application resulted in a stronger hyperpolarisation of resting membrane potentials (RMP) in human L3 PCs compared to L2 PCs. In addition, NAAG increased the threshold of the action potentials (APs) generation, resulting in more current needed to elicit the first spike (rheobase). Lastly, upon activation of mGluR3, human PCs fired fewer APs at the same input compared to mouse PCs. In contrast, mGluR3 activation did not affect intrinsic excitability of mouse PC neurons, while it reduced paired pulse ratios of evoked excitatory synaptic transmission. These data show that mGluR3 activation reduces excitability in L2/3 neuronal circuitry in both mouse and human cortex, but through distinct mechanisms. Our findings suggest a presynaptic mode of action in mouse PCs, while it acts postsynaptically in human PCs. Moreover, mGluR3 acts more prominently in human cortical L3 compared to cortical human L2 PCs.

Disclosures: A. Galakhova: None. **T.S. Heistek:** None. **S. Idema:** None. **N. Verburg:** None. **H.D. Mansvelder:** None. **N.A. Goriounova:** None.

Poster

PSTR382

G-Protein Coupled Receptors

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.19/B29

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

Support: Slovenian Research and Innovation Agency (ARIS) Grant P3-310
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Title: Age-associated alterations in neuronal and glial calcium signaling and glucose metabolism in Drosophila brain

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Abstract: In ageing and neurodegeneration, glucose utilisation in the brain is reduced, which probably contributes to the progression of cognitive decline. This may be due to dysfunction of the noradrenergic system, which controls brain metabolism as well as learning and memory formation. The release of noradrenaline from noradrenergic neurons triggers intracellular increases in Ca^{2+} and cAMP signalling in brain cells through the activation of adrenoceptors. In astrocytes, at least in *in vitro* systems, noradrenergic signalling increases glucose uptake, glycogen degradation and facilitates aerobic glycolysis with the end product lactate. It is assumed that the latter is transported from astrocytes to neurons to serve as fuel during increased brain activity and to promote cognition. Whether noradrenergic activation, which controls neuronal and glial metabolism, is impaired in aged brains is unclear and was investigated in this study.
We expressed fluorescent sensors for Ca^{2+}, cAMP, free glucose and lactate in young and aged *Drosophila* brains selectively in neurons or glial cells to measure the changes in these intracellular second messengers and metabolites upon stimulation with octopamine (an analogue of noradrenaline in invertebrates). Octopamine elicited Ca^{2+} signalling in neurons and glial cells in young but not in old brains, suggesting age-related impairment of intracellular Ca^{2+} signalling. Furthermore, octopamine stimulation triggered neuron-specific increases in intracellular cAMP and lactate in an age-independent manner, suggesting that aerobic glycolysis occurs mainly in neurons. Interestingly, despite the absence of aerobic glycolysis in glial cells, young brains showed an increase in cytosolic glucose triggered by octopamine, which was not observed in old brains. This increase is most likely the result of glucose uptake from the extracellular space. Both neurons and glial cells were able to take up extracellular glucose and lactate to a similar extent. However, in neurons from aged brains, glucose uptake was reduced. Importantly, these neurochemical changes in aged brains were associated with neurodegenerative lesions. Overall, these results suggest that neurons, but not glial cells, are the primary site of regulated

aerobic glycolysis in *Drosophila* brains. In aged brains, octopaminergic Ca^{2+} signalling, regulation of glial glucose uptake and glucose delivery to neurons were impaired, which may contribute to the age-related cognitive-like deficits.

Disclosures: A. Horvat: None. **U. Cerne:** None. **R. Zorec:** None. **N. Scholz:** None. **N. Vardjan:** None.

Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

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Topic: I.04. Physiological Methods

Support: NIH Grant R01 NS118648/NS/NINDS

Title: Optogenetic identification of the laminar origin of sensory-evoked high gamma ECoG signals

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Abstract: Electrocorticography (ECoG) measures cortical surface electrical potentials (CSEPs) with high temporal and spatial resolution in both humans and animals, making it a unique translational methodology. Sensory-evoked CSEPs in the sensory cortex display peaks of activity in the high gamma band (65 -170 Hz) that are spatially localized to ~1 cortical column. However, the neural origin of the signals recorded by ECoG are not fully understood. Observational studies have found correlations between ECoG signals and single unit firing across all layers. In contrast, biophysical modeling studies predict that, while neurons in all layers contribute to some extent, pyramidal (PYR) neurons in deep layers (L) are the primary generating source of sensory-evoked ECoG high-gamma. To causally test the laminar origins of high gamma activity recorded by ECoG, we utilized layer-specific optogenetic suppression while recording ECoG signals in anesthetized mice.
We used custom designed, high-density μECoG to record neural activity from whisker primary somatosensory cortex (wS1) during stimulation of contralateral whiskers. Single whisker deflections evoked a brief peak of high gamma activity, topographically localized to the whisker's cortical column. To test whether L2/3 or L5 pyramidal (PYR) neurons are the main sources of this signal, we optogenetically suppressed either cell type by viral expression of GtACR2.0 in wS1 of Drd3-Cre (L2/3 PYR) or Rpb4-Cre (L5 PYR) mice. Suppression of L5 PYR cells caused the strongest reduction in sensory-evoked high gamma power (-22.3% ± 3.2,

mean ± sem at 96 Hz, N = 6 mice), while optogenetic suppression of L2/3 PYR cells induced a weaker, broadband suppression of the sensory-evoked signal (-13.3% ± 2.4, mean ± sem from 10 to 170 Hz, N = 5 mice). Thus, despite L5 PYR somata being further away from the surface recording site, L5 PYR activity contributes more than L2/3 PYR activity to sensory-evoked high-gamma signals.

Disclosures: P. Garderes: None. **K.E. Bouchard:** None. **D.E. Feldman:** None.

Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

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Topic: B.07. Network Interactions

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Research to Prevent Blindness

Title: Deep learning approach to identify prefrontal inputs to visual areas using their simultaneously recorded local field potentials

Authors: *H. AHN[1], K. CLARK[2], G. WENG[3,4], A. AKBARIAN[2], B. NOUDOOST[2], N. NATEGH[1,4];
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Abstract: Understanding the role of prefrontal feedback in the processing of visual information is crucial for deciphering the neural basis of visual functions at the integration of sensory and cognitive factors. Achieving this goal necessitates simultaneous recording of signals from both prefrontal and visual cortices and the identification of prefrontal signal components that are transmitted to and utilized in visual areas. This study particularly aims to capture the information communicated between the frontal eye field (FEF) part of the prefrontal cortex (PFC) and area V4 by identifying the components of FEF signals that affect V4 neural responses. We recorded local field potentials (LFPs) in the FEF area simultaneously with the LFPs of a population of V4 neurons, measured using linear array electrodes, while monkeys performed a visually guided saccade task. Using a deep learning framework, we extract the oscillatory components within FEF LFPs that are sent to V4 during saccadic eye movements. Our model leverages neural network architectures that combine convolutional neural networks (CNNs) and transformer-based models to extract the oscillatory features capturing the temporal, spatial, and spectral

dynamics of the FEF and V4 LFP signals. This integration enables a comprehensive analysis of FEF and V4 interactions in a lower dimensional oscillatory feature space, which is essential for tracing the shared components communicated between the two areas. By identifying the FEF sources contributing to the oscillatory properties of V4 responses, this approach can enhance our understanding of the nature of interactions between prefrontal and sensory areas and their functional roles. Our results will also demonstrate the feasibility of generative neural network frameworks for modeling the spatio- and spectro-temporal dynamics of large-scale LFP data across multiple channels, trials, and brain regions. Such a framework would enable significant advances toward understanding the neural mechanisms underpinning several neural phenomena associated with brain oscillations, including inter-areal synchrony and spike phase coding.

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Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

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Title: Characterization of Locus Coeruleus Regulation of Arousal States Through a Machine-learning Approach

Authors: *E. WEISS, A. LIU, Q. WANG;
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Abstract: The Locus Coeruleus (LC) is a complex nucleus responsible for regulating arousal throughout the brain. Therefore, it is essential to understand how the LC-NE system controls cortical states. Both cortical EEG and pupil size can be used as indices of arousal, changing non-linearly during spontaneous arousal fluctuations and in direct response to increased LC activation. Given the richness of information encoded in EEG spectral bands, this study first aimed to quantify the relationship between pupil size and the EEG spectrum using machine learning approaches during spontaneous fluctuations of arousal state. Additionally, we aimed to assess how this relationship changes when the LC is activated. Using optogenetics to selectively activate the LC, we recorded both pupil and cortical EEG in awake, head-fixed mice. By observing different spectral patterns in both quiet wakefulness and heightened LC-active states,

we quantified how EEG indexed these cortical states. To further uncover the complexity hidden within-session-wide pupil dynamics, we developed a variational autoencoder network that seamlessly encoded time-delay embedded pupil size into its latent dynamics, subsequently reconstructing it back into each canonical EEG frequency band (Delta, Theta, Alpha, Beta, and Gamma). This approach demonstrated that network-level cortical activity can be accurately modeled from low-dimensional representations of time-delay embedded pupil size. Our results reveal a latent space reflecting the functional relationship between pupil size and cortical EEG spectral band power, with LC activation augmenting the intrinsic arousal dynamics present in spontaneously occurring cortical states. This study proposes new ways to probe the influence of the LC on cortical EEG and pupil dynamics, offering insight into the hidden manifold of arousal using non-invasive indicators like pupil size and EEG spectral power.

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Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

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Research to Prevent Blindness

Title: Extracting the oscillatory features of local field potentials using neural networks

Authors: *A. KOVALEV[1], K. CLARK[2], G. WENG[3], A. AKBARIAN[2], B. NOUDOOST[2], N. NATEGH[1];
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Abstract: Local field potential (LFP) signals reflect a combination of multiple oscillatory and non-oscillatory neural sources. Identifying the constituent oscillatory components within measured LFPs is crucial for characterizing the information conveyed by these signals. Standard frequency-filtering techniques have been widely used for decomposing LFP signals into bandpassed oscillatory components. However, these methods do not account for the spatiotemporal dynamics of LFP signals, thereby providing a limited view on the various sources

underlying the LFP generation. In this study, we develop a neural network framework that leverages the spatiotemporal structures in multichannel LFP data to extract interpretable oscillatory components contributing to LFP generation. LFPs from 16 channels were recorded simultaneously using linear electrode arrays in visual area V4 of macaque monkeys performing a visually guided saccade task with visual probes. Employing an autoencoder-decoder framework, the LFPs are projected onto spatiotemporal features learned by convolutional neural networks in an unsupervised setting. Hyperparameters are tuned to optimize reconstruction performance. Subsequently, the learned spatiotemporal features can be used to extract oscillatory components from the raw multichannel LFPs in each trial. Furthermore, recordings over multiple trials are used to account for the trial-by-trial variability of the identified spatiotemporal features. Using this approach, the neural network model effectively decomposes LFP signals into a small number of oscillatory components. We assess the capability of this unsupervised neural network approach in identifying a concise and robust set of oscillatory components that capture the spatiotemporal dynamics of large-scale LFPs with substantially fewer dimensions than the original data. The LFP decomposition framework can also serve as a means to measure the timing of simultaneously recorded spiking activity relative to these component oscillations rather than to the bandpassed LFP components. This approach can have significant implications for a robust measurement of phase coding.

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Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

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Title: Effect of anesthesia-induced LFP bursting and suppression on auditory evoked broadband gamma activity

Authors: *A. N. SIMMONS[1,2,3,4], A. NOURMOHAMMADI[2,3,4], T. XIE[2,3,4], J. T. WILLIE[1,2,3,4], P. BRUNNER[1,2,3,4];
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Abstract: Under deep anesthesia, a pattern of semi-periodic neuronal bursting followed by periods of suppressed activity called burst suppression is widely observed across subjects. While anesthesia depth has been observed to correlate with changes in stimulus-evoked response characteristics, the separate impact of bursting and suppression observed during anesthesia-induced burst suppression on these responses is not well characterized. Previous work has found that broadband gamma electrocorticography (ECoG) responses to auditory stimuli during general anesthesia can be used to identify cortical areas associated with language function. The aim of this study is to determine the effect of anesthesia-induced burst-suppression on auditory evoked broadband gamma (BBG) responses. We analyzed auditory evoked potential (AEP) data recorded from 11 adult neurosurgical patients diagnosed with refractory epilepsy. As part of their standard treatment procedure, all patients were implanted with subdural electrode grids that partially or fully covered the superior temporal gyrus (the main anatomical region involved in auditory processing). Participants underwent passive language mapping while awake and under deep anesthesia while AEPs were measured using the implanted ECoG electrodes. All participants understood English and had no history of hearing impairments. Each trial consisted of a 0.7 second auditory stimulus followed by a 1 second silent (baseline) period. Auditory stimuli consisted of 32 words and 32 non-words delivered through over-ear headphones. BBG activity (70 - 170 Hz) was analyzed for each recording channel by bandpass filtering the signal and extracting the BBG envelope. Channels showing significant responses to auditory stimuli were then identified through a correlation analysis and Wilcoxon rank-sum test between baseline and task BBG activity. Separating bursting and suppression state trials in recording channels that were found to be auditorily sensitive under anesthesia revealed that across patients, bursting state trials had a higher BBG response than suppression state trials on average. This difference was statistically significant across trials both at the individual and group level for both word and non-word stimuli. This result aligns with our hypothesis that AEP related BBG activity may be affected by burst suppression. It is possible that the semi-periodic bursting that appears under deep anesthesia are indicative of simultaneous, semi-periodic increases in excitability. Consequently, the results of this investigation may have significance in improving our mechanistic understanding of how and why burst suppression arises.

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Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

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Title: Characterization of human neocortical layer dynamics during the transition from anesthesia-induced unconsciousness to wakefulness

Authors: *M. S. BREAULT[1], W. MUNOZ[2], E. N. BROWN[3], Z. WILLIAMS[4];
[1]MIT, Cambridge, MA; [2]Neurosurgery, MGH, HMS, Massachusetts Gen. Hosp., Boston, MA; [3]MGH & MIT, Cambridge, MA; [4]Harvard Univ., Chestnut Hill, MA

Abstract: The human brain undergoes dynamic changes when transitioning from an unconscious state mediated by general anesthesia to wakefulness. Indeed, the transitions between these states, globally observed across the neocortex, are caused in part by intricate interactions between cortical and thalamic connections organized into six layers. These layers consist of varying cell types with unique inputs and outputs. This is evident by their distinct neural activity from one layer to another. Until now, only limited information has been available about the role these individual layers play during different anesthetic states. To this end, we recorded local field potential (LFP) and spiking activity from all layers of the human neocortex during anesthetic-induced unresponsiveness, emergence, and wakefulness in ultra-fine spatial and temporal resolution using Neuropixel probes to observe changes in layer dynamics. We identified the spectral features and patterns of LFP for each layer during these three anesthetic states. We observed complete synchronous activity across layers during deep unconsciousness. Layer-specific activity then diverges into coupled synchrony that alternates between layers during sedation and emergence. This fades to independent activity as wakefulness ensues. These dynamics were quantified using neural correlates and metrics related to synchrony. Similar observations were made for the spiking activity. Finally, we built a multinomial logistic regression model based on these key features that could classify the anesthetic states based on neural activity. We show the importance of changes within and between layers dynamics to restore consciousness and the functional architecture dictating the connectivity between layers. This work offers a direct look at changes in layer-specific cortical activity during the transitions from anesthetic-mediated unconsciousness to wakefulness.

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Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

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Title: Timed Up and Go test shows cortical and subcortical power modulation in Parkinson's disease patients

Authors: *J. H. MARKS, K. H. LOUIE, J. E. BATH, J. BALAKID, H. FEKRI AZGOMI, D. D. WANG;
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Abstract: The Timed Up and Go (TUG) test is a common clinical assessment of mobility and balance for patients with movement disorders, including Parkinson's Disease (PD). The test involves different types of movements including postural changes, straight line walking, and turning that may indicate PD progression and fall risk. However, there is limited research investigating cortical and subcortical neurophysiological changes during the test. In this study, we analyze pallidal and sensorimotor cortical local field potentials (LFPs) from PD patients during the TUG test using data streamed from their implanted Medtronic Summit RC+S neurostimulators.

Three PD patients (two male and one female) consented to participate in this research as part of a larger study. All three patients are implanted with deep brain stimulation (DBS) devices targeting the globus pallidus interna (GPi). Two are implanted bilaterally and one is implanted unilaterally on the left side. The patients also have subdural cortical paddles placed over the primary motor (M1) and premotor (PM) cortices. LFP data recorded from each patient during the TUG test was aligned with gait data from wearable kinematic Delysis Trigno and Xsens Awinda sensors. The data was segmented into different TUG events (standing, first bout of walking, turning, second bout of walking, and sitting), then spectral analysis was conducted using the continuous wavelet transform. An ANOVA test was performed comparing spectral power between TUG events, then corrected for multiple comparisons.

We observed significant changes in both cortical and subcortical LFP power modulation in the beta (12-30 Hz) and low gamma (31-50 Hz) band frequencies. Decreases in beta power between standing and turning, standing and the second bout of walking, and standing and sitting were observed in the GPi and M1. In terms of low gamma power, an increase was seen between the first bout of walking and sitting in the GPi, while a decrease was seen between standing and sitting in M1. Additionally, we noted increased modulation between the alpha (7-11 Hz) and beta band frequencies in the transition from sitting to standing up.

We found that there are consistent, event-related changes in GPi and motor cortex low frequency

LFP power in PD patients during the TUG test. These results provide an exploratory look into the neurophysiology of the TUG task and may be used to better understand PD progression and fall risk.

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Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.08/B37

Topic: B.07. Network Interactions

Title: Comparison analysis between the evoked potentials (EPs) generated by the peripheral nerve stimulation (PNS) of median nerves and deep brain stimulation (DBS) in the PPN

Authors: *Y. SUN[1], A. SEYYED MOUSAVI[1], T. D. SANGER[2,1];
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Abstract: For movement disorders such as essential tremor, Parkinson's disease, and dystonia, there are two main stimulation treatments: The peripheral nerve stimulation (PNS), which is a noninvasive treatment, and deep brain stimulation (DBS), which is an invasive treatment. For DBS, the pedunculopontine nucleus (PPN) of the brainstem is a recent treatment target for movement disorders. Current results show that PNS of median nerve generates robust evoked potentials (EPs) in PPN, which indicates PPN receives sensory information from the periphery. Present studies have shown that DBS and PNS have partial modulatory effects on certain targets, as well as local disruption of abnormal signals. This leads to the hypothesis that indirect stimulation of PPN via the median nerve may be operating through a similar mechanism. We will examine and compare the EPs in PPN due to median nerve stimulation and direct electrical stimulation of PPN.During the Neuromodulation Monitoring Unit (NMU), we temporarily implant AdTech MM16C depth leads into multiple targets, including PPN, which allows us to deliver stimulation by macro-contacts and simultaneously record the local brain signals from micro-contacts to detect the EPs. The PNS of the median nerve was given in bursts of 100 ms at 3 to 50 Hz and current motor threshold alternating with 100 ms without stimulation, and DBS in PPN was given in bursts of 100 ms at 25 to 85 Hz and 3 volts alternating with 100 ms without stimulation. DBS of PPN in burst patterns may help to identify the different therapeutic effects by comparing responses with PNS. We also perform continuous DBS at 25 to 85 Hz with 3 volts.Results show that PNS of the median nerve can generate all visible pulses at the frequencies tested and evoked response with longer EPs' dispersion and response delay in PPN, which may be due to variability in axonal and synaptic transmission. However, EPs generated by PNS have peak-to-peak values that are equal or slightly decreased compared to EPs generated by

directly stimulation in PPN. We conclude that since PNS of median nerve produces responses in PPN with magnitudes that are consistent with the response to intracranial (DBS) electrical stimulation, a similar mechanism may be responsible for indirect stimulation of PPN via median nerve. These results suggest the possibility that noninvasive PNS could have effects similar to DBS in some patients. Further investigation is needed to understand the role of PPN and to determine whether median nerve stimulation may have similar clinical effects to DBS in PPN, which could be effective in patients with certain movement disorders.

Disclosures: Y. Sun: None. **A. Seyyed Mousavi:** None. **T.D. Sanger:** None.

Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

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Program #/Poster #: PSTR383.09/B38

Topic: B.07. Network Interactions

Title: Localized Impact of 185 Hz deep brain stimulation on intracranial neural activity patterns in pediatric dystonia

Authors: *R. SOROUSHMOJDEHI[1], M. KASIRI[2], J. NATARAJ[3], A. SEYYED MOUSAVI[1], T. D. SANGER[4,5];
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Abstract: Deep Brain Stimulation (DBS) is a recognized treatment for dystonia and specifically the 185 Hz stimulation frequency is commonly used clinically to stimulate the GPi. However, the mechanisms by which different stimulation frequencies impact brain activity are not fully understood. This study investigates the effects of various DBS frequencies on brain activity in 13 pediatric patients with dystonia. Temporary stereoelectroencephalography (sEEG) leads with both macro and micro contacts were implanted in the internal globus pallidus (GPi), subthalamic nucleus (STN), Ventral Anterior (VA), and Ventral Oralis (VO). Stimulation was delivered through macro contacts in each designated region, while micro contacts recorded data during stimulation, enabling simultaneous stimulation and recording from both the stimulated and other regions. Stimulation frequencies of 55, 85, 185, and 250 Hz were tested. Power spectral densities (PSDs) for frequencies below 50 Hz were calculated to identify changes in the delta, theta, alpha, beta, and low gamma bands relative to baseline, where no stimulation was applied. Our findings indicate a general increase in brain power during stimulation, independent of the frequency or location of the stimulation. Notably, stimulation of one region at 185 Hz significantly increased theta power in that region, compared to other stimulation frequencies. This phenomenon occurred in all tested regions, demonstrating a distinct, localized enhancement. These findings

underscore the potential for frequency-specific responses in DBS therapy, enhancing our understanding of its mechanisms and reinforcing the distinct therapeutic advantages of 185 Hz stimulation in achieving localized neural modulation.

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Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

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Program #/Poster #: PSTR383.10/B39

Topic: B.07. Network Interactions

Title: Infra-slow oscillations in human intracranial electrophysiology and the oscillatory hierarchy

Authors: *S. GOULIS[1], M. VERWOERT[2], M. OTTENHOFF[2], P. KUBBEN[2], M. ROBERTS[3], S. TEN OEVER[3], G. VALENTE[3], M. WIBRAL[1,4], C. HERFF[1,2], V. G. VAN DE VEN[1,3]; [1]Ctr. for Integrative Neurosci., [2]Dept. of Neurosurgery, Mental Hlth. and Neurosci. Res. Inst., [3]Dept. of Cognitive Neuroscience, Fac. of Psychology and Neurosci., [4]Dept. of Microeconomics and Publ. Economics, Sch. of Business and Econ., Maastricht Univ., Maastricht, Netherlands

Abstract: Infra-slow neural oscillations (<0.2 Hz) have long been suggested to play a role in top-down information processing, but the phenomenon has remained understudied. Previous non-invasive imaging methods (M/EEG, fMRI) and invasive intracranial (ECoG) studies in humans have shown infra-slow oscillations supporting network connectivity during resting-states and sleep vs. wakefulness. However, there is limited intracranial electrophysiological evidence about task-dependent connectivity of infra-slow oscillations and its relation to the oscillatory hierarchy above 1Hz, which is commonly considered the oscillatory domain of cognition and behavior. In this study, we explored the properties of infra-slow oscillations in intracranial depth EEG recordings of 33 epileptic patients undergoing epileptic monitoring while completing a wide variety of tasks. Recording contacts with statistically significant infra-slow oscillatory power were identified across all tasks, with most prominent locations in medial cortical and subcortical areas, including hippocampus, orbitofrontal cortex, putamen and insula. In relation to local processing, multiple contacts demonstrated statistically significant phase-amplitude-coupling between infra-slow phase and delta (1-3 Hz), theta (4-8 Hz), gamma (35-70) or broadband high gamma (BHG, 70-150 Hz) power, while a small subset of contacts revealed simultaneous multi-level phase amplitude coupling between all band pairs (gamma to BHG not included). Lastly, cross-contact connectivity analysis revealed temporally coherent pairs of contacts in the infra-slow range with significant lags in the order of seconds and most prominently in areas that corresponded to task demands, precluding the possibility they are a

result of a common artifact. In summary, we herewith expand via intracranial depth electrophysiology the spatial map of prominent infra-slow oscillatory activity in the human brain during wakefulness in a large number of subjects, demonstrate that oscillatory power across the oscillatory hierarchy is coupled to the infra-slow phase, and show spatial patterns of inter-areal coherence in the infra-slow range. These findings lend support to the idea that infra-slow oscillations may represent a broad organizational timescale on which state-dependent brain activity is orchestrated.

Disclosures: S. Goulis: None. **M. Verwoert:** None. **M. Ottenhoff:** None. **P. Kubben:** None. **M. Roberts:** None. **S. Ten Oever:** None. **G. Valente:** None. **M. Wibral:** None. **C. Herff:** None. **V.G. Van de Ven:** None.

Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.11/B40

Topic: B.07. Network Interactions

Title: Neural signatures and neuromodulation in a subject experiencing inhibition control deficits following temporal resection

Authors: *L. MATTAR[1], S. SHAH[2], J. ADKINSON[1], R. MATHURA[3], A. J. WATROUS[1], Y. ZHANG[1], L. CHAMAKURA[3], D. OSWALT[6], K. R. BIJANKI[4], S. R. HEILBRONNER[2], S. A. SHETH[1], G. BANKS[1], E. BARTOLI[5];
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Abstract: Title: Neural signatures and neuromodulation in a subject experiencing inhibition control deficits following temporal resection
Introduction
This case study focuses on a male subject suffering from drug resistant epilepsy and Kluver-Bucy syndrome (KBS) like symptoms, namely the inability to inhibit inappropriate sexual behavior. KBS symptoms developed following a left temporal resection.
Objective
We aimed to determine if the subject's lack of inhibitory control could be assessed through an emotional Go/No-go paradigm, and if neuromodulation of the prefrontal and insular cortex could influence inhibitory control abilities via a top-down mechanism.
Methods
Data was collected while the subject was undergoing stereo-electroencephalographic evaluation of epilepsy. Inhibition control was assessed through a Go/No-go task containing subject specific

KBS triggering provocative imagery and neutral imagery as a control. Initially, the patient performed the task without stimulation to obtain a baseline. Afterwards, the subject repeated the task with bipolar stimulation at 130 Hz to either the right inferior frontal gyrus (rIFG) or left anterior insula. Neural activity (power in 20-60 Hz) recorded bilaterally from multiple contacts placed in the fusiform gyrus and amygdala was analyzed in nonoverlapping 100 ms bins using nonparametric testing with false-discovery rate correction. Response accuracy and image type were tested for independence via chi-squared test.

Results

At baseline, neural activity in the left fusiform gyrus differed significantly between neutral and provocative imagery (200-300 ms after image onset, $p<0.05$). Analysis of the Go/No-go response scores showed lower accuracy in association to provocative imagery ($p<0.001$). Accuracy during neutral trials did not significantly differ across experiments, while accuracy during provocative trials improved with repeated exposure and particularly with rIFG continuous stimulation. The neural activity difference between neutral and provocative images found at baseline was also abolished over the course of stimulation.

Conclusions

Our results suggest that neuromodulation of the rIFG and repeated exposure therapy could be a potential treatment modality for controlling the patient's KBS-like symptoms, and improve emotional regulation abilities in general.

Disclosures: L. Mattar: None. **S. Shah:** None. **J. Adkinson:** None. **R. Mathura:** None. **A.J. Watrous:** None. **Y. Zhang:** None. **L. Chamakura:** None. **D. Oswalt:** None. **K.R. Bijanki:** None. **S.R. Heilbronner:** None. **S.A. Sheth:** None. **G. Banks:** None. **E. Bartoli:** None.

Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.12/B41

Topic: B.07. Network Interactions

Support: National Natural Science Foundation of China

Title: Brain wide temporal dynamics of mice social behavior

Authors: *H. WANG[1,2], P. LAN[2,1], X. LI[2,1], H. JIANG[2,3,1], J. LUO[2,1];
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Abstract: Social behavior in mammals requires innate motivation, parallel processing of multisensory information, retrieval of history social experiences and other neural mechanisms. When animals engage in social interactions, procedures mentioned above are accomplished by

brain-wide parallel processing mechanism to reach appropriate social decisions. To reveal how brain cope with the complex and temporally dynamical social information, we recorded LFP (local field potential) signals in 13 brain regions during social interaction and object exploration in mice, calculated the temporal-frequency dynamics and region-region synchronizations. First, We found that brain wide low gamma(30~50Hz) and high gamma(50~100Hz) oscillation took place during social investigation, and theta oscillation power elevated during novel object exploration. Meanwhile, high gamma oscillation was significantly higher during interacting with female mice than that with male mice, and both low gamma and high gamma oscillation power was higher during social context than novel object context. Second, to explore regional synchronizations related to behavior, we compared the Phase Lag Index (PLI) between baseline and behavioral phases. Our findings suggest that the amygdala could serve as a central hub in social behavior. This is based on the observation that the amygdala was predominantly involved in connections with a higher PLI during behavioral phases compared to baseline, particularly in social contexts as opposed to novel object contexts. Taken together, this study reveals multi-brain region LFP temporal-frequency dynamics and functional network during social interaction and object exploration contexts.

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Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

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Program #/Poster #: PSTR383.13/B42

Topic: B.07. Network Interactions

Support: Pomona College

Title: Fast and slow brain oscillations report distinct forms of neural activity

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Abstract: Action potentials, also known as spikes, isolated from single neurons, are thought to provide the most accurate readout of brain activity. In contrast, local field potentials (LFPs), often referred to as brain waves, which reflect the electrical activity of a large number of neurons located near an extracellular electrode, are easier to measure than spikes but seem to offer only coarse-grained readouts of behavior. Previously, we discovered that spatiotemporal patterns in LFPs recorded from the rat hippocampus contain astonishingly precise information about the rat's current location within its environment. However, the question of what the relationship is

between the information contained in spikes and LFPs remains to be fully detailed. To answer this question, we attempted to predict the spiking of individual hippocampal neurons, known as place cells, from LFPs in multiple frequency bands. We found that both low-frequency (<~20 Hz) and high-frequency (>~200 Hz) LFPs can be used to predict the spiking of place cells but in very different ways. At high frequencies, the LFPs measured at electrodes near the cell are highly predictive because they detect the spiking of the cell itself. At low frequencies, anatomically distributed LFP patterns are highly predictive because they detect activity in large assemblies of neurons with which the place cell co-activates. Our work shows how information embedded in various spatial scales of neural activity is reflected at distinct temporal scales of the LFP.

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Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

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Topic: B.07. Network Interactions

Support: KIST Intramural Grant (2N74510)
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Title: Understanding behavioural pinpoints based on network properties of transient oscillations in large brain regions.

Authors: *C. LEE[1], K. LEE[2], J. CHOI[1,3];
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Abstract: The study of brain oscillations has significantly advanced our understanding of neural mechanisms and facilitated a variety of experimental approaches and conceptual frameworks, such as 'communication through coherence' (Pascal Fries. 2015, *Neuron*). This hypothesis proposes that selective communication between brain regions is achieved through coherence in oscillation frequency. However, during the analysis process, averaging effects can potentially mask the temporal and spatial properties inherent in brain oscillation patterns, including sustained and transient in the temporal domain, and local and global in the spatial domain. To mitigate these issues, we analysed spatiotemporal data from NeuroPixels recordings (Steinmetz et al. 2019, *Nature*). In this study, we focus on transient and anatomically constrained patterns of activity as indicators of brain state, specifically examining burst networks constructed from phenomena known as transient oscillations. This approach preserves the integrity of temporal and spatial features by using non-averaged data across trials or subjects, and distinguishes

between local and global bursts for a more accurate and comprehensive understanding of neural dynamics. To construct a network based on co-occurring bursts, burst detection was performed using a broadband method to avoid initial frequency bias. Multi-region, multi-frequency bursts were detected from local field potential data using empirical mode decomposition (Huang, N. E. et al. 1998, *Proc. R. Soc. Lond. A*) and wavelet spectrograms. We also compared current source density with conventional analysis methods to distinguish local bursts from global bursts and to determine the timing of stimulus and behavioural changes. The directionality and community structure of the network was investigated by using channels corresponding to individual regions as nodes and the co-occurrence of observed transient oscillations between them as links. Influence measures and node connectivity were also used as an index for comparison with previous circuit study results. Our results identified features of co-occurring bursts that correspond to spontaneous states and individual behavioural phases. We confirmed that the density of co-occurring phenomena shows region and frequency specificity. In addition, co-occurring burst networks were found to reflect different behavioural phases. A residual hypothesis is that globally synchronising distributed brain regions may be observed, decoding behavioural transitions. This integrated perspective aims to open new avenues for understanding neural and behavioural correlates.

Disclosures: C. Lee: None. **K. Lee:** None. **J. Choi:** None.

Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

Location: MCP Hall A

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Program #/Poster #: PSTR383.15/B44

Topic: B.07. Network Interactions

Support: CRCNS/BRAIN R01NS115327
NIBIB T32EB025816
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Title: Capturing cortical state switching dynamics in the presence of modulating inputs

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Abstract: Cortical areas undergo fluctuations in background network activity that profoundly influence function moment-to-moment. These cortical state changes are strongly shaped by modulatory inputs from the thalamus, basal forebrain, and other cortical areas. While cortical states are commonly identified with ad hoc thresholding approaches operating on the spectral content of the local field potential (LFP), there have been recent efforts to model state switching

in cortex in a more principled manner with Hidden Markov Model (HMM)-based frameworks. However, the HMM framework does not model how inputs arising from other brain areas modulate state switching dynamics in cortex. To incorporate extracortical inputs in a model of cortical state switching dynamics, we use the primary somatosensory cortex (S1) of the awake, head-fixed mouse as a model system. Initially, we characterize how optogenetic manipulations of the ventral posteromedial nucleus of the thalamus (VPM) affect LFP signatures of cortical state in S1. In agreement with previous literature, our preliminary findings show that VPM excitation induces a rapid shift from cortical deactivation to cortical activation. Based on preliminary results, we are developing a Partially Observable Markov Decision Process (POMDP) approach for modeling cortical state switching in the presence of modulating inputs. In essence, POMDPs are extended HMMs with a set of different state transition matrices associated with distinct system inputs. We are exploring the application of POMDPs to cortical state modeling in two contexts. Firstly, we are evaluating the use of optical drive as the modulatory input to the POMDP in contexts where optogenetics are being used to steer the activity of a natural modulator of cortical state (i.e., VPM). This would allow for cortical state modeling under different regimes of tightly controlled modulatory activity and set the stage for adaptive control of cortical state. Secondly, in future work we will explore the use of spontaneously occurring whisking activity as the POMDP input. Whisking is a behavioral readout of thalamic, cholinergic, and other modulating signals, so its inclusion could lead to improvements in cortical state modeling during changing behavior. Finally, we are developing a software tool (implemented in Bonsai) for real-time cortical state inference using an HMM-based fit of cortical state switching, which will later extend to POMDP models. Overall, our work extends a cortical state modeling framework to include highly relevant cortical modulators, leading to more flexible models and opening the possibility of adaptive control of cortical state with optogenetics.

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Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

Location: MCP Hall A

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Topic: B.07. Network Interactions

Support: DBT/WELLCOME TRUST INDIA ALLIANCE
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Title: Distinct extracellular signatures of chemical and electrical synapses impinging on active dendrites differentially contribute to ripple-frequency oscillations

Authors: *R. SIRMAUR, R. NARAYANAN;
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Abstract: During slow-wave sleep and awake quiescence states, the hippocampus manifests signature activity patterns in the local field potentials (LFPs) known as sharp-wave ripples (SWRs). SWRs have been implicated in memory consolidation and are characterized by slow negative deflections (sharp waves) coupled with high-frequency oscillatory patterns (ripples). One of the several models for ripple generation postulates that the generation of basal dendritic spikes in response to the synchronous afferent activity from CA3 as well as the recurrent CA1 inputs onto basal dendrites together contribute to ripple generation. Here, we quantitatively assessed this postulate by studying the impact of active basal dendritic currents, generated as responses to recurrent and afferent inputs, on LFPs in the *stratum oriens* of CA1. We recorded LFPs using a 3D electrode array spanning the basal dendrites of a biophysically and morphologically realistic conductance-based model of a CA1 pyramidal neuron. We employed this setup to address two fundamental questions on the relationship between active dendrites and LFPs. First, we assessed the impact of active and passive basal dendrites, activated through either chemical synapses or gap junctions, on LFPs. We found striking differences between LFP signatures of inputs through chemical *vs.* electrical synapses, with excitatory chemical synapses yielding a sink near the synaptic location and electrical synapses manifesting as a source. Our analyses show that LFP signatures were qualitatively and quantitatively different for passive *vs*. active dendritic structures, with distinct spectral profiles in LFPs associated with oscillatory inputs through chemical *vs*. electrical synapses. Second, with specific reference to ripples, we evaluated the impact of four kinds of inputs on LFPs for active and passive basal dendritic models: randomly timed afferent inputs, precisely timed excitatory recurrent inputs through chemical or electrical synapses occurring in Gaussian pattern across time, and Gaussian-patterned inhibitory inputs impinging on basal dendrites. We detected ripple-frequency oscillations in LFPs when the basal dendrites were innervated by Gaussian-patterned inhibitory inputs, but not with randomly timed inputs, with quantitative differences in ripple power associated with passive *vs* active dendrites. Our analyses unveil a dominant mediatory role of Gaussian-patterned inhibition in ripple generation, with recurrent excitations through chemical synapses and gap junctions in conjunction with return-current contributions from active dendrites playing regulatory roles in determining ripple characteristics.

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Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

Location: MCP Hall A

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Topic: B.07. Network Interactions

Support: NIH R21 MH125242
VA I01 BX004500
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VA IK6 BX005714

Title: Respiratory-entrained phase-amplitude coupling in mouse salience network-associated brain regions and attention in a signaled reaction time task.

Authors: E. B.-L. MANESS[1], M. G. MACIVER[1], J. CHOI[2], B. KOCSIS[3], R. E. STRECKER[1], J. T. MCKENNA[1], *J. M. MCNALLY[1];
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Abstract: Phase-amplitude coupling (**PAC**) between two different frequencies of neural oscillations has been shown to be involved in a range of cognitive processes, including attention. Such coupling is believed to reflect synchronization between local and/or global networks in the brain. Recent findings suggest that the phase of respiratory rhythms can enhance the amplitude of cortical local field potential (**LFP**) activity, not just in olfaction-related areas, but regions involved in attention and salient information processing as well. However, the incidence and importance of respiration-coupled oscillatory activity in sustained attention and how such activity changes across behavioral states are not well understood. Here, we utilized simultaneous respiratory and multi-site LFP electrode recordings in mice during performance of a self-initiated rodent psychomotor vigilance task that requires attention and measures signaled reaction times. In doing so, we compared time-frequency analysis with PAC in brain regions associated with attention and the processing of salient sensory signals. The analysis determined the role of slower rhythms, which correspond to the rate of respiration (1-8 Hz), in modulating LFP activity in higher frequency bands pertinent to signal detection and attentional performance (beta: 15-30 Hz; low-gamma, 30-80 Hz; mid-gamma, 80-120 Hz; high-gamma, 120-200 Hz). Time-frequency analysis of our data showed significant suppression of high-gamma activity and augmentation of beta and lower-frequency gamma power during the attentional effort preceding the visual cue in cortical areas included in the salience network, such as the prelimbic and anterior insular cortices, as well as the basal forebrain, a subcortical region recently shown to modulate salience and default network activity. Additionally, beta and gamma PAC were found at respiration-consistent phase frequencies in these areas, but *not* in the hippocampal CA1 subregion, during attention. This PAC was not observed during inattentive periods (reward retrieval). Additionally, faster signaled reaction times were associated with slower respiratory rate and greater coupling between respiration and mid-gamma oscillations compared to trials with slower reaction times. Taken together, these findings suggest that respiratory entrainment of fast cortical and subcortical oscillations enhances attentional performance and supports goal-directed behavior.

Disclosures: E.B. Maness: None. **M.G. MacIver:** None. **J. Choi:** None. **B. Kocsis:** None. **R.E. Strecker:** None. **J.T. McKenna:** 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. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Merck MISP. **J.M. McNally:** None.

Poster

PSTR383

Mechanisms and Significance of Brain Oscillations

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: B.07. Network Interactions

Support: National Research Foundation of Korea grant (2N74510, Development of Original Technology of Collective Brain Science)
KIST Grant funded by the Korean goverment (2E32901)
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Title: Multi-frequency oscillations implement complex spatio-temporal patterns of inter-regional communication

Authors: *J. KIM[1,2,3], J. CHOI[4,5], D. BATTAGLIA[6,7];
[1]KIST, KAIST, Seoul, Korea, Republic of; [2]Koea Institute of Science and Technology (KIST), Seoul, Korea, Republic of; [3]Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea, Republic of; [4]Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of; [5]University of Science and Technology (UST), Daejeon, Korea, Republic of; [6]Univ. of Strasbourg, Strasbourg, France; [7]Centre national de la recherche scientifique (CNRS), Paris, France

Abstract: Cognitive tasks require the flexible communication between multiple brain regions, which may be facilitated by neural oscillations. Several studies have emphasized the role of transient oscillatory coherence in a specific frequency band (e.g. gamma) to mediate directed communication. However, oscillations in the brain occur simultaneously in multiple frequency bands, which dynamically cooperate to perform the cognitive tasks. In this study, we systematically investigate multi-frequency oscillation patterns and their effect on inter-region communication by constructing computational spiking networks models of structurally coupled local regions oscillating at different frequencies. We consider simulated network activity at different synchrony levels, ranging from asynchronous to edge-of-synchrony and more strongly synchronous regimes. We firstly identify multi-frequency oscillations patterns (MFOPs) that can transiently emerge during spontaneous dynamics. These MFOPs are characterized by the joint occurrence of oscillatory bursts across multiple frequencies and regions. Remarkably, we find that as an effect of inter-regional excitatory or inhibitory interactions, oscillatory burst at fast or slow frequencies can arise in all populations, irrespectively of their natural local resonance frequency. Secondly, we use information theoretical analyses to extract the Information Routing Patterns (IRPs) associated to each type of MFOP. We reveal that the joint occurrence of oscillatory bursts at different frequencies leads to a boosting of directed information transfer, particularly enhanced at one or more specific latencies within the slower oscillation cycles. Each

MFOP map to a different spatio-temporal motif of transfer boosting. Therefore, the variety of possible MFOPs gives rise to a rich dictionary of emergent IRPs, in which regions can exchange information multiple times in alternating directions, on time-scales different from the ones initially hardwired at the neuronal resonance level. Overall, our computational analyses predict that interacting faster and slower frequency oscillations can impact information routing in much more complex ways than postulated by current theories of frequency-multiplexing of directed communication or gating via cross-frequency coupling. They also emphasize that inter-regional information transfer is an active computation, not limited to the passive reception of information from a source region but also encompassing the active integration within the target region of multiple information chunks received at different times.

Disclosures: J. Kim: A. Employment/Salary (full or part-time):; Korea Institute of Science and Technology (KIST). **J. Choi:** A. Employment/Salary (full or part-time):; Korea Institute of Science and Technology (KIST). **D. Battaglia:** None.

Poster

PSTR384

Microglia Function Within Neural Circuits

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.01/B48

Topic: B.09. Glial Mechanisms

Support: NIH Grant U01-AA029969
NIH Grant F31AA030712
Charles Lafitte Foundation for Research in Psychology & Neuroscience

Title: Immune signaling modulation impacts developmental microglial-parvalbumin interactions in the male hippocampus

Authors: *J. DZIABIS[1], I. JONATHAN[1], B. HORVATH[1], G. ZHANG[1], C. J. SMITH[2], M. PATTON[1], D. M. NGUYEN[1], M. JAMMULA[1], B. DEVLIN[1], S. MONROE[3], S. BILBO[1]; [1]Duke Univ., Durham, NC; [2]Psychology and Neurosci., Boston Col., Chestnut Hill, MA; [3]Neurobio., Duke Univ., Durham, NC

Abstract: As the resident macrophages of the brain, microglia perform many immune roles, such as releasing inflammatory factors and phagocytosing debris. However, microglia also perform indispensable roles in development, often using the same immune language and functions to direct the healthy maturation of synaptic connections, circuits, and ultimately behavior. Therefore, it is unsurprising that aberrant inflammation during development may contribute to brain changes through microglial mechanisms. Here, we sought to experimentally modulate microglial response to an immune challenge early in life. We were interested in the role that microglia may play in the development of parvalbumin+ interneurons (PVIs). PVIs are GABAergic cells with high metabolic needs and a long maturation period, making them highly

susceptible to early life challenges. Indeed, PVIs are a common cell type impacted in disorders with neurodevelopmental origins and immune etiology. Despite this, little is known about how and when microglia interact with PVIs in the context of healthy development or during immune challenge. We hypothesized that in the context of early life inflammation, loss of microglial pro-inflammatory signaling would be beneficial for developing PVIs. We administered lipopolysaccharide (LPS) at postnatal day (P)4 to male and female mice that either had intact microglia (control) or a constitutive loss of MyD88 (cKO), a critical co-adaptor for most toll-like receptors, in microglia (Cx3cr1+ cells). Loss of microglial-MyD88 blunted the release of cytokines and chemokines acutely in response to LPS. Male mice without microglial-MyD88 had significantly more PVIs across the hippocampus in adulthood, an effect that was exacerbated by P4 LPS. The same pattern was found by electrophysiology; male cKO mice that received P4 LPS early had increased sIPSC amplitudes relative to controls. This suggested that baseline microglia inflammatory signaling may play a role in regulating the development of PVIs in a sex-specific manner. To determine when microglia may be controlling future PVI density, we compared control and cKO microglia during the second postnatal week and found that male cKO microglia are more phagocytic than controls, but do not preferentially eliminate excitatory presynaptic material at P12. Instead, cKO males that received P4 LPS have significantly more inhibitory presynaptic material in their microglial lysosomes. Future work will identify how cKO microglia are transcriptionally distinct from controls, both at baseline and in response to LPS, to better understand the mechanisms that lead to inappropriate microglial-interneuron interactions in development.

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Poster

PSTR384

Microglia Function Within Neural Circuits

Location: MCP Hall A

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Program #/Poster #: PSTR384.02/B49

Topic: B.09. Glial Mechanisms

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NSF Grant 1943906

Title: Microglia surveillance responds to neuronal activation and sustained intracortical microstimulation

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Abstract: Microglia play a significant role in intracortical microstimulation (ICMS) through the formation of the glial scar around implanted electrodes. However, the effects of ICMS on the activity and surveillance of microglia have not been previously explored. Thus, we investigated how microglia processes respond to ICMS. Microglia morphology and neuronal calcium activity were labeled using CX3CR1-GFP/jRGECO transgenic mice (N=4 mice - 2 m, 2 f, n=32 cells). 4-channel Michigan probes were inserted into Layer 2/3 of the right visual cortex and activity under anesthesia was recorded using two-photon microscopy during ICMS (1 hr, 10 Hz, 10 uA). Given that microglia surveillance consists of process extensions and retractions, we measured the proportion and speed of these movements to determine if stimulation induced any changes. Across all time points, extensions and retractions occurred with equal frequency (One-Way ANOVA, $p=0.48$) and an average movement speed of 0.769±0.0035 um/min (n=32). Retractions were 6.1% faster than extensions across all timepoints (0.736±0.006 vs 0.781±0.006 um/min, Two-Way ANOVA, $p<0.001$). However, the speed of retractions also increased by 9.71% from pre-to post-stimulation (0.78 vs 0.85 um/min, Kruskal-Wallis, $p<0.001$). This increase in retraction speed could suggest that sustained stimulation affected microglia surveillance. To assess the effects of the electric field, we quantified the angle of process movements relative to the electrode site. Extensions and retractions demonstrated a non-uniform distribution of angles, with movements generally toward the electrode (K-S, $p<0.001$ and $p<0.005$). However, across all timepoints, extensions within 150 um were more directed toward the electrode (Two-Way ANOVA, $p<0.001$) while retractions were directed away (Two-Way ANOVA, $p<0.001$). Thus, the presence of the electrode without stimulation may be affecting microglia surveillance. We also assessed microglia activity related to activated neurons. Although there were no significant changes pre-, post-, and during stimulation, processes within 20 um of activated neurons moved faster compared to distant processes (Two-Way ANOVA, $p<0.05$). Extensions of processes within 20 um of activated neurons were also preferentially directed toward the neuron compared to process extensions further away (Two-Way ANOVA, $p<0.05$) while stimulation had no effect ($p=0.92$). We have demonstrated that the retraction speed of microglia processes increased after electrical stimulation, the speed of process movements is increased by neuron activation, and the direction of process movement is affected by proximity to the electrode and neurons.

Disclosures: C. Preszler: None. **K.C. Stieger:** None. **K. Chen:** None. **T.D. Kozai:** None.

Poster

PSTR384

Microglia Function Within Neural Circuits

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.03/B50

Topic: B.09. Glial Mechanisms

Support: NIH Grant R35NS132326
NIH Grant K99NS126417

Title: Chemogenetic activation of microglial Gi signaling decreases microglial surveillance and impairs neuronal synchronization

Authors: *S. ZHAO, L. WANG, Y. LIANG, J. ZHENG, L.-J. WU;
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Abstract: Microglia actively survey the brain and dynamically interact with neurons to maintain brain homeostasis. Microglial Gi-protein coupled receptors (Gi-GPCRs) play a critical role in microglia-neuron communications. However, the impact of temporally activating microglial Gi signaling on microglial dynamics and neuronal activity in the homeostatic brain remains largely unknown. In this study, we employed Gi-based Designer Receptors Exclusively Activated by Designer Drugs (Gi-DREADD) to selectively and temporally modulate microglial Gi signaling pathway. By integrating this chemogenetic approach with *in vivo* two-photon imaging, we observed that exogenous activation of microglial Gi signaling transiently inhibited microglial process dynamics, reduced neuronal activity, and impaired neuronal synchronization. These altered neuronal functions were associated with a decrease in interactions between microglia and neuron somata. Altogether, this study demonstrates that acute, exogenous activation of microglial Gi signaling can regulate neuronal circuit function, offering a potential pharmacological target for neuromodulation through microglia.

Disclosures: S. Zhao: None. **L. Wang:** None. **Y. Liang:** None. **J. Zheng:** None. **L. Wu:** None.

Poster

PSTR384

Microglia Function Within Neural Circuits

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.04/B51

Topic: B.09. Glial Mechanisms

Support: NIH Grant: 1 F32 MH32MH 124298- 01A1
URMC SPIN Grant
NIH Grant: R01 MH127737

Title: Dopaminergic signaling regulates microglial surveillance and adolescent plasticity in the frontal cortex

Authors: *R. D. STOWELL[1], K. H. WANG[2];
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Abstract: Adolescence is a sensitive period for frontal cortical development and cognitive maturation. The dopaminergic (DA) mesofrontal circuit is malleable in response to changes in

adolescent experience and DA activity. However, the cellular mechanisms behind this plasticity remain unexplored. Microglia, the resident immune cells of the brain, serve diverse roles in developmental circuit refinement and respond to specific neurotransmitter signals. With a combination of transgenic, optogenetic, and viral labeling methods in mice, we investigated if microglia respond to changes in mesofrontal DA signaling *in vivo*. Using *in vivo* two-photon microscopy, we tracked fluorescently labeled microglia and DA axons in the M2 frontal cortical region pre- and post- stimulation of DA activity. After 2-hours of wheel running, which activates DA neurons, microglia exhibit increased arborization and parenchyma occupation. This effect was unique to adolescent frontal, but not visual, cortical microglia. Furthermore, microglia exhibit a biphasic response to optogenetic stimulation of DA axons, characterized by an initial reduction in surveillance of the parenchyma during DA release, and a subsequent extension of processes post-stimulation. After stimulation, microglia contact the axonal backbone prior to new bouton formation. Pharmacological manipulation of either D1-type receptors (D1R) or D2R perturbs the biphasic microglial response to DA axon stimulation, blocks bouton plasticity, and attenuates microglial contacts with boutons. Further, blocking D2R signaling in adult mice reinstates microglial surveillance of DA axons and axonal plasticity. Our results show that DA signaling regulates microglial surveillance and axonal plasticity uniquely in the adolescent frontal cortex.

Disclosures: R.D. Stowell: None. **K.H. Wang:** None.

Poster

PSTR384

Microglia Function Within Neural Circuits

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.05/B52

Topic: B.09. Glial Mechanisms

Support: NIDA Grant 5R01DA039062-08

Title: Capturing the effects of cannabinoid signaling on microglial motility through live-cell imagining of ex vivo slice cultures

Authors: *N. MOIN AFSHAR[1], S. ASHTON[2], M. M. MCCARTHY[3];
[1]Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Psychology, Univ. of Maryland, Col. Park, College Park, MD; [3]Dept. of Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Cannabis use during pregnancy has increased in the United States concomitant with increased positive perceptions of cannabis and its main psychoactive component Δ9-tetrahydrocannabinol (THC). Despite its growing prevalence, investigations of the effects of gestational cannabis exposure have identified differences in cognitive, attentive, and emotional processing as well as deficits in social interaction, together impacting multiple facets of the

child's life (e.g. PMID: 32965490). Understanding of how developmental THC exposure affects later-life behavior is limited, but one potential mechanism is that THC exposure may disrupt critical processes normally mediated by endocannabinoid signaling. Preclinical research indicates endocannabinoid signaling within the developing amygdala mediates microglial phagocytosis of astrocytic progenitors. Specifically, the amygdala of neonatal male rats has a higher endocannabinoid tone and greater rate of microglia-mediated phagocytosis compared to that of female rats. This process is important for appropriate masculinization of social play circuitry and is mimicked in female rats developmentally exposed to THC. The mechanism by which THC modulates microglial phagocytic activity has not been identified; however, we have demonstrated that microglia within the medial amygdala of males display increased interaction with surrounding cells—and newborn cells in particular—compared to those in the female (PMID: 30827729). This sex difference in the interactome of microglia may contribute to the greater rates of phagocytosis in males than females. We hypothesize that cannabinoid signaling in microglia in both sexes promotes increased motility resulting in increased surveillance and subsequent identification of pro-phagocytic markers on neighboring cells. To test this hypothesis, we employ *ex vivo* slice cultures from the SD-Tg(Iba1-EGFP)Mmmc transgenic rat, giving us the ability to perform live imaging of endogenously fluorescent microglia within a native brain structure (PMID: 34417284). We predict that exposure of brain slices to exogenous cannabinoids such as THC will increase microglia motility. This experimental approach will allow for further dissection of the signaling cascade mediating the effects of THC as well as the ability to investigate the role of various pro- and anti-phagocytic signals on microglia activity. This work furthers understanding of how perinatal THC exposure impacts the developing brain.

Disclosures: N. Moin Afshar: None. **S. Ashton:** None. **M.M. McCarthy:** None.

Poster

PSTR384

Microglia Function Within Neural Circuits

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.06/B53

Topic: B.09. Glial Mechanisms

Support: Burroughs Wellcome Fund Career Award at the Scientific Interface

Title: Unveiling the Impact of Glial Cells on Neuronal Dynamics: Insights from Synaptic Ensheathment

Authors: N. GARCIA, *G. HANDY;
Univ. of Minnesota, Minneapolis, MN

Abstract: Recent years have witnessed a growing body of experimental evidence highlighting the active role of glial cells in modulating neuronal dynamics. Glial cells, including astrocytes and microglia, have been found to influence various aspects of neuronal function, such as

regulating neurotransmitter concentrations and ion buffering. However, the mechanisms through which glial cells communicate with and affect neighboring neurons remain unclear.
In this study, we focus on recent experimental findings indicating that glial cells physically wrap around synapses, a process known as "ensheathing," which can disrupt the flow of neurotransmitters between pre- and post-synaptic terminals. We expand upon a previous microscale model of the synaptic cleft to explore how different strengths of synaptic ensheathment impact synaptic communication. Our results align with a prior study (Handy and Borisyuk, 2023), suggesting that ensheathment accelerates synaptic transmission while reducing its strength and reliability. However, we find that the previous model underestimates this effect, as glial cells can effectively switch off synaptic connections.
Building on these findings, we introduce an "effective" glial cell model that can be integrated into large-scale neuronal networks. Specifically, we consider a network with highly heterogeneous synaptic time constants, where the degree of astrocytic proximity parametrizes synaptic time constants. Unlike previous studies that assumed normal parameter distributions, our model uses parameters drawn from distinct distributions. We apply this framework to large networks of exponential integrate-and-fire neurons, extending linear response theory to analyze not only firing rate distributions but also noise correlations across the network.
Finally, we demonstrate the utility of our model by reproducing recent findings from Haruwaka et al. (2024), which showed that microglial ensheathment leads to post-anesthesia hyperactivity of excitatory neurons. We also explore critical network thresholds that enable this effect to manifest.

Disclosures: N. Garcia: None. **G. Handy:** None.

Poster

PSTR384

Microglia Function Within Neural Circuits

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.07/B54

Topic: B.09. Glial Mechanisms

Support: NIH Grant NS120609
NIH Grant EY019277
NIH Grant NS099973
NIH Grant NS114480

Title: Microglial β2-adrenergic signaling modulates microglial surveillance and injury response dynamics in a region specific manner

Authors: *M. B. STOESSEL[1], A. N. VU[2], R. D. STOWELL[2], A. K. MAJEWSKA[2];
[1]Univ. of Rochester, Rochester, NY; [2]Neurosci., Univ. of Rochester, Rochester, NY

Abstract: Microglia interact with neurons in an activity dependent manner to influence neuronal plasticity. This interaction has been shown to in part depend upon long range neuromodulatory cues to such as norepinephrine (NE). NE is of particular interest in that it affects neuronal plasticity in an activity dependent manner with noradrenergic tone itself depending greatly upon arousal. Microglia express the β2 adrenergic receptor (β2-AR) at higher levels than other cells in the nervous system, and stimulation of this receptor in cortical microglia produces robust decreases in microglial motility, surveillance, and interaction with cortical neurons. Taken together, such evidence presents a potential mechanism connecting microglial dynamics, neuronal plasticity, and behavioral state. Whether microglial β2-AR signaling is conserved across microglial populations in different brain regions remains an open question. Given the differences in the physiology and transcriptional profile between cortical and cerebellar microglia. We show that cerebellar microglia respond to β2-AR stimulation in a similar manner to that of their cortical counterparts, reducing their process ramification and surveillance. We report subtle alterations to the microglial focal injury response, though interestingly, we find that β2-AR stimulation increases somal motility during the focal injury response. Despite the robust decrease in surveillance elicited by β2-AR stimulation in cerebellar microglial and the hypothesized link between microglial motility and synaptic plasticity, we found no changes in cerebellar learning when central β2-ARs were chronically stimulated. Our results highlight the effects of noradrenergic signaling on microglia which could alter their interactions with the cerebellar microcircuit.

Disclosures: M.B. Stoessel: None. **A.N. Vu:** None. **R.D. Stowell:** None. **A.K. Majewska:** None.

Poster

PSTR384

Microglia Function Within Neural Circuits

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.08/B55

Topic: B.09. Glial Mechanisms

Support: K08NS114170
R01NS129609

Title: $P2y_{12}$ receptors are critical for th regulation of neuronal excitability by satellite microglia

Authors: *A. B. FEICHTENBINER[1], K. SYTSMA[2], R. O'BOYLE[3], C. B. RANSOM[4], A. NOLAN[2];
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Abstract: Microglia, the primary mediators of innate immune activation in the brain, are increasingly recognized as key modulators of neuronal activity. Prolonged activation of the innate immune system can impede repair in traumatic brain injury (TBI), and it is not understood how microglia's impact on neuronal activity might contribute or protect. One microglial subtype that appears to be critical in the regulation of neuronal excitability is the perineuronal satellite microglia (Sat-MG). These microglia are juxtaposed adjacent to neurons with their soma and processes entwined around the neuronal cell body. Our previous work demonstrated that Sat-MG suppress neuronal excitability in control conditions but lose this ability with chronic TBI. We have also found that there is an associated decrease in expression of $P2Y_{12}$ receptors with chronic TBI; however, the role of this receptor in satellite microglia-associated neuronal regulation of excitability has not yet been characterized. To investigate this potential relationship, we utilized transgenic mice with GFP-labeled microglia (Tmem119-EGFP) to obtain whole cell patch clamp recordings for neurons both near and away from satellite microglia in the presence and absence of a $P2Y_{12}$ receptor antagonist (PSB0739). Our data indicate that blocking the P2Y12 receptor reverses the Sat-MG-associated reduction in excitability found in control conditions, while also increasing hyperexcitability in the network, similar to chronic TBI and supporting our hypothesis that reduction of P2Y12Rs in microglia may contribute to loss of neuronal regulation and cognitive dysfunction after TBI.

Disclosures: A.B. Feichtenbiner: None. **K. Sytsma:** None. **R. O'Boyle:** None. **C.B. Ransom:** None. **A. Nolan:** None.

Poster

PSTR384

Microglia Function Within Neural Circuits

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.09/B56

Topic: B.09. Glial Mechanisms

Support: Schmitt Program in Integrative Neuroscience (SPIN) Grant
NSF Grant 2150799

Title: Towards Deciphering the Mechanism of Microglia-Driven Remodelling in Brain Extracellular Space Using SFA-FRAP

Authors: *S. R. PAUL[1], T. ELIAS[2], A. K. MAJEWSKA[3], E. BROWN[2];
[1]Univ. of Rochester, Rochester, NY; [2]Biomed. Engin., Univ. of Rochester, Rochester, NY;
[3]Neurosci., Univ. of Rochester, Rochester, NY

Abstract: Learning and memory require dynamic movement of different diffusible molecules and structural changes of dendritic spines, crucial for synaptic plasticity and the remodeling of neural connections. However, being a physical barrier, the extracellular matrix (ECM) of the brain can affect this movement. We hypothesize that microglia are master manipulators of the

ECM due to the remarkable motility of microglial processes, and the ECM-degrading enzymes that they produce. We utilize acute brain slices from *Cx3cr1*^{CreER}*Ai9-tdTomato* mice and apply a quantitative optical method, Two-Photon Fluorescence Recovery After Photobleaching with Spatial Fourier Analysis (2P-SFA-FRAP), which we have been developing and optimizing specifically for this investigation, to measure diffusive transport within ECM while manipulating microglial morphology and dynamics. Our objective is to assess whether microglia actively modulate ECM hindrance, as measured by diffusive transport, and if so, to elucidate the precise mechanism through which they accomplish such modulation. We have measured diffusion coefficients in cortical acute brain slices which are recovered in 2000kDa and 500kDa FITC-Dextran. We have shown that 2D Fourier Transformed 2P-SFA-FRAP data in FITC-Dextran solution and *in situ* in recovered brain slices is well fit by a single exponential model which allow the determination of a diffusion coefficient. The diffusion coefficient of 2000kDa FITC-Dextran *in situ* in brain slices ($D = 3.78 \pm 0.62$ $\mu m^2/s$, $n = 3$) is significantly lower than in free solution ($D = 6.31 \pm 1.13$ $\mu m^2/s$, $n = 3$). When comparing the diffusion coefficients between tracers of different sizes, we noted an approximately twofold increase in diffusion coefficient for the 500kDa tracer compared to that of the 2000kDa tracer, suggesting a complex relationship *in situ* compared to Stokes-Einstein relationship they follow in free solution. We are currently optimizing the 2P-SFA-FRAP technique to extract data in a reliable and replicable way and then continue to collect data for at least 4 more tracer sizes, covering a range of hydrodynamic radii (RH) from ~0.4 to 20 nm. We aim to characterize the ECM while manipulating the microglia dynamics through purinergic and adrenergic stimulation. Results from this study are anticipated to shed light on the mechanisms underlying microglia-ECM interactions and provide insights into potential therapeutic strategies for neurological disorders associated with aberrant synaptic plasticity.

Disclosures: S.R. Paul: None. **T. Elias:** None. **A.K. Majewska:** None. **E. Brown:** None.

Poster

PSTR384

Microglia Function Within Neural Circuits

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.10/B57

Topic: B.09. Glial Mechanisms

Support: NIH Grant RO1GM134104
Cosmos Club Foundation Cosmos Scholar Grant
Uniformed Services University Graduate Student Research Award Grant

Title: Determining actin cytoskeleton's role in iC3b-mediated synaptic pruning in mice

Authors: *S. G. PAULSON, J. D. ROTTY;
Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD

Abstract: Microglia are the resident immune cells of the central nervous system (CNS). They respond to phagocytic cues to help maintain homeostasis within the brain. These phagocytic cues include members of the complement cascade, a pro-inflammatory protein cascade responsible for helping label and clear foreign bodies and debris. Complement proteins such as iC3b are also used during learning and memory to label excess dendritic spines for phagocytosis in a process called synaptic pruning. Our initial studies use iC3b as an *in vitro* model substrate to understand how microglia use actin-dependent cellular protrusions to sense and respond to the iC3b phagocytic cue. Using primary murine microglia and actin cytoskeleton targeting drugs, we utilized a novel confinement assay to model the higher confinement levels found in the CNS and examined our cells' movement and response to phagocytic cues under the influence of confinement. Preliminary confinement data suggests that microglia phagocytize more efficiently and migrate more persistently during confinement than they do in a 2D culture on glass. Using a mouse model that allows for targeted knockout of the actin cytoskeleton component Arp2/3 in microglia, our next steps are to use hippocampal slice cultures to understand how iC3b-mediated phagocytosis works within an *in vivo* context. Loss of the Arp2/3 complex may impair iC3b-mediated synaptic pruning through rendering microglia unable to sense the iC3b cue, as has been shown in our preliminary data using the BV2 microglial cell line. This change in synaptic pruning may lead to changes in neuronal structure, and cognitive functions such as learning and memory. Through use of the Morris Water Maze, rotarod, and fear conditioning behavioral assays, the impact of microglial inability to sense iC3b on dendritic spines is being determined. Our overall goal is to define the molecular mechanism and *in vivo* contribution of microglial actin-based complement-sensing protrusions to CNS function.

Disclosures: S.G. Paulson: None. **J.D. Rotty:** None.

Poster

PSTR384

Microglia Function Within Neural Circuits

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.11/B58

Topic: B.09. Glial Mechanisms

Support: STI 2030-Major Projects 2022ZD0204704, 2021ZD0201704
National Natural Science Foundation of China 82071311
Shanghai Municipal Science and Technology Major Projects 22ZR1413700, 2018SHZDZX01

Title: Hdac3 expression in microglia is required to maintain microglial homeostasis and normal synaptic pruning during development

Authors: *Y. YUAN, H. CAO, X. CHEN, J. LI, C. WANG, X. PEI, L. MAO, Y. GAO;
State Key Lab. of Med. Neurobio., MOE Frontier Ctr. for Brain Sci., and Inst. of Brain Sci., Fudan Univ., Shanghai, China

Abstract: The inhibition of HDAC3 expression in microglia results in specific suppression of pro-inflammatory microglial proliferation post-stroke, thereby promoting post-stroke neurofunctional recovery. Particularly, the subtype-specific proliferation inhibition by HDAC3 is associated with PU.1, a pivotal transcription factor during microglial development. This study primarily focuses on elucidating the role of HDAC3 in microglial cell development and the consequences of HDAC3 knockout-induced microglial homeostatic imbalance on neurons. We performed subcutaneous injections of tamoxifen in $HDAC3^{fl/fl}CX3CR1CreERT^{+/-}$ mice at P0 to induce specific HDAC3 knockout in developing microglia (HDAC3-miKO-D). During developmental stages, we observed a significant reduction in the number of Iba1-positive cells in the HDAC3-miKO-D group compared to the wildtype group. Interestingly, in the adulthood of the HDAC3-miKO-D group, we identified an increase in Iba1-positive cells independent of Cre nuclear entry, along with sustained pro-inflammatory activation of these cells across different time points compared to other groups. Immunostaining for macrophage and microglia-specific markers further revealed that these additional Iba1-positive cells predominantly expressed markers of macrophages, suggesting an increased infiltration of macrophages in the brain. This infiltration resulted from the enhanced proliferation of Iba1-positive cells, particularly within the brain's macrophage population, rather than disruption of the blood-brain barrier. At the structural and functional levels of neurons, HDAC3 knockout delayed early myelination maturation without impacting white matter structure and function in adulthood. Dil and Golgi staining revealed a significant reduction in dendritic spine density, particularly in the thin dendritic spine, a mature spine type associated with learning and memory, in adult knockout mice. This reduction was attributed to excessive synaptic engulfment by Iba1-positive cells. Consequently, HDAC3-miKO-D resulted in impaired memory function and pronounced anxiety-like behavior in adult mice, while not affecting motor function. In conclusion, this study provides new insights into the role of HDAC3 in regulating morphological, numerical, and functional changes in microglia during development and highlights the impacts of developmental microglial changes on neuronal structure and cognitive function in adulthood.

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Poster

PSTR384

Microglia Function Within Neural Circuits

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.12/

Topic: B.09. Glial Mechanisms

Support: NIH R35GM124977-05

Title: Unraveling the dynamics of pi3k/akt-mediated microglial phagocytosis: insight into microglial polarization

Authors: *E. KRUEGER[1], R. SETHI[2], P. KEKENES-HUSKEY[3];
[1]Cell and Mol. Physiol., Loyola Univ. Chicago, Maywood, IL; [2]Loyola University-Chicago, Maywood, IL; [3]Cell and Mol. Physiol., Loyola University-Chicago, Maywood, IL

Abstract: Microglia, the resident macrophages of the central nervous system (CNS), surveil the brain parenchyma and phagocytose deleterious materials to ensure the health of the CNS. Microglial phagocytosis involves intricate cytoskeletal rearrangement driven by receptor activation and downstream intracellular communication. Among the signaling cascades that control microglial function, the PI3K/Akt pathway stands out as a key regulator, steering microglia towards an anti-inflammatory phenotype characterized by enhanced phagocytosis. While the influence of the PI3K/Akt axis on microglial polarization is recognized, the kinetics, magnitude, and feedback inhibition governing this signaling cascade remain incompletely understood. To address these gaps, we implemented an *in vitro* system using BV2 microglia to evaluate the effects of the activation of the PI3K/Akt axis on microglial phagocytosis. By introducing various effectors, we elucidated their individual roles in modulating this signaling cascade and subsequent phagocytosis. Furthermore, we established a computational model of PI3K/Akt-mediated phagocytosis to predict how distinct components of the PI3K/Akt axis orchestrate microglial behaviors and understand what intrinsic timescales govern its regulation of phagocytosis. By delineating the dynamics of PI3K/Akt-mediated microglial phagocytosis regulation, we aim to uncover fundamental insights into the mechanisms underlying microglial function and its implications for CNS health and disease.

Disclosures: E. Krueger: None. **R. Sethi:** None. **P. kekenes-huskey:** None.

Poster

PSTR384

Microglia Function Within Neural Circuits

Location: MCP Hall A

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Program #/Poster #: PSTR384.13/B59

Topic: B.09. Glial Mechanisms

Support: NIMH R01MH119164
NIMH R01MH118332

Title: Ketamine as a Novel Strategy for Normalizing Microglial-Mediated Synaptic Pruning in a Mouse Model of Early Life Adversity

Authors: *C. BOWERS[1], A. KAFFMAN[1], S. AHMED[2], L. GIULIANO[3];
[1]Psychiatry, Yale Univ., New Haven, CT; [2]Psychiatry, Yale Med. Sch., New Haven, CT;
[3]Psychiatry, Yale Univ., Orange, CT

Abstract: Early Life Adversity (ELA) impairs hippocampal development and function in humans and rodents. Most of the research in mice has primarily focused on the mechanisms underlying cognitive and synaptic impairments in adult animals, with very few studies exploring this question in adolescent mice. In addressing this issue, we have recently shown that prepubescent pups at postnatal day 17 (P17), subjected to the limited bedding (LB) paradigm exhibit significant deficits in microglial-mediated synaptic pruning in the hippocampus. These deficits were associated with reduced synaptic connectivity and abnormal contextual fear conditioning in P33 adolescent mice. Chemogenetic activation of microglia during the second week of life normalized microglia-mediated synaptic pruning at P17 and corrected the synaptic and cognitive deficits observed in adolescent mice. These findings suggest that pharmacological interventions that enhance microglial-synaptic pruning during this critical period may be able to correct the synaptic and contextual fear conditioning abnormalities observed in adolescent LB mice. Given the ability of a low dose of ketamine to impact microglial function, we tested the effects of 10 mg/kg of ketamine intraperitoneally on microglial-mediated synaptic pruning in vivo and ex vivo in P17 pups exposed to control and LB conditions. Preliminary data showed that ketamine injection rapidly normalized microglial volume and the capacity to phagocytose synaptic material in LB pups but not control (CTL). This impact was seen within one hour post injection and was associated with rapid mTOR phosphorylation and Trem2 expression in microglia. These results suggest a novel role for ketamine in rescuing microglial-mediated synaptic pruning during a critical period of hippocampal development in mouse models of ELA as well as uncovering potential direct effects of ketamine on the microglia.

Disclosures: C. Bowers: None. **A. Kaffman:** None. **S. Ahmed:** None. **L. Giuliano:** None.

Poster

PSTR384

Microglia Function Within Neural Circuits

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Topic: B.09. Glial Mechanisms

Support: NIDA Grant 5R01DA039062-08

Title: Microglial response to THC exposure at varying timepoints across development

Authors: *A. PHAM[1], M. M. MCCARTHY[2];
[1]Univ. of Maryland, Baltimore, Baltimore, MD; [2]Dept. of Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: As a result of increased legalization and medicalization of cannabis, its use during pregnancy has increased in the US and Canada. Cannabis use has also increased among adults with children present in the home, creating the potential for exposure to the primary psychoactive component of cannabis, Delta-9-tetrahydrocannabinol (THC), at varying timepoints across development. Preclinical work in rodents finds that androgens program endocannabinoid tone which in turn regulates microglial phagocytosis of early astrocyte progenitors only in the developing amygdala (PMID:30827729). This results in more microglia-mediated phagocytosis in males compared to females which sculpts sex specific social play circuitry. Females exposed to THC postnatally, a partial agonist for endocannabinoid receptors, see increases in microglial phagocytosis and subsequent increases in social play to that of control male levels. Interestingly, preliminary data from our lab has shown that offspring exposed to THC prenatally have a decreased total number of microglia with no changes in frequency of phagocytic microglia in multiple brain regions. Microglia also have a wide range of morphologies and actively modify their processes and soma in response to varying stimuli. A transitioning morphology between ameboid and ramified has been associated with phagocytosis, but there is a lack of standard classification, making it difficult to link a specific morphology to a particular role. We seek to address this deficit using a combination of fractal analysis and principal component analysis for unbiased morphological classification to determine if THC exposure at varying time points across development influences morphology. We hypothesized that the timing of THC exposure results in distinct changes in microglia number, frequency of phagocytosis, and morphology. Through image analysis using MicroglialMorphology for FIJI (https://doi.org/10.1101/2023.11.03.565581), we found that exposure to THC induced brain-region and sex specific changes in microglial morphology. Exposure to THC postnatally resulted in a higher number of ameboid microglia in the amygdala in both females and males (2way ANOVA treatment; $P = <0.001$), and interestingly an increase in the number of transitioning microglia only in males (2way ANOVA interaction; $P = 0.035$). We seek to use these results to emphasize the importance of unbiased tools to interrogate changes in microglial morphology in response to stimuli and to determine the difference in microglial response to THC exposure prenatally, postnatally, or perinatally.

Disclosures: A. Pham: None. **M.M. McCarthy:** None.

Poster

PSTR384

Microglia Function Within Neural Circuits

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.15/B61

Topic: B.09. Glial Mechanisms

Support: NIMH K99/R00 Pathway to Independence Award #MH120327
NARSAD Young Investigator Grant #31308

Whitehall Foundation Grant #2022-08-051
NSF Graduate Research Fellowship Program

Title: Early-life adversity alters microglia-mediated synaptic refinement of corticotropin-releasing hormone-expressing neurons in the central nucleus of the amygdala

Authors: *H. D. LICHTENSTEIN[1], M. K. SEQUEIRA[2,3], J. L. BOLTON[1];
[1]Georgia State Univ. Neurosci. Inst., Atlanta, GA; [2]Neurosci. Inst., Emory Univ., Decatur, GA; [3]Georgia State University Neuroscience Institute, Atlanta, GA

Abstract: Early-life adversity (ELA) is a risk factor for developing neuropsychiatric disorders like depression. The central nucleus of the amygdala (CeA) is a stress-sensitive region of the brain that is also involved in reward-related behaviors. We recently showed that ELA provokes anhedonia-like behaviors in males, mediated by corticotropin-releasing hormone (CRH) overexpression in the CeA. In another stress-sensitive brain region, we find that diminished microglial engulfment of excitatory synapses onto CRH+ neurons in the paraventricular nucleus of the hypothalamus (PVN) is a key mechanism that contributes to the altered stress responsivity of male mice exposed to ELA. Interestingly, these two stress-sensitive regions of the brain have different developmental timelines, suggesting the possibility of different sensitive periods for intervention in the CeA compared to the PVN. There may also be sex-specific effects of ELA and different developmental trajectories between the sexes at baseline. Here, we test the hypotheses that 1) microglial synaptic pruning is impaired in the CeA by ELA and 2) this enduringly impacts the synaptic inputs to CeA-CRH+ neurons. To test these hypotheses, we induced ELA using limited bedding and nesting from postnatal days (P)2-10. We employed male and female single- (CRH-tdTomato+) and double- (CX3CR1-GFP+; CRH-tdTomato+) reporter mice, in conjunction with immunohistochemistry to label pre- and post-synaptic excitatory and inhibitory puncta, and confocal microscopy with IMARIS software for data collection and analysis, to assess synapse number and microglial synaptic pruning. We find that ELA increases excitatory and decreases inhibitory synaptic inputs onto CeA-CRH+ neurons at P14 in males, but not females, and this effect persists through at least P24, weeks after the ELA experience has ended. Our synapse engulfment data indicates that ELA inhibits microglial phagocytosis of excitatory synapses on CRH+ neurons specifically in the CeA. Analysis of microglial engulfment of inhibitory synaptic puncta and the timeline of synaptic pruning is ongoing. Our future work will investigate if these impairments can be prevented by chemogenetically manipulating microglial dynamics during development.

Disclosures: H.D. Lichtenstein: None. **M.K. Sequeira:** None. **J.L. Bolton:** None.

Poster

PSTR384

Microglia Function Within Neural Circuits

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.16/B62

Topic: B.09. Glial Mechanisms

Support: NIH Grant U01-AA029969
NIH Grant F31AA030712
Duke Dean's Summer Research Fellowship Grant
Duke Psychology & Neuroscience Vertical Integration Program Grant

Title: Investigating the role of microglia pro-inflammatory signaling on early life adversity-induced brain and behavior outcomes

Authors: *G. ZHANG[1], J. DZIABIS[2], D. M. NGUYEN[3], S. BILBO[2];
[1]Duke Univ., Cary, NC; [2]Duke Univ., Durham, NC; [3]Duke Univ., Simpsonville, SC

Abstract: Early life adversity (ELA) is a known risk factor for psychopathology. In both human and animal models, ELA can impact circuit development, leading to long-term changes in emotional regulation, reward processing, and neuroendocrine response. Microglia, the resident immune cells of the brain, have emerged as a cell type of interest in understanding the etiology of ELA brain differences. In this study, we sought to determine if blunting microglial pro-inflammatory signaling would be protective in the context of ELA. We used a transgenic mouse line with a constitutive loss of MyD88 in Cx3cr1-expressing cells (in the brain, microglia). MyD88 is a critical protein in signal transduction pathways for response to many inflammatory agents, such that loss of MyD88 leads to blunting of NF-kappaB-dependent gene expression. To model ELA, we used the limited bedding and nesting (LBN) paradigm, where nesting resources for mothers are reduced from postnatal day (P)4-10. Mothers reared pups with (CON) and without (cKO) microglial MyD88 expression in LBN or control conditions. Offspring were weighed across development, and ultrasonic vocalizations were recorded in the second postnatal week. Both male and female CON and cKO weights were acutely reduced by LBN, with male effects lasting longer, though all changes were resolved by P28. Interestingly, LBN-cKO males on average consistently weighed the least of any group. LBN increased the number and length of ultrasonic vocalizations (USVs) in both male and female offspring, but loss of MyD88 was protective in males only, where cKO-LBN males called closer to control-reared levels. Finally, brain tissue was collected at P28 to look at microglia and parvalbumin interneuron (PVI) endpoints. PVIs are fast-spiking GABAergic cells and a common cell type impacted by ELA. Mature PVIs are surrounded by perineuronal nets (PNNs), which are extracellular matrix depositions that stabilize synapses. In the male prefrontal cortex, we observed a significant interaction of treatment and genotype on the density of PNN+ PVIs, where LBN increased density in CONs, but LBN-cKO males had similar density as control-reared pups. Continued work will determine if LBN-cKO microglia differentially interact with PNN components at P28, as well as investigate the same endpoints in females and in other brain regions.

Disclosures: G. Zhang: None. **J. Dziabis:** None. **D.M. Nguyen:** None. **S. Bilbo:** None.

Poster

PSTR384

Microglia Function Within Neural Circuits

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.17/B64

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01AA025591

Title: Time course of microglia depletion using CSF1R inhibitor PLX5622 in male and female rats

Authors: *E. R. CARLSON, S. A. COLLINS, K. NIXON;
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Abstract: Microglia, the hallmark neuroimmune effector cells of the brain, and their reactivity are thought to underlie brain damage in a variety of neurodegenerative conditions, including alcohol use disorder. One approach to establish the role of microglia in alcohol-induced neurodegeneration is to remove microglia and measure subsequent damage. The CSF1R inhibitor PLX5622 is an established tool for microglia depletion in mice, but infrequently used in rats. Here, we conducted a pilot study to determine the efficacy of PLX5622 in depleting microglia across dose and time via subcutaneous injection, a route not previously reported. Subcutaneous injection results in less trauma and risk of damage to internal organs, which are important considerations when frequent doses are required. Adult male and female Sprague-Dawley rats were treated with PLX5622 or vehicle (25 or 50 mg/kg; MedChemExpress), by subcutaneous injection (s.c. every 12 h), for three (50 mg/kg only), five (50 mg/kg only), or seven days. Rats were transcardially perfused with saline 12 h after the last dose of PLX, then brains were post-fixed in PFA for 24 h and sectioned at 40 µm on a vibrating microtome in 1:12 series. Immunohistochemistry for the microglia marker IBA1 (Wako) was conducted to quantify depletion. Automated counts of microglia were performed using an ImageJ macro on images acquired in the hippocampus and rhinal cortex to estimate % depletion. Seven days of 25 mg/kg PLX5622 resulted in 33% (hippocampus) to 55% (rhinal cortex) microglial depletion, while seven days of 50 mg/kg PLX depleted microglia 89% (hippocampus) to 92% (rhinal cortex). Shorter duration of 50 mg/kg PLX resulted in less effective microglial depletion, with three days of treatment resulting in 59% (hippocampus) to 69% (rhinal cortex) and five days yielding 77% (hippocampus) to 85% (rhinal cortex). Observationally, fewer (~10% less) microglia were seen in females compared to males after 7 days of 50 mg/kg PLX, but this study was not powered to detect sex differences. These data indicate pharmacological depletion of microglia is possible by subcutaneous administration of PLX5622 in male and females rats, with the most efficacious dosing paradigm being 50 mg/kg PLX5622 for 7 days twice daily.

Disclosures: E.R. Carlson: None. **S.A. Collins:** None. **K. Nixon:** None.

Poster

PSTR384

Microglia Function Within Neural Circuits

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.18/B65

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: University of Catania research grant PIAno inCEntivi RIcerca (PIACERI) Ateneo 2020/2022
PRIN 2017XZ7A37
PRIN 2022 E53D23011300006

Title: Potential neuroprotective role of neurosteroids acting through ionotropic receptors in microglia

Authors: *M. CHISARI[1], M. BARRACO[2], A. G. COPANI[3], M.-A. SORTINO[1];
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Abstract: Allopregnanolone is a natural neurosteroid known to modulate current of neuronal $GABA_A$ and NMDA receptors. Besides that, it was identified as positive modulator of ionotropic purinergic (P2X) receptors and primarily the subtype P2X4. It is known that this receptor might increase the microglia release of brain-derived neurotrophic factor (BDNF), leading to neuroprotective effects. However, little is known about P2X4 current regulation by allopregnanolone and its potential role in neuroinflammation through this mechanism. P2X4 receptors are endogenously expressed in BV2 microglia cell line and are activated by a wide range of ATP concentrations (3-100 μM). Performing patch-clamp experiments in BV2 cells, we found that allopregnanolone can potentiate P2X4 current in a concentration-dependent manner. At a sub-saturating ATP concentration (5 μM), potentiation by allopregnanolone occurs at the nanomolar range (1-100 nM), instead at saturating ATP concentration (50 μM), a small but significant increase in current is observed with high allopregnanolone concentration (10 μM). It is known that P2X4 receptors are present in lysosomes and their exocytosis, by fusion to the plasma membrane, was described as an important feature to promote synaptic plasticity and to increase myelin phagocytosis mediated by microglia. In order to link the increase in current mediated by allopregnanolone and the P2X4 receptor trafficking, we made a construct of P2X4 receptor tagged with the yellow fluorescent protein (P2X4-YFP). We expressed this construct in HEK cells and we confirmed current potentiation data observed in BV2 cells. Afterwards, we performed live cell imaging experiments and we found that P2X4-YFP colocalizes with the lysosomal marker lysotracker red DND-99 (1 nM, incubation 1 min at RT) in BV2 cells. Using the lysosomal inhibitor NH_4Cl (250 μM for 10 min), red fluorescence by lysotracker was dimmed meanwhile P2X4-YFP fluorescence increased, suggesting disruption of lysosomes and potentially higher P2X4 receptors trafficking towards the plasma membrane. Finally, we exposed P2X4-YFP transfected BV2 cells to ATP (5 μM) and allopregnanolone (100 nM) and we found an increase in fluorescence exclusively when cells were exposed to both drugs.Taken together, data suggest that the P2X4 increase in current by co-stimulation with allopregnanolone is likely due to new receptors inserted in the plasma membrane while lysosomes are fusing to it. Such mechanism suggests a neuroprotective role of neurosteroids mediated by microglia.

Disclosures: M. Chisari: None. **M. Barraco:** None. **A.G. Copani:** None. **M. Sortino:** None.

Poster

PSTR384

Microglia Function Within Neural Circuits

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.19/B66

Topic: B.09. Glial Mechanisms

Support: Startup funds from Emma Lundberg lab
NIH#P30AG066515
Knight Initiative funding

Title: Development of Spatial Proteomics Assays: Exploring Microglial States across Neuroinflammatory, Neurodegenerative and Normal Tissues

Authors: *A. MARTINEZ CASALS[1], J. NIRSCHL[2], A. ISAKOVA[3], C. SMURTHWAITE[4], E. LUNDBERG[1,5];
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Abstract: Microglia are resident cells of the central nervous system considered the brain's guardian system, monitoring the parenchymal environment and acting as the first line of defense. Their heterogeneity has been described from several perspectives, focusing on the microglia ratio/density in the tissue, regional differences, cell morphology/state (homeostatic vs responding), and sex-dependent; emphasizing the importance of microglial heterogeneity. Despite the supportive data indicating the presence of putative microglial states, much remains unknown about the proteomic profile diversity and its spatial distribution in the human aging brain. Exploring the aging brain, at the protein level using antibody-based method, entails accurate antibody validation in tissues from different conditions to encompass the different microglial states. In this study, we aim to characterize microglia heterogeneity using a highly multiplexed spatial proteomics approach for deeper phenotyping at single-cell resolution, across normal, neuroinflammatory (HHV6-leukoencephalitis, LE) and neurodegenerative (Alzheimer's disease, AD) hippocampus tissues. This application provides comprehensive profiling by detecting multiple proteins simultaneously, allowing for a holistic characterization of the protein landscape within formalin-fixed paraffin embedded samples. We have deployed PhenoCycler Fusion (PCF) technology to enable detection of a large number of proteins using DNA-barcoded antibodies: we have established a meticulous pipeline to generate an antibody panel targeting microglia (TMEM119), macrophage/microglia (CD11B, CD68, IBA1, CD163), markers from peripheral immune cells (CD4, CD14, CD45, HLA-DR), neurons (MAP2, NECAB1, NEUN), astrocytes (GFAP), oligodendrocytes (MBP), endothelial cells (CLAUDIN5, CD31) and markers to be used as control in the aging brain (e.g. amyloid-β, α-synuclein-P, tau-P). The PCF output

results comprise high-dimensional images which are further cell segmented and downstream analyzed for cell clustering/annotation. We have found defined cell clustering separation between the different main cell types and, as expected, peripheral immune cells have been primarily observed in perivascular regions. Regarding microglia, CD163-positive cells have shown a distinctive ameboid morphology in the LE hippocampus compared with the ramified shape in the AD hippocampus; TMEM119 cells have been observed positive in associated plaque. These results provide a robust antibody panel to phenotype the human brain aging, essential for a better understanding of alterations during disease.

Disclosures: A. Martinez Casals: None. **J. Nirschl:** None. **A. Isakova:** None. **C. Smurthwaite:** None. **E. Lundberg:** None.

Poster

PSTR385

Neuro-Oncology: Mechanisms, Models, and Methods

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.01/B67

Topic: B.11. Neuro-Oncology

Support: NCI T32

Title: Elucidating the Role of Microglia in Remodeling Neuron-Glioma Circuitry

Authors: *R. MANCUSI[1], M. MONJE[2];
[1]Stanford Univ., Stanford, CA; [2]Neurol., Stanford Univ., Stanford, CA

Abstract: Pediatric high-grade gliomas (pHGG) represent the leading cause of cancer-related deaths in children. Neuronal activity drives pHGG growth and progression through activity-regulated paracrine growth factors and synaptic integration of gliomas into neural circuits. In turn, pHGGs increase the excitability of neurons to promote neuron-glioma interactions. It remains unclear how glial cell types that play important roles in neural circuit assembly and refinement in the healthy nervous system may participate in this process to foster tumor-promoting, hyperexcitable neural networks in pHGG. We hypothesize that microglia - the resident immune cells of the central nervous system which play important roles in neural circuit refinement and remodeling in development and disease - may contribute to hyperexcitable neural circuitry that promotes pHGG growth. Our current work has investigated if and how microglial-mediated circuit refinement may be altered by pHGG and contribute to tumor-promoting neuronal hyperexcitability. To further elucidate microglia-mediated circuit refinement in the pHGG tumor microenvironment, we explored both excitatory and inhibitory synaptic engulfment by microglia in patient-derived xenograft models of diffuse intrinsic pontine glioma (DIPG). We utilized high-resolution confocal microscopy analysis to reveal tumor-specific, activity-regulated differences in excitatory and inhibitory synaptic engulfment by microglia which may affect neuron-glioma network excitability. Complementary single cell transcriptomics studies indicate

concordant tumor-specific, activity-regulated changes in microglia-neuron signaling which may drive differential excitatory and inhibitory synaptic engulfment by tumor-associated microglia. Further studies will explore strategies to target these tumor-promoting microglia-neuron interactions in pHGG.

Disclosures: R. Mancusi: None. **M. Monje:** None.

Poster

PSTR385

Neuro-Oncology: Mechanisms, Models, and Methods

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.02/B68

Topic: B.11. Neuro-Oncology

Title: Neurotransmitter-activated GPCR expression in glioblastoma multiforme: Assessment with validated recombinant monoclonal antibodies

Authors: C. CHEN, A. BALL, Y. HSIEH, Y. CHIU, C.-K. HUANG, S.-C. HUANG, S.-R. WU, C. LIU, L. CHIA-NAN, *C.-Y. LIN;
GeneTex, Irvine, CA

Abstract: Glioblastoma multiforme (GBM) is the most common and lethal form of brain cancer, with a high rate of recurrence and therapeutic resistance to chemo- and immunotherapies. Hence, identifying new drug targets for GBM is an urgent necessity. G-protein coupled receptors (GPCRs), comprising a superfamily of transmembrane receptors situated on the cell surface, are associated with diverse physiological functions and have been implicated in the pathophysiological mechanisms underlying GBM. Therefore, these GPCRs have generated significant promise as potential targets for novel GBM therapies. However, the limited availability of reliable antibodies directed against these GPCRs hinders both GBM research and the development of GPCR-directed agents. In this study, we examined the expression and cellular distribution of GPCRs in GBM, with an emphasis on neurotransmitter-activated and inflammation-related GPCRs, using highly specific GPCR antibodies from GeneTex. We validated these antibodies by knockdown/knockout, target overexpression, tissue control slides, and GPCR arrays, and then performed immunohistochemistry. In comparison to the normal brain and cerebellum, GBM specimens exhibited a significant upregulation of histamine receptor H1 (HRH1) expression, accompanied by diverse morphological changes in HRH1-positive cells. Somatostatin receptor 3 (SSTR3) expression was also markedly augmented in GBM cells. Furthermore, elevated levels of dopamine receptors D1 and D4 (DRD1 and DRD4) were detected in GBM cells with characteristics akin to granule cells, while dopamine receptor D2 (DRD2) manifested extensive expression in certain GBM samples. Intriguingly, the purinergic receptor P2RY2 exhibited widespread expression despite its relatively lower expression. In addition to neurotransmitter-activated GPCRs, the expression of chemokine receptors CXCR4

and CXCR7 was notably increased in GBM. Surprisingly, the adhesion G protein-coupled receptor E5 (ADGRE5, CD97) was observed to exhibit a restricted spatial distribution within GBM. Our results suggest that these validated GPCR recombinant monoclonal antibodies can contribute to defining expression-related descriptive criteria for GBM classification and perhaps reveal potential opportunities for targeted therapies.

Disclosures: C. Chen: A. Employment/Salary (full or part-time):; GeneTex. **A. Ball:** A. Employment/Salary (full or part-time):; GeneTex. **Y. Hsieh:** A. Employment/Salary (full or part-time):; GeneTex. **Y. Chiu:** A. Employment/Salary (full or part-time):; GeneTex. **C. Huang:** A. Employment/Salary (full or part-time):; GeneTex. **S. Huang:** A. Employment/Salary (full or part-time):; GeneTex. **S. Wu:** A. Employment/Salary (full or part-time):; GeneTex. **C. Liu:** A. Employment/Salary (full or part-time):; GeneTex. **L. Chia-nan:** A. Employment/Salary (full or part-time):; GeneTex. **C. Lin:** A. Employment/Salary (full or part-time):; GeneTex.

Poster

PSTR385

Neuro-Oncology: Mechanisms, Models, and Methods

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: B.11. Neuro-Oncology

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NSERC Grant DGECR-2022-00190
NSERC Grant RGPIN-2022-03734
CIHR Grant OGB-185739
Cole Foundation Transition Award

Title: Targeting NMDA receptors to fight glioblastoma recurrence, a strategy tested in organoids

Authors: *N. PARADIS-ISLER[1], Y. TANAKA[1,2];
[1]Maisonneuve-Rosemont Hosp. Res. Ctr., Montreal, QC, Canada; [2]Medecine, University of Montreal, Montreal, QC, Canada

Abstract: Glioblastoma (glioma grade IV) is the most frequent and deadliest primary cancer of the central nervous system (CNS). Glutamate, which is the predominant excitatory neurotransmitter in the CNS, also acts as a factor that promotes survival, proliferation and migration of glioblastoma cells. *N*-methyl-*D*-aspartate (NMDA) receptors, a type of ionotropic glutamate receptors present at the surface of many different cell types in the CNS, have also been found to be expressed by glioblastoma cells. NMDA receptors have gained attention as potential targets for glioma and glioblastoma adjuvant therapy. Whether inhibiting NMDA receptor activity can help prevent glioblastoma recurrence and which NMDA receptor antagonists are good candidates remain however unclear. We sought here to validate whether inhibiting NMDA receptor activity can mitigate glioblastoma cell proliferation and migration in 3D cell models

recapitulating features of tumor and neural tissue microenvironments. We assessed the effects of various NMDA receptor antagonists on the growth of spheroids, tumor models, formed with the glioblastoma cell lines LN-229, T98G or U-87 MG. We found that spheroids from all cell lines used exhibited some sensitivity to at least one of the NMDA receptor antagonists tested. To test the effects in conditions more closely mimicking neural tissue, we generated cortical organoids through directed differentiation of human embryonic stem cells and later grafted fluorescent protein expressing glioblastoma cells. We found that NMDA receptor blockade was associated with a general decrease in glioblastoma cell abundance in cortical organoids, to a degree depending on glioblastoma cell lines and antagonists tested. Our results overall validate that inhibition of NMDA receptor activity slows glioblastoma cell growth and invasion in tumoral and neural microenvironments. These findings further support the potential of NMDA receptor antagonists already approved for the treatment of psychiatric and neurological disorders, such as major depressive disorder and aAlzheimer disease, to be repurposed as adjuvant treatment for glioblastoma. The work presented here offers more generally a proof of principle for an approach to test preclinically various candidate drugs for glioma treatment.

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Poster

PSTR385

Neuro-Oncology: Mechanisms, Models, and Methods

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.04/B70

Topic: B.11. Neuro-Oncology

Support: CRC 1057382

Title: Amino acid neurotransmitter reprogramming of the tumour microenvironment and metabolism in glioblastoma

Authors: *H. L. DENIS[1], J. MÉRONÉ[2], V. FORT[3], G. KHELIFI[4], V. WATTERS[4], L. BERTHIAUME[2], E. AUDET-WALSH[2], S. HUSSEIN[4], M. RICHER[1];
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Abstract: Glioblastoma (GBM) is the most aggressive type of primary brain cancer, affecting children and adults alike. The prognosis is poor (less than 15 months) and current treatments are not effective in slowing disease progression. It is therefore critical to develop more effective therapies that improve survival and reduce side effects for these lethal brain cancers. The role of amino acids such as gamma-aminobutyric acid (GABA) and glutamate in neurotransmission is

well-established. These effects are mediated through various neuronal ligand-gated ion channels, G-protein-coupled receptors, synthetizing enzymes and transporters. We recently highlighted the existence of a novel, neurotransmitter-related glioma subgroup (NT-1) which is characterized by the overexpression of GABA, glutamate and calcium genes[1,2]. Indeed, we revealed that lower expression of GABA signaling genes was correlated with increased aggressivity in GBMs[1,2]. Overall, these findings support the existence of operational and potentially actionable neurotransmitter signaling pathways in adult gliomas. It is clear now that invading GBM cells reprogram, locally, various aspects of their non-neoplastic microenvironment. How this invasion impacts on amino acid neurotransmission and metabolism to alter the dynamics of GBM progression is unknown. We propose to address these important questions by combining transcriptomic, histologic and metabolomic analyses along with experimental studies in a human cerebral organoids (hCO) invasion model that better recapitulate brain cancer biology. Our project will provide new insight about: (1) neurotransmitter-related actionable targets which may ultimately improve survival and quality of life for GBM patients; (2) mechanisms by which both neurotransmitter-related genes and metabolic reprogramming take place following infiltration of the brain by GBM cells. **(1) Nguyen, et al.** (2022) « Deciphering of Adult Glioma Vulnerabilities through Expression Pattern Analysis of GABA, Glutamate and Calcium Neurotransmitter Genes », J Pers Med / **(2) Nguyen, et al.** (2020) « A machine learning analysis of a 'normal-like' IDH-WT diffuse glioma transcriptomic subgroup associated with prolonged survival reveals novel immune and neurotransmitter-related actionable targets », BMC Med.

Disclosures: H.L. Denis: None. **J. Méroné:** None. **V. Fort:** None. **G. Khelifi:** None. **V. Watters:** None. **L. Berthiaume:** None. **E. Audet-Walsh:** None. **S. Hussein:** None. **M. Richer:** None.

Poster

PSTR385

Neuro-Oncology: Mechanisms, Models, and Methods

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.05/B71

Topic: B.11. Neuro-Oncology

Support: Cancer Research Society

Title: Exploring neurotransmitter signaling pathways in glioblastoma

Authors: *M. RICHER[1], H. DENIS[2];
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Abstract: Background. Glioblastomas (GBM) constitute the majority of malignant primary brain tumors, and cannot be cured. The identification of cancer cell survival- and proliferation-associated mechanisms may help the identification of new therapeutic targets. Several lines of

evidence suggest that gamma-aminobutyric acid (GABA) and glutamate, along with other molecules pertaining in neurotransmitter signaling, are involved in gliomagenesis. Their communication networks within the tumor microenvironment (TME) remain nonetheless poorly defined. We hypothesize that the modulation of pathways related to GABA, glutamate, and calcium signaling may control tumoral aggressivity. Here, we aimed at (1) identifying vulnerable biomarker genes for GBM, and (2) assessing their validity in *in vitro* and clinical studies. **Methods**. scRNAseq data of nine resected new-diagnostic, and five recurrent IDH-wildtype GBM was used to characterize cell populations, and gene expression of the glioma TME using a novel algorithmic cell type identification approach. Vulnerabilities were identified through gene mutability analysis following GABA, glutamate, and calcium gene extraction. A GABA-treated GBM cell line (U87) was bulk RNA sequenced, then differential and enrichment analysis were performed. A cohort comprising 50 resected GBM samples was utilized to associate extracted new genes, as well as previous markers C5AR1, VGAT, GAD1, and GABAB, to histologic characteristics including necrosis, angiogenesis, and infiltration. Immunohistochemical alignment between our gene targets and clinical markers, such as MIB1 for proliferation, was performed. **Results**. scRNAseq data revealed strong cellular heterogeneity within the tumoral environment, with specific differences in cellular populations as the tumor evolved. Neurotransmission expression was highly dependent on cell and tumor type. However, a group of a few dozen genes exhibited notable regulatory patterns. Some transcriptional responses varied enough to represent possible vulnerabilities. RNAseq data *in vitro* suggested that GABA regulate the transcription of cancer-associated pathways, such as survival and metabolism. RNAseq and scRNAseq comparison showed a strong modulation of the identified genes in the TME, correlating with histopathological analyses showing that GABA is increased in MIB1-rich zones. **Conclusion**. This study introduces GABA-, glutamate-, and calcium-associated biomarkers as potential GBM therapeutic vulnerabilities.

Disclosures: M. Richer: None. **H. Denis:** None.

Poster

PSTR385

Neuro-Oncology: Mechanisms, Models, and Methods

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.06/B72

Topic: B.11. Neuro-Oncology

Title: The Spatio-Temporal Landscape of Synaptic Diversity Across Pediatric Brain Tumors

Authors: *C. LO CASCIO[1], C. OLIVEIRA DE BIAGI JUNIOR[2], E. LUNA MELENDEZ[3], G. ALENCASTRO VEIGA CRUZEIRO[2], M. FILBIN[2];
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Abstract: Pediatric brain tumors are the most common solid tumors in children and adolescents and represent the leading cause of cancer-related deaths in this age group. Numerous studies on the biology of pediatric brain tumors have provided extensive insight into the genetic and epigenetic aberrations driving tumorigenesis and treatment resistance. However, targeting of these mechanisms have not yet translated into successful therapeutic breakthroughs due to the failure to fully capture and characterize tumor cell interactions with their microenvironment. Recent paradigm-shifting studies have elucidated that normal neurons promote proliferation of cancer-stem like in a subset of childhood brain cancers via direct glutamatergic and GABAergic synaptic neurotransmission. These discoveries have since paved the way for the burgeoning field of cancer neuroscience – the study of how reciprocal interactions between the central nervous system and cancer cells influences oncogenesis and malignant growth. While functional synapses between neurons and brain cancer cells are known to stimulate tumor cell proliferation, the full breadth of synaptic heterogeneity present across all subtypes of pediatric brain tumors, and how this may vary across tumors arising in different anatomical brain regions and age groups, remains undefined. To this end, we analyzed publicly available single-cell transcriptomic datasets to characterize the diversity of neurotransmitters and cognate receptors expressed in pediatric brain tumors that harbor distinct neurodevelopmental origins: diffuse midline glioma H3K27-altered (n=50), ependymoma (n=60), and medulloblastoma (n=40). We leveraged our datasets to infer tumor-specific neuron-to-cancer cell communication networks, followed by comparative analyses of neurotransmitter-related genes expressed by predefined tumor cell states. We found that expression of genes and networks related to glutamatergic, GABAergic, cholinergic, and serotonergic neurotransmission varied widely across all brain tumor subgroups, and identify specialized cancer cell states within each tumor that are enriched for synaptic programs in region-specific manner. All together, we developed a comprehensive single-cell atlas that will serve as a unique resource to further investigate the diversity and spatio-temporal landscape of synaptic communication across different pediatric brain tumors. Unraveling the complex role of synaptic signaling in cancer cell growth has the potential to unveil a novel subset of therapeutic targets, with the ultimate outcome to improve outcomes for children affected by these lethal brain tumors.

Disclosures: C. Lo Cascio: None. **C. Oliveira de Biagi Junior:** None. **E. Luna Melendez:** None. **G. Alencastro Veiga Cruzeiro:** None. **M. Filbin:** None.

Poster

PSTR385

Neuro-Oncology: Mechanisms, Models, and Methods

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.07/B73

Topic: B.11. Neuro-Oncology

Title: Synaptic gene expression profile across pediatric and adult brain tumors reveals a differential tumoral integration with the neuronal networks

Authors: *L. MERINO GALAN[1], S. ARORA[2], A. RAJENDRAN[3,1], E. HOLLAND[4], S. S. PATTWELL[1,2,5];
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Abstract: Brain tumors intricately interact with their microenvironment, where the dynamic interplay between neurons and cancer cells plays a fundamental role in brain cancer pathophysiology. Neuronal activity emerges as a key driver influencing glioma growth and progression through synaptic communication and paracrine signaling. Beyond gliomas, various tumors within and outside the central nervous system exhibit altered neuron-tumor cell communication, impacting synaptic function and protein dynamics, neuroimmune crosstalk and providing a milieu favorable to tumor progression. This underscores the critical need to understand the synaptic foundations of brain tumors. This study visualizes and compares synaptic processes and synaptic receptor gene expression across various brain tumor types, contrasting findings with normal brain tissue. We constructed a comprehensive Brain-UMAP reference, incorporating data from 702 adult gliomas, 802 pediatric tumors, and 1409 samples of a healthy normal brain from TCGA, CGGA and GTex databases. Differential gene expression analysis and SynGO analysis were conducted across 3 adult glioma subtypes, 21 pediatric brain tumor types, and healthy brain samples. Furthermore, GSVA analysis employing SynGO pathways was performed on the batch-corrected normalized gene counts, and the resulting scores were visually represented on the landscape. Kaplan-Meier curves were generated based on survival data and synaptic gene expression status. Our findings reveal distinct synaptic pathway enrichments and survival associations for specific synaptic genes across different cancer subtypes. Notably, aggressive IDH-WT adult gliomas exhibit up-regulation in *Synaptic target regulation* and *Postsynaptic neurotransmitter diffusion trapping* GO terms, with poor prognosis associated with high expression of *GRIA1, GABRR2, CHRNA9, HTR3A and P2RX1* receptors. Conversely, astrocytomas and oligodendrogliomas show increased expression in *Regulation of retrograde trans-synaptic signaling by endocannabinoids* and *Anterograde axonal protein transport,* with poor prognosis associated with low expression of *GRIA1, GABRB3, CHRNA2* and *HTR2A* in astrocytoma. To summarize, this study provides a comprehensive overview of the synaptic components involved in diverse brain tumor types, offering new insights into the brain tumor pathophysiology, which warrants future research. Understanding these synaptic mechanisms will lay the foundation for developing targeted therapeutic strategies aimed at disrupting the tumor cell communication with the surrounding microenvironment, thereby advancing brain tumor treatment strategies.

Disclosures: L. Merino Galan: None. **S. Arora:** None. **A. Rajendran:** None. **E. Holland:** None. **S.S. Pattwell:** None.

Poster

PSTR385

Neuro-Oncology: Mechanisms, Models, and Methods

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.08/B74

Topic: B.11. Neuro-Oncology

Title: Fetal derived cells within the maternal brain: a lifelong contribution to a mother's neuronal environment

Authors: *M. R. HATHAWAY[1], J. M. HEMENWAY[1], H. L. JAGANA[1], S. S. PATTWELL[1,2,3];
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Abstract: During pregnancy, it has been documented that a mother and her fetus exchange cells that remain within her body for decades. The introduction of a small percentage of cells into the body from a genetically distinct host if referred to as microchimerism (Mc). Fetal cells transferred to the mother in this way have been found in peripheral blood as immune cells and integrated within organs as tissue-specific cells. It has been shown the Mc cells can be transferred to siblings from previous pregnancies, twins, and that pregnancies need not be carried to term for cell transfer to occur. In parous mice, fetal cells have been found in all the maternal organs, with heavier density of fetal cells found in the lungs and liver. These fetal Mc cells have also been shown to make their way to the brain, but the overall effect of these cells on disease is currently only theorized. The overall goal of our project is to explore the role of fetal Mc cells within the maternal brain by breeding female B6 (JAX® #000664) mice with eGFP (JAX® #003291) males to track the passage of fetal-derived GFP positive cells between dames and their offspring. After successful breeding, the same female will be bred with an RFP (JAX® #000664) positive male. This will allow us to not only track and compare how many fetal cells the dame is receiving during pregnancy, but also will allow us to examine the litter of RFP bred pups to determine how many, if any, GFP positive cells are transferred during gestation. Fetal Mc cells have been observed to travel to injured areas of the brain and display morphological similarities to local cell types. Once there they also express immunocytochemical markers seen in surrounding neural cell types. Our preliminary data shows a small percentage of fetal Mc cells within the brain tumor microenvironment of women with GBM who have previously had sons. To further explore how the fetal Mc cells interact with the brain tumor microenvironment, we will generate glioblastoma multiforme (GBM) in parous mice via intracranial implantation and brains will be analyzed via flow cytometry, immunohistochemistry, and spatial profiling to further elucidate the role of fetal Mc cells in the maternal brain, uncovering novel neurobiology of this fascinating biological phenomenon.

Disclosures: M.R. Hathaway: None. **J.M. Hemenway:** None. **H.L. Jagana:** None. **S.S. Pattwell:** None.

Poster

PSTR385

Neuro-Oncology: Mechanisms, Models, and Methods

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.09/B75

Topic: B.11. Neuro-Oncology

Support: NIH/NCI 5K22CA258953

Title: Elucidating the link between TrkB.T1-initiated Ca^{2+} mobilization and Rho GTPase activation in neurodevelopment and neuro-oncology

Authors: *H. L. JAGANA[1], M. SHABAR[1], L. MERINO GALAN[1], J. M. HEMENWAY[1], M. R. HATHAWAY[1], A. RAJENDRAN[2], S. ARORA[4], L. RUBIO[6], N. RECHE-LEY[7], S. S. PATTWELL[1,5,3];
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Abstract: The neurotrophin receptor TrkB and its ligand brain derived neurotrophic factor (BDNF) are critical for proper neural development. The TrkB.T1 splice variant has been shown to be involved in glial development and exhibits altered expression in many neurological diseases and several cancers. TrkB.T1 stimulation by BDNF initiates a signaling cascade that has been shown to increase intracellular Ca^{2+} in astrocytes. In addition, TrkB.T1 has been shown to associate with Rho-GDI1 leading to morphological changes in astrocytes. Both Ca^{2+} transients and Rho GTPases regulate neural developmental pathways resulting in cytoskeletal changes. Although links between TrkB.T1 and Ca^{2+} signaling and TrkB.T1 with Rho GTPase activity have been individually identified, a gap in how they interact to maintain TrkB.T1 specific signaling and the implications of such signaling throughout the lifespan remains. We hypothesize that TrkB.T1 specific Ca^{2+} mobilization and Rho activation are linked, and together are indicative of neurotrophic contributions to development and dysregulation in neurological disorders and cancers. Affinity purification mass spectrometry was utilized to identify both direct and indirect interacting proteins of TrkB.T1, full length-TrkB (TrkB.FL), and a GFP control. Gene Ontology (GO) analysis of these TrkB.T1 binding proteins revealed statistically significant terms related to GTPase activity, GTP/GDP Binding, Ca^{2+} mobilization, and Ca^{2+} ion binding. The Ca^{2+} inhibitor, 2-APB, and the Rho/ROCK pathway inhibitor, Y-27632, were used to measure each pathway's influence on the other. A G-LISA Rho activation assay further was utilized to measure changes in active Rho after Ca^{2+} inhibition. Changes in intracellular Ca^{2+}, fluorescently marked by Fluo-4AM, were observed post ROCK inhibition. Lastly, we explored if Rho inhibition and Ca^{2+} inhibition elicit changes on downstream processes in a linked manner. Although TrkB.T1's dominant negative role and initial studies investigating its binding partners and signaling capabilities have been conducted, follow up studies on direct interactors of TrkB.T1 and the downstream consequences of these signaling cascades are desired. Teasing out TrkB.T1's specific signaling pathways, apart from its TrkB.FL counterpart, can offer great insight into its role throughout development and its dysregulation in disease.

Disclosures: H.L. Jagana: None. **M. Shabar:** None. **L. Merino Galan:** None. **J.M. Hemenway:** None. **M.R. Hathaway:** None. **A. Rajendran:** None. **S. Arora:** None. **L. Rubio:** None. **N. Reche-Ley:** None. **S.S. Pattwell:** None.

Poster

PSTR385

Neuro-Oncology: Mechanisms, Models, and Methods

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.10/B76

Topic: A.03. Stem Cells and Reprogramming

Title: Evaluating the therapeutic potential of novel ntrk agonists in high-risk neuroblastoma

Authors: *D. JOHNSON[1], T. SCHLICHTHAERLE[4], N. EDMAN[4], S. ARORA[5], N. KATIYAR[2], D. BAKER[4], S. S. PATTWELL[3];
[2]BTCCCR, [3]Human Biol., [1]Seattle Children's Res. Inst., Seattle, WA; [4]Univ. of Washington, Seattle, WA; [5]Fred Hutch, Seattle, WA

Abstract: Neuroblastoma is the most common extra-cranial tumor diagnosed in children within the first two years of life. Although low-risk and intermediate -risk neuroblastoma patients have a survival rate of 90-95%, high-risk neuroblastoma patients have a survival rate of approximately 50%. To improve the survival rate in high-risk patients, our group focuses on tropomyosin receptor kinases (TRKs); a family of tyrosine kinases that have been heavily implicated in crucial developmental processes, such as differentiation, that are typically hijacked in cancer. Using de novo agonists specifically designed against TrkA, our group is investigating the fundamental biological role of TrkA receptors in mediating tumorigenesis in neuroblastoma. Preliminary results show that our TrkA agonists cause a significant decrease in TrkA expression in several neuroblastoma cell lines which we hypothesize is due to internalization and degradation of TrkA upon activation as we also observe increases in pERK after agonist treatment. Phenotypically, we observe that neuroblastoma cell line SK-N-SY5Y(SY5Y), lose their neuroblast-like phenotype and begin to exhibit significant neurite outgrowth when treated with TrkA agonists, suggestive of enhanced differentiation. Moreover, these agonists induced more neurite outgrowth compared to our positive control, retinoic acid (RA), which has been previously shown to cause neurite elongation in SY5Y and is currently used as a retinoid therapy to differentiate neuroblastoma cells. With these results, we hypothesize that modulating TrkA activity via highly specific agonists may provide new insight into therapies for neuroblastoma and potentially, other cancers. Moreover, our research provides, for the first time: (1) a TrkA agonist specifically designed to bind and activate TrkA receptors, (2) show that activating TrkA with our agonists cause neurite elongation in SY5Y cells with similar efficiency as RA, thereby (3) providing novel insight into potentially uncovering a new TrkA-induced differentiation mechanism in neuroblastoma, and ultimately, (4) highlighting a new avenue for treating neuroblastoma.

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Poster

PSTR385

Neuro-Oncology: Mechanisms, Models, and Methods

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.11/B77

Topic: B.09. Glial Mechanisms

Support: NIH Grant R01ES033892
Support from the Center for Biomedical Discovery and the Mayo Clinic Cancer Center

Title: Hv1 proton channels promote myeloid infiltration and glioma progression

Authors: *J. ZHENG[1,2], L. WANG[3], S. ZHAO[3], K. AYASOUFI[4], A. J. JOHNSON[4,2], L.-J. WU[5,6,7];
[1]Ctr. for Neuroimmunology and Glia Biol., UT Hlth. Houston, Houston, TX; [2]Neurology, Mayo Clinic, Rochester, MN; [3]Grad. Sch. of Biomed. Sci., Mayo Clin., Rochester, MN; [4]Immunol., Mayo Clin., Rochester, MN; [5]Neurol., Mayo Clin., Rochester, MN; [6]Immunology, Mayo Clinic, Rochester, MN; [7]Center for Neuroimmunology and Glia Biology, UT Health Houston, Houston, TX

Abstract: Myeloid cells, constituting up to 50% of the total tumor mass in glioblastoma (GBM), contribute to tumor progression and immunosuppression. The re-education of myeloid cells including microglia and macrophages is an emerging approach of treating GBM. In this context, our focus centers on the voltage-gated proton channel Hv1, an ion channel predominantly expressed in myeloid cells, shaping their physiological functions. Our bioinformatics analysis revealed a significant correlation between elevated Hv1 expression within the tumor mass and an unfavorable disease prognosis among patients. Utilizing a leading immunocompetent GBM mouse model, we demonstrated that Hv1 knockout mice (*Hv1*$^{-/-}$) exhibit a decelerated glioma progression and prolonged survival. By utilizing in vivo two-photon imaging, we observed the early infiltration of myeloid cells into the tumor mass, engaging in intimate interactions with tumor cells. Furthermore, RNA sequencing of sorted myeloid cells revealed that *Hv1*$^{-/-}$ myeloid cells exhibit increased MHCII expression and enhanced anti-tumor profiles. Full-spectrum flow cytometry analysis confirmed reduced monocyte/macrophage infiltration and heightened immune responses against the tumor in *Hv1*$^{-/-}$ mice. Together, our results demonstrated that Hv1 controls myeloid infiltration, highlighting its potential as a novel therapeutic target for GBM treatment.

Disclosures: J. Zheng: None. **L. Wang:** None. **S. Zhao:** None. **K. Ayasoufi:** None. **A.J. Johnson:** None. **L. Wu:** None.

Poster

PSTR385

Neuro-Oncology: Mechanisms, Models, and Methods

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.12/B78

Topic: B.11. Neuro-Oncology

Support: NIH Grant R01 CA269365

Title: Screening for dominant pathways underlying mitochondria transfer to GBM cells from neutrophils associated with tumor progression

Authors: *N. FILIPPOVA, X. YANG, L. B. NABORS;
Neurol., UAB, Birmingham, AL

Abstract: $TREM1^+$ - myeloid-derived microenvironment is mostly represented by immunosuppressive neutrophils (PMNs) in immunocompetent mouse GBM models; the analysis of spatial TREM1 distribution pointed to peri-necrotic proinflammatory loci in both human and mouse GBM architectures. TREM1 overexpression significantly shortened patient survival based on data presented in R2: genomic platform. Using differentiated human neutrophils, we discovered mitochondria transfer between neutrophil-like differentiated HL-60 cells and human GBM Xenolines. The mechanisms of mitochondria transfer can be coordinated by direct cell-to-cell interaction leading to complete cell fusion or temporal TNT formations, or through the mitochondria extracellular release and endocytosis to the acceptor cells. Our preliminary data suggests that interaction between PMNs and cancer cells can be specifically initiated by TREM1/surface-actin interaction which is favorable during cancer cell metaphase to anaphase transition and proinflammatory stress. To delineate the dominant mechanisms of the mitochondria transfer between neutrophils and cancer cells, we employed genome-wide screening using human GBM Xenolines transduced with lentiviral CRISPR genome-wide knockdown library to search for genes essential for mitochondria transfer in control and at the mimic proinflammatory conditions. GBM genome-wide knockdown cells expressing EGFP were treated with neutrophils containing pre-labeled mitochondria (red fluorescence prob) at different GBM /neutrophils ratios. GBM cells with acquired mitochondria ($EGFP^+RFP^+$ cells) were detected by flow cytometry technique and separated from GBM cells unable to acquire mitochondria ($EGFP^+RFP^-$). Genomic DNAs were isolated from both pools of cells, DNA amplicons corresponding to gRNAs have been generated and sequenced, and the data will be presented. On the cellular level, cancer cells with acquired from neutrophil mitochondria proliferated significantly faster compared to cells incapable of acquiring mitochondria. Similar experiments were also performed with the SRI42127-resistant GBM cells (isolated after prolonged treatment with an inhibitor of HuR protein dimerization SRI42127); these cells exhibited an increase in stemness and were mostly represented by cells with FAM98B gene knockdown leading to about 700 folds of growth advantage compared to the background cells. In

conclusion, our data suggests that neutrophils may be a significant source of mitochondria transfer to disease-affected microenvironment, particularly to GBM cells, and may play a significant role in glioma progression.

Disclosures: N. Filippova: None. **X. Yang:** None. **L.B. Nabors:** None.

Poster

PSTR385

Neuro-Oncology: Mechanisms, Models, and Methods

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.13/B79

Topic: B.11. Neuro-Oncology

Support: Supported by Ministry of Health of the Czech Republic, grant nr. NU23-08-00307

Title: Galectin-positive glioblastoma exosomes: new biomarkers and targets for glyconanotherapeutics

Authors: *P. KRUPA[1], O. BATKIVSKA[2], O. JANOUSKOVA[3];
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Abstract: GBM accounts for half of all primary malignant brain tumors associated with high morbidity and mortality. Early diagnosis and treatment of GBM is exceptionally difficult and despite current multimodality treatment the survival remain only 1-2 years. The identification of new rational therapeutic targets and reliable biomarkers are thus a vital issue. The tumor microenvironment fundamentally affects the course of the disease. Extracellular vesicles/ exosomes play an important role in cell-to-cell communication in tumor tissue, modulating tumorigenesis and anti-tumor immune response. The composition of exosomes can reflects pathological changes. Galectins, proteins binding B-galactosides, are strongly expressed in the tumor cells and suggest prognosis and clinical outcome in patients with GBM. These lectins play an important role in the processes of cell proliferation, migration, vascularization or oncogenic signaling and anti-tumor immune response. Their spread by exosomes and the associated modulation of tumor progression, influence on the anti-tumor immune response and the ability of exosomes to transfer them through the blood-brain barrier are unexplored topics, as well as the regulation of their production by newly designed glyco-nanotherapeutics. We present first results from prospectively collected data of patients who underwent surgery due to glioblastoma with subsequent adjuvant radiochemotherapy. We optimized the method of exosomal isolation using the ultracentifugation from 20 ml of blood. Subsequently we characterized the exosomes using the nanoparticles track analysis (NTA) and particle size distribution (DLS) with respect of

exosomal count and size. We detected typical exosomal markers CD63, CD9 and CD81 and galectines 1,3,8 or 9 using western blotting. Our results indicate no significant differences in obtained number of exosomes between 1 hour, 2 hours and 3 hours of ultracentrifugation. We did not observed significant difference in expression of exosomal markers but expression of galectin, gal 8, and gal 9 were significantly reduced in the exosomes isolated from blood of glioblastoma patients obtained before treatment and after adjuvant therapy when compared to healthy controls. The expression of gal 3 and gal 1 showed reduced trend in glioblastoma patients but not achieve significancy in evaluate group. These unique observations can open new possibilities to monitore development of diseases and together with future experiments aimed to the exosome impact to immune cells and migration can help to understand the role of communication vesicles and microenvironment in glioblastoma progression.

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Poster

PSTR385

Neuro-Oncology: Mechanisms, Models, and Methods

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Program #/Poster #: PSTR385.14/B80

Topic: B.11. Neuro-Oncology

Support: NIH R01 NS123562
Sontag Foundation

Title: Investigation of cellular plasticity of surgically resected glioblastoma tissues after engraftment into region-specific brain organoids

Authors: *T. BHATIA[1], S. GANTA[1], A. SING[1], A. KING[1], C. SOJKA[1], K. HOANG[2], R. READ[3], J. OLSON[2], S. A. SLOAN[1];
[1]Emory Univ., Atlanta, GA; [2]Neurosurg., Emory Univ., Atlanta, GA; [3]Pharmacol. and Chem. Biol., Emory Univ., Atlanta, GA

Abstract: Glioblastoma multiforme (GBM) is an aggressive and universally fatal primary brain tumor. Recent transcriptomic analyses suggest that neoplastic cells in these tumors adopt neural progenitor cell-like (NPC-like), oligodendrocyte progenitor cell-like (OPC-like), astrocyte-like (AC-like), and mesenchymal-like (MES-like) fates. Neoplastic GBM cells can also readily transition between these distinct cell states, which may contribute to their therapeutic defiance. Thus, there is a profound need to identify and target the molecular regulators of GBM cell state transitory behaviors. Here, we focused on how GBM cell states are influenced by microenvironmental variables using a fully human model system. We acutely isolated patient-derived GBM cells from neurosurgical resections and engrafted these cells into region-specific human brain organoids (mimicking the dorsal forebrain, ventral forebrain, midbrain, and hindbrain). To determine changes in GBM cell state signatures upon engraftment into each

human CNS niche, we performed paired single-cell RNA sequencing on GBM cells pre-engraftment as well as 14-days post-engraftment. We found that GBM cells mainly adopt an NPC-like signature within the organoid microenvironment. This effect was surprisingly consistent across all four brain regions, suggestive of common extrinsic factors underlying these transcriptomic changes. Furthermore, GBM cells converged on this NPC-like state irrespective of the cell state of the parent tumor prior to engraftment, implying that the organoid microenvironment actively impacts the plasticity of GBMs. To further support our findings, we also report that this NPC-skewed effect is recapitulated when engrafting patient-derived glioma cell lines into all four region-specific brain organoids. Thus, the dominance of this shared GBM cell state within organoids suggests that there are shared extrinsic factors favoring an NPC identity across all four regions. We are now working to identify these extrinsic signals since GBMs may rely on them to fulfill their plastic potential, adapt to the microenvironment, and evade or resist therapeutics.

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Poster

PSTR385

Neuro-Oncology: Mechanisms, Models, and Methods

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.15/B81

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01CA268125

Title: Spontaneous activity induced by acute chemotherapy-induced neuropathy in rat

Authors: *T. COPE, S. N. HOUSLEY, P. NARDELLI;
Biol. Sci., Georgia Inst. of Technol., Atlanta, GA

Abstract: Acute neuropathic pain, encompassing dysesthesia and paresthesia often results early in chemotherapy utilizing standard of care anticancer agents including taxanes and platinum-based compounds, e.g. oxaliplatin (OX). Evidence points to spontaneous or unprovoked spiking of primary somatosensory neurons as an underlying pathophysiology. With the goal of gaining more detailed characterization and mechanistic understanding of SA, we designed preclinical studies of adult Wistar rats treated with OX (30mg/kg ip) one day before recording spiking activity from low threshold mechanoreceptors (LTMRs) responsible for encoding propriosensory and tactile information. Our electrophysiological study *in vivo* maintained neurons intact and undamaged by commonly used recording techniques that alter neuronal excitability. SA measured as erratic firing in the absence of overt stimulation was rarely observed in wild type rats, but present in 45% of muscle LTMRs. Only 20% of glabrous skin LTMRs exhibited SA, largely because that sample was dominated by rapidly adapting receptors that exhibited SA half

as often as slowly adapting receptors. SA firing spanned rates comparable to those encoding naturalistic mechanical stimuli, and were, therefore, sufficient to create sensory illusions and disrupt normal propriosensory and tactile behaviors. By applying spike triggered averaging, we demonstrated placed SA's neuronal epicenter neither in DRG nor along axons traversing the peripheral nerve, but instead near to or at normal zone(s) of mechanosensory evoked action potential initiation. These non-ectopic sites seem most relevant in examining molecular events and targeting treatment for SA.

Disclosures: T. Cope: None. **S.N. Housley:** None. **P. Nardelli:** None.

Poster

PSTR386

Systemic Factors and Brain Function

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR386.01/B82

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant R01 MH119165

Title: Dna methylation in the inflammatory genes after gastrointestinal surgery and diagnostic ability of post-operative delirium

Authors: *T. YAMANASHI[1], Y. NISHIZAWA[2], N. KAJITANI[1], M. IWATA[1], G. SHINOZAKI[3];
[1]Tottori Univ., Tottori, Japan; [2]Osaka Med. and Pharmaceut. Univ., Osaka, Japan; [3]Stanford Univ., Palo Alto, CA

Abstract: The pathophysiological mechanisms of postoperative delirium (POD) are still unclear, and there is no reliable biomarker to differentiate those with POD from those without. Therefore, there is an urgent need to better elucidate the pathophysiology of delirium, especially at the molecular level. Previously, we collected pre- and post-operative blood from epilepsy patients undergoing neurosurgery and found that neurosurgery significantly altered many DNA methylation (DNAm) levels at CpG sites on the TNF, IL1B, and IL6 genes. Furthermore, it was found that the Inflammatory Methylation Index (IMI) based on postoperative DNAm levels at the selected five CpG sites may be a potential tool for detecting delirium with moderate accuracy; the area under the curve (AUC) value was 0.84 (Yamanashi T. et al., Transl Psychiatry. 2021). Although these findings provided further evidence for the potential role of epigenetics and inflammation in the pathophysiology of delirium, it would be difficult to automatically apply these results to POD because the subjects in this previous study were relatively young and had undergone direct surgical insults to their nervous system through brain resection. In the current study, we evaluated DNAm levels in peripheral blood mononuclear cells (PBMC) from elderly patients scheduled for surgery for gastric, colorectal, pancreatic, cholangiocarcinoma, and duodenal cancer. A psychiatrist assessed subjects the day before

surgery and from the day after surgery through the third postoperative day using the Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 and the Delirium Rating Scale-Revised (DRS-R)-98. Genome-wide DNAm analysis was performed using the Infinium HumanMethylationEPIC BeadChip Kit. DNAm levels of PBMC samples from 17 subjects, including 6 subjects who developed POD, were analyzed. Several DNAm levels at CpG sites of the TNF, IL1B, and IL6 genes changed the day after surgery, but approached preoperative levels by the third day of surgery. We also calculated the IMI from the DNAm levels of the five CpG sites that were selected in our previous study on the day after surgery. The IMI in the POD group was higher than that in the non-POD group (p=0.038). The AUC based on IMI from the current cohort was 0.81 (95% CI: 0.56-1.00). These data are consistent with our previous investigations in the neurosurgical cohort and provide strong evidence for the involvement of inflammation-related epigenetic signals in the pathogenesis of POD. This study was approved by the Clinical Research Review Committee of Tottori University Hospital (Reference No. 1704B007).

Disclosures: T. Yamanashi: None. **Y. Nishizawa:** None. **N. Kajitani:** None. **M. Iwata:** None. **G. Shinozaki:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); pending patents, No. PCT/US19/51276, pending patents, No. PCT/US21/63166, U.S. Provisional Patent, No. 62/731599.

Poster

PSTR386

Systemic Factors and Brain Function

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR386.02/B83

Topic: C.01. Brain Wellness and Aging

Support: Kunin Chair in Women's Healthy Brain Aging

Title: Negative association of cognitive performance with blood serum neurotoxicity and its modulation by human herpes virus 5 (HHV5) seropositivity in healthy women

Authors: *L. JAMES[1], P.-E. C. TSILIBARY[2], E. WANBERG[3], A. P. GEORGOPOULOS[4]; [1]Univ. of Minnesota/Minneapolis VAHCS, Minneapolis, MN; [2]Neurosci., Univ. of Minnesota, Minneapolis, MN; [3]Mayo Col. of Med., Rochester, MN; [4]Univ. of Minnesota, Minneapolis, MN

Abstract: Identification of early influences on cognitive decline is of paramount importance in order to stem the impacts of decrements in cognitive functioning and to potentially intervene. Thus, here we focused on 132 healthy adult women (age range 26-98 yr) to a) determine whether factors circulating in serum may exert neurotoxic effects *in vitro*, b) evaluate associations between serum neurotoxicity and cognitive performance, and c) assess the influence of neurotropic human herpes virus (HHV) seroprevalence and other factors on apoptosis and cognitive performance. The results documented that the addition of serum from healthy adult

women to neural cell cultures resulted in apoptosis, indicating the presence of circulating neurotoxic factors in the serum. Furthermore, apoptosis increased with age, and was associated with decreased cognitive performance. Stepwise regression evaluating the influence of 6 HHVs on apoptosis and cognitive function revealed that only HHV5 (cytomegalovirus; CMV) seropositivity was significantly associated with apoptosis and cognitive decline, controlling for age. These findings document neurotoxic effects of serum from healthy women across the adult lifespan and suggest a unique detrimental influence associated with CMV seropositivity.

Disclosures: L. James: None. **P.C. Tsilibary:** None. **E. Wanberg:** None. **A.P. Georgopoulos:** None.

Poster

PSTR386

Systemic Factors and Brain Function

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR386.03/Web Only

Topic: C.01. Brain Wellness and Aging

Support: U19AG078109

Title: Type 2 diabetes mellitus is associated with faster rates of neurodegeneration in an ethnoracially diverse cohort of cognitively unimpaired older adults.

Authors: *A. A. TSIKNIA[1], K. V. WHEELER[2], N. LEE[1], B. HALL[1], A. W. TOGA[3], S. O'BRYANT[5], K. YAFFE[6], M. PETERSEN[7], W. J. MACK[4], R. PALMER[8], L. AKSMAN[10], A. CLARK[11], R. NANDY[9], M. N. BRASKIE[1];
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Abstract: The association between type 2 diabetes mellitus (T2DM) and higher Alzheimer's disease (AD) risk may be driven by neurodegeneration. However, prior longitudinal studies linking T2DM to neuroimaging markers of neurodegeneration have mostly examined clinical samples of non-Hispanic white adults, making their findings difficult to generalize to the wider

population. We examined whether T2DM is associated with neurodegeneration in a community-based sample of 1095 cognitively unimpaired older adults (50-90 years; 727 female; 242 diabetic; 455 Hispanic; 67 non-Hispanic Black; 573 non-Hispanic white) from the Health and Aging Brain Study - Health Disparities cohort. To obtain T1-weighted MRI scans, participants were scanned on either a 3T Siemens MAGNETOM Skyra scanner or a 3T Siemens MAGNETOM Vida scanner at baseline. Participants had at least one follow-up scan over a median follow-up of 2.25 years. We used linear mixed effects models with participant- and MRI scanner-specific slopes and intercepts testing for an interaction between T2DM and years from baseline on cortical thickness in 34 mean bilateral Freesurfer-derived regions and left and right hippocampal volume, covarying for age at baseline and self-reported sex. Analyses of hippocampal volume additionally covaried for intracranial volume. In regions exhibiting faster neurodegeneration in diabetics compared to non-diabetics, analyses were repeated with additional covariates for income, education, abdominal circumference, dyslipidemia, hypertension, and *APOE*4 positivity, first in the full sample and then separately within each sex. T2DM was associated with faster thinning of the caudal middle frontal, cuneus, paracentral, pars opercularis, precuneus, superior frontal, superior parietal and supramarginal (standardized βs ranged from -0.08 to -0.05; FDR-corrected *P*s <=0.04), and the effect sizes were similar and remained significant in the fully adjusted model. The association between T2DM and faster cortical thinning was evident in female (standardized βs ranged from -0.12 to -0.07; FDR-corrected *P*s <=0.02), but not male participants (standardized βs ranged from -0.04 to -0.004; FDR-corrected *P*s >0.05), but there was no significant three-way interaction between T2DM, sex, and time or between T2DM, ethnoracial group, and time on cortical thickness in any of the regions. Our findings demonstrate that T2DM is associated with faster neurodegeneration of frontal, parietal and occipital regions, independently of socioeconomic factors, comorbidities, and genetic AD risk.

Disclosures: A.A. Tsiknia: None. **K.V. Wheeler:** None. **N. Lee:** None. **B. Hall:** None. **A.W. Toga:** None. **S. O'Bryant:** None. **K. Yaffe:** None. **M. Petersen:** None. **W.J. Mack:** None. **R. palmer:** None. **L. Aksman:** None. **A. Clark:** None. **R. Nandy:** None. **M.N. Braskie:** None.

Poster

PSTR386

Systemic Factors and Brain Function

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR386.04/B84

Topic: C.01. Brain Wellness and Aging

Support: Wellcome Trust
Medical Research Council
NIHR
Berrow Foundation

Title: The influence of APOE, Klotho and sex on cognition across ageing: a UK Biobank study

Authors: *K. SHIBATA, C. CHEN, X. TAI, S. G. MANOHAR, M. HUSAIN;
Univ. of Oxford, Oxford, United Kingdom

Abstract: Carrying the apolipoprotein E (APOE) ε4 allele is associated with an increased risk of developing Alzheimer's Disease. By contrast, the Klotho-VS heterozygous variant has been associated with longevity and improved human cognition. Genotyping is therefore of high clinical utility to characterise and predict dementia and cognitive decline. However, the impact of the APOE and Klotho genes on cognition and brain structure remains subject to debate, especially across different stages of ageing. Furthermore, antagonistic pleiotropy is associated with the APOE gene, where certain variants of this gene provide developmental benefits early in life but later cause detrimental effects. We used the UK Biobank, the largest cohort of healthy genotyped subjects reported on to date (N = 320,861 for cognition and N = 37,976 for structural MRI), to assess the sex and age-related effects of APOE and Klotho. The sample size afforded by the UK Biobank enabled the targeted investigation of effects mediated by carrying two copies of the APOE ε4 gene (N = 7,854). A composite measure of cognition was calculated using principal component analysis on 9 cognitive tasks. Healthy homozygous carriers of APOE ε4 showed overall lower cognitive scores compared to ε3 carriers, especially at older ages. This gene subgroup also had smaller hippocampi and amygdala. Critically, sex and age differentially modulated the decline in cognition, with young (40-50 years) female homozygous ε4 carriers showing a cognitive advantage over female ε3 carriers. This effect was not observed in male homozygous ε4 carriers compared to male ε3 carriers. A slower rate of decline in cognition was observed in APOE ε2 carriers compared to ε3 carriers across both sexes. By contrast, Klotho-VS heterozygosity, which was expected to mediate protective effects on cognition, did not affect cognition or brain volume, regardless of APOE genotype, sex, or age. Overall, we replicated the detrimental effects of APOE ε4 at later-age, and showed a sex-dependent antagonistic pleiotropy effect in young carriers of the APOE ε4 gene. Looking ahead, it is important to further characterise Klotho's effects on the ageing brain and to systematically investigate the role of genetic factors in cognitive functions within extensive cohort studies.

Disclosures: K. Shibata: None. **C. Chen:** None. **X. Tai:** None. **S.G. Manohar:** None. **M. Husain:** None.

Poster

PSTR386

Systemic Factors and Brain Function

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR386.05/B85

Topic: C.01. Brain Wellness and Aging

Title: Sex-specific signatures of dementia risk factors and the impact of reproductive aging in females

Authors: *A. MUKORA[1], M. COSTANTINO[2], O. PARENT[1], G. A. DEVENYI[3], M. CHAKRAVARTY[4];
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Abstract: Treating modifiable risk factors of dementia, obesity, hypertension, and metabolic disorders, may delay or prevent maladaptive neurodegeneration.[1] Research has demonstrated different impacts of the factors on males and females, prompting exploration of sex-specific profiles of dementia risk.[2] In females, the risk of cognitive decline is greatly increased after the menopause transition, which has been linked to alterations of brain structure, particularly in areas involved in higher cognitive processes.[3] In this study, we investigated sex-specific relationships, including menopause, between dementia risk factors and the brain in age-matched cohorts. Leveraging cross-sectional data from the UK Biobank, we selected a cohort of 28,636 (age 45-82, 50.4% female/49.6% male) participants and collated data related to modifiable risk factors. Regional cortical thickness measures were derived from T1-weighted structural MRI scans. Using Partial Least Squares (PLS) analysis in a sex-specific manner, we examined latent variables (LVs) representing linear combinations that maximize covariance between brain and behavior variables. PLS found 5 significant LVs ($p < 0.05$) in females and 4 LVs in males, with LV2 in both sexes showed similar cortical thickness fluctuations, but differing behavioral associations, notably with exercise and education. Stratifying females by menopausal status (premenopausal, postmenopausal, postmenopausal after surgical menopause) revealed differing brain-behavior relationships. Sub-analysis of females, age-matched by menopausal status, found one significant LV that explained 60.89% covariance and is associated with widespread cortical thickness increases, disturbed sleep, and elevated blood pressure. Repeated analysis with the addition of age-matched males found one significant LV that explained 59.84% covariance and is associated with widespread cortical thickness increases and an increased household size. These results underscore the nuanced interplay of sex and dementia risk offering insights for tailored prevention strategies.
References:1. Livingston et al., 2020, Lancet. 2. Sindi et al., 2021, Alzheimer’s & dementia. 3. Mosconi et al., 2021, Sci Rep.

Disclosures: A. Mukora: None. **M. Costantino:** None. **O. Parent:** None. **G.A. Devenyi:** None. **M. Chakravarty:** None.

Poster

PSTR386

Systemic Factors and Brain Function

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR386.07/B86

Topic: C.01. Brain Wellness and Aging

Support: NSTC Grant 111-2320-B-037 -011 -MY3

Title: Exercise might improve the Blood-Brain Barrier leakage and structural alteration via mitochondrial biogenesis enhancement in hypertensive mouse model

Authors: Y.-S. CHANG[1], C. LEE[2], Y.-Y. WU[3], ***Y.-H. SHIH**[4];
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Abstract: Exercise might improve the Blood-Brain Barrier leakage and structural alteration via mitochondrial biogenesis enhancement in hypertensive mouse model Ying-Shuang Chang [1,4], Chu-Wan Lee[2], Yi-Ting Wu[3], Yao-Hsiang Shih[4]
[1]Graduate Institute of Medicine, college of medicine, Kaohsiung medical university, Kaohsiung, Taiwan[2]Department of Nursing, National Tainan junior college of nursing, Tainan, Taiwan[3]Department of Nursing, Tzu Hui Institute of Technology, Taiwan[4]Department of anatomy, college of medicine, Kaohsiung medical university, Kaohsiung, Taiwan
Objective: Blood-brain barrier (BBB) impairment is associated with several neurodegenerative diseases, such as Alzheimer's disease and frontotemporal dementia. For decades, many studies have reported that exercise can potentially ameliorate neurodegeneration. Recently, we showed exercise could reduce BBB leakage in a modified two-kidney one-clip (2K1C) hypertensive animal model. Thus, we hypothesize that exercise can restore the BBB alteration in hypertensive animal models.**Methods:** We use the modified two-kidney, one-clip (2K1C) surgery to induce hypertension in C57BL/6 mice, then we make the mice execute the moderately intense treadmill exercise for five weeks after hypertension-induced three weeks later. After that, the mice brains were collected for further immunofluorescence staining and immunoblotting to determine the BBB structure and related protein alteration.**Results:** We found that 2K1C mice showed spatial memory impairment and hippocampus IgG leakage, and exercise can restore both alterations. Lectin staining reveals that exercise could restore the decreased endothelial cells. In addition, the double staining with S100B and Claudin-5 indicated that exercise could increase the perivascular astrocyte and tight junction protein in the 2K1C hypertensive animal model. Furthermore, 2K1C surgery increased the AQP-4 protein expression level, which exercise can also restore. The immunoblotting showed Nrf1 and COXIV levels recovered in the exercise-hypertensive animal group, which means the exercise-enhanced mitochondrial biogenesis might be the potential mechanism for BBB restoration.**Conclusion:** Exercise can alleviate the BBB leakage by restoring the BBB structure impairment, which might be via mitochondrial biogenesis enhancement.

Disclosures: Y. Chang: None. **C. Lee:** None. **Y. Wu:** None. **Y. Shih:** None.

Poster

PSTR386

Systemic Factors and Brain Function

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR386.08/B87

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant R01AG073223-01
Lisa Dean Moseley Foundation

Title: Improved function and blood brain barrier properties in naturally aged mice systemically transplanted with multipoint stem cells

Authors: *C. RUGEL[1,2], S. THOMPSON[1,2], J. CURTIN[1], E. YANG[3], M. LAVASANI[1]; [1]Shirley Ryan AbilityLab, Chicago, IL; [2]Physical Medicine & Rehabilitation, Northwestern University, Chicago, IL; [3]Neurobio., Northwestern Univ., Chicago, IL

Abstract: During natural aging, breakdown of the blood brain barrier (BBB), such as reduction in endothelial cell size and glia/vascular coverage, impairs the exchange of nutrients, signaling molecules, and metabolic waste that regulates a healthy neural environment. In motor areas of the brain, this homeostatic disruption can lead to loss of coordinated movements and increased risk of falls and injuries. Previously, we have found that intraperitoneal transplantation of our unique adult multipotent muscle-derived stem/progenitor cells (MDSPCs), isolated from young mice, doubles lifespan, delays age-associated motor dysfunction, and induces neovascularization in a mouse model of progeria (a disease of accelerated aging). In this study, we seek to further clinical translation by investigating the impact of young MDSPC systemic transplantation on 2-year-old naturally aged female mice (NA-IP, n=10). After transplantation, NA-IP mice showed improved motor function and neurovascular markers compared to their saline-injected counterparts (NA-C, n=10). NA-IP mice demonstrated greater voluntary physical activity and running speeds during open field testing and reduced muscle fatiguability during four-limb hang testing. Immunohistochemistry of the motor cortex was used to visualize elements of the BBB, including CD31+ endothelial cells, GFAP+ glia, and AQP4+ water channels in astrocyte endfeet. Image analysis revealed NA-IP mice had significantly more CD31+ vascular area and AQP4+ vascular overlap, suggesting improved metabolic waste clearance. In addition, increased vascular/glial overlap indicated improved barrier integrity of the brain parenchyma in NA-IP mice compared to NA-C. Furthermore, these changes were more pronounced in deeper layers of the motor cortex compared to more superficial layers. These findings, related to the BBB permeability, are of particular interest as we have previously determined that our systemically transplanted MDSPCs do not engraft in the brain. This strongly implies a therapeutic secretome-related mechanism, supported by analysis of the secretome from MDSPCs isolated from young mice, which secrete increased amounts of multiple pro-angiogenesis proteins compared to MDSPCs isolated from aged mice. Thus, in the final aim of this project, we will use mass spectrometry to analyze brain and serum proteomics between NA-IP and NA-C mice.

Disclosures: C. Rugel: None. **S. Thompson:** None. **J. Curtin:** None. **E. Yang:** None. **M. Lavasani:** None.

Poster

PSTR386

Systemic Factors and Brain Function

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR386.09/B88

Topic: C.01. Brain Wellness and Aging

Support: PA Department of Health SAP #4100083102 to ACS
U19 AG074866
R24 AG073190

Title: Diffusion-weighted mri connectivity assessment of healthy aging trajectories of the marmoset brain

Authors: *D. SZCZUPAK[1], R. BHIK-GHANIE[1], B. ZHANG[2], D. PAPOTI[3], V. P. CAMPOS[4], L. DUBBERLEY[1], T. K. HITCHENS[5], F.-C. YEH[1], D. J. SCHAEFFER[6], S. J. SUKOFF RIZZO[7], A. C. SILVA[8];
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Abstract: The common marmoset (Callithrix jacchus) is an important animal model in neuroscience and neurological diseases (e.g., Alzheimer's disease - AD), as they present primate-specific evolutionary features such as an expanded frontal cortex. Here, we aim to characterize healthy aging trajectories of the structural cortical connectivity (white matter fibers) for a population of marmosets. We imaged a cohort of 19 marmosets (13 males, 6 females) across the lifespan (8 to 89 months) using a dedicated 9.4T 30cm bore MRI scanner (Bruker BioSpin Corp, Billerica). The animals were anesthetized under isoflurane anesthesia. High-resolution (500 µm isotropic) diffusion-weighted structural MRI were acquired. The brain images were aligned and registered to the Marmoset Brain Mapping V2 template, the brain was segmented using the MBM white matter atlas, and voxel-based-morphometry was used to quantify regional white matter volume in the left and right hemispheres. Furthermore, we used DSI STUDIO to perform the whole-brain tractogram and calculate network-based-statistics (NBS) and correlate them with age. With our small (but increasing) sample size, we were able to note interesting trends in NBS. Assortativity, Smallworldliness and Hierarchy seems to decrease with age while network efficiency increases, showing the maturation processes of white matter across development. We aim to increase our sample size in order to identify the entire marmoset age trajectory and its impact to white matter connectivity. Our work is the first to thoroughly describe the normal aging of the marmoset brain, a valuable model for age-related neuropathologies (e.g., AD). This

research will set the normal parameters for marmoset aging and will be vital for generating transgenic marmoset models for AD, which is the goal of the MARMO-AD consortium.

Disclosures: D. Szczupak: None. **R. Bhik-Ghanie:** None. **B. Zhang:** None. **D. Papoti:** None. **V.P. Campos:** None. **L. Dubberley:** None. **T.K. Hitchens:** None. **F. Yeh:** None. **D.J. Schaeffer:** None. **S.J. Sukoff Rizzo:** None. **A.C. Silva:** None.

Poster

PSTR386

Systemic Factors and Brain Function

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR386.10/B89

Topic: C.01. Brain Wellness and Aging

Support: DOD Grant #PR21767

Title: High-definition Brain Network (HDBN) delineation of CDKL5 Deficiency Disorder (CDD) in Mice

Authors: *N. W. COULSON, D. WEST, D. CORTES, K. SCHWAB, T. BECKER-SZURSZEWSKI, S. HARTWICK, C. LO, Y. WU;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: High-definition Brain Network (HDBN) delineation of CDKL5 Deficiency Disorder (CDD) in Mice

Authors: Noah W. Coulson[1], Dalton West[1], Devin Raine Everaldo Cortes[1, 2, 3], Kristina E. Schwab[1, 3], Thomas Becker-Szurszewski[1, 3], Sean Hartwick[1, 3,], Cecilia Lo[1], Yijen L. Wu[1, 2, 3]
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Disclosures: Noah W Coulson: None. Dalton West: None. Devin Raine Everaldo Cortes: None. Kristina E. Schwab: None, Thomas Becker-Szurszewski: None. Sean Hartwick: None. Cecilia Lo: None. Yijen L. Wu: None.

CDKL5 Deficiency Disorder (CDD) is an X-linked neurodevelopmental disorder characterized by developmental delay, intellectual disabilities, and early-onset refractory epilepsy. One major gap in CDD research is that *Cdkl5* knockout (KO) mice do not display epilepsy, making it infeasible to use epilepsy for therapeutic screening. The cognitive functions are performed by the "information highway," the brain network. The objective of this study is to delineate brain network architecture underlying the cognitive impairment of CDD in *Cdkl5* knockout (KO) mice. We have developed a robust high-definition brain network (HDBN) analysis as the

surrogate endpoint, using high-definition fiber tracking (HDFT) with diffusion tensor imaging (DTI) MRI followed by network topological analysis with graph theory to delineate brain network in *Cdkl5* KO mice. The ex-vivo mouse brains were subjected to the magnetic resonance (MR) diffusion tensor imaging (DTI). A 72-region autosegmenting atlas was used to analyze the neural tractography and connectomes for the strength between the regions of interest (ROIs) and connectivity organization. We found altered brain network architecture in neuronal pathways relevant to CDD, including cortex for cognition, sensory, motor, and executive functions; hippocampus for contextual learning and memory; cerebellum for motor functions; amygdala for fear and anxiety; hypothalamus for homeostasis; and superior colliculus for auditory, visual, and somatosensory function. HDBN can sensitively map brain information highway architecture underlying neurological presentations in *Cdkl5* KO mice. *Cdkl5* KO mice showed altered brain network. HDBN can be sensitive surrogate endpoint for sensitive phenotyping to guide mechanistic investigation and therapeutic development.

Disclosures: N.W. Coulson: None. **D. West:** None. **D. Cortes:** None. **K. Schwab:** None. **T. Becker-Szurszewski:** None. **S. Hartwick:** None. **C. Lo:** None. **Y. Wu:** None.

Poster

PSTR386

Systemic Factors and Brain Function

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR386.11/B90

Topic: C.01. Brain Wellness and Aging

Support: The Phil & Penny Knight Initiative for Brain Resilience provided grant number KCG-116

Title: Endocannabinoid metabolism as a driver of brain aging

Authors: *C. PORTER[1,4,5,6], P. M. KLEIN[2,4,7,6], C. WANG[3,4,7,6], J. S. FARRELL[8,1,4,7,6], K. I. ANDREASSON[3,4,7,9,6], I. SOLTESZ[1,4,7,6];
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Abstract: Chronic inflammation is a known pathological mechanism that underlies cognitive decline in the aging brain. We recently identified that peripheral inflammatory signaling via the lipid messenger prostaglandin E_2 (PGE_2) increases with age, while treatment that reduces myeloid PGE2 EP2 receptor signaling can prevent brain inflammation and restore cognitive

function to a more youthful state. There is also an emerging understanding that the endocannabinoid signaling molecule, 2-arachidonyl glycerol (2-AG), is a major substrate for the generation of PGE_2 in the brain and other organs. We therefore hypothesized that elevated endocannabinoid signaling in aging promotes increased PGE_2 generation, and that reducing 2-AG to PGE_2 metabolism would help reduce brain inflammation and improve cognitive function. Using 2-photon imaging of hippocampal neuronal activity in awake and behaving mice running on treadmills with cued belts, we find that calcium activity coupling to endocannabinoid release remains similar between young adult (3-6 months) and aged (21-24 months) animals. Furthermore, administration of the monoacylglycerol lipase (MAGL) antagonist JZL 184 (16 mg/kg, i.p.), which inhibits 2-AG hydrolysis and eventual conversion to PGE_2, was associated with enhanced and prolonged 2-AG transients across all groups. With chronic administration of JZL across 6 weeks (16 mg/kg, 3 times per week), spatial memory performance in a Barnes maze task in treated old mice was trending towards levels seen in young mice. We are continuing to assess the impacts of chronic JZL treatment on tissue-specific PGE_2 expression, brain cytokine levels and expression of inflammatory markers on microglia. Overall, we believe that targeting upstream mechanisms that link brain endocannabinoid signaling to inflammatory mechanisms has great potential to improve cognitive function in the aging brain.

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Poster

PSTR386

Systemic Factors and Brain Function

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR386.12/B91

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant 1R21NS125509-01A1

Title: Amplifying cerebrospinal fluid drainage by neck muscle stimulation

Authors: *A. S. REGNIER-GOLANOV[1], E. V. GOLANOV[2], G. W. BRITZ[2];
[1]Houston Methodist Hosp., Houston, TX; [2]Neurosurg., The Methodist Hosp., Houston, TX

Abstract: Excessive accumulation of cerebrospinal flow (CSF) known as hydrocephalus affects about 1 million Americans of all ages, which is especially high in children (88 per 100,000) and older (175 per 100,000) populations. Hydrocephalus is frequently observed following central nervous system insults such as subarachnoid hemorrhage and traumatic brain injury. CSF has numerous important functional roles including maintenance of ionic and pH balance; waste removal; distribution of humoral factors; and providing a cushion for the brain. Thus, CSF homeostasis is critically important for normal brain function. Several exit routes for the CSF outflow have been identified: the arachnoid granulations, paravascular and paracranial nerves

pathways and meningeal lymphatics. In the current project, we aim to leverage the drainage system of the CSF flow to accelerate the CSF efflux. Studies showed that up to 50% of CSF drains through the brain lymphatics and then the cervical lymph nodes. We propose that contraction of the neck muscles, by compressing the underlying cervical valve-equipped lymph vessels and nodes, is capable of accelerating CSF drainage resulting in the clearance of CSF excess and waste metabolites. According to Monroe-Kellie doctrine, ICP is a proxy measurement for a change in the intracranial CSF volume. Here we show that, in naive mice, a 5Hz intraburst - 0.1Hz interburst train of bipolar pulses (1 Volt, 1 msec) induced a continuous intracranial pressure (ICP) decrease of -22.2%±6.0 (mean±SD; n=2 mice with 2 repeats/mice) followed by a rebound of +45.8%±3.0 from the maximum decrease during stimulation, but when delivered at 0.2Hz, ICP decreases to a maximum of -41.4%%±6.5 (n=3 mice with 4 repeats/mice) and showed a minimal rebound (+19.5%±21.3) that resolved 6-mins post-stimulation. Additional data showed that 6Hz intraburst - 0.2Hz interburst decreased ICP to -27.8%±14.4 (n=3 mice with 3 repeats/mice) and showed a rebound of +20.2%23.6. Recent work from (Yoon et al., 2024) showing the presence of valves in the cervical lymphatics of *Prox1-GFP* lymphatic reporter mice further support the present study. Taken together, the present results bring robust preliminary evidence of our proof-of-concept.

Disclosures: A.S. Regnier-Golanov: None. **E.V. Golanov:** None. **G.W. Britz:** None.

Poster

PSTR386

Systemic Factors and Brain Function

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Topic: C.01. Brain Wellness and Aging

Support: 5R25NS114326 NIH/NINDS (Hainline, Forlano, Stewart - MPIs; Serri, Jones - Scholars)
Erich K. Lang Fellowship in Urology Research, Department of Urology, SUNY Downstate Health Sciences University (Adetunji - Fellow)
2023 SUNY Downstate Health Sciences University SVPR Seed Grant (Orman, Weiss - MPIs)
1R03AG075644 NIH/NIA (Orman - PI)

Title: A Void-Spot Assay to Determine Urination Patterns in the Fruit Bat Carollia perspicillata

Authors: R. KOLLMAR[1], A. ADETUNJI[2], M. SERRI[3], N. JONES[3], J. WEISS[2], ***M. STEWART**[4], R. ORMAN[5];
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Pharmacol., Downstate Med. Ctr., Brooklyn, NY; [5]Physiol. & Pharmacol., SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY

Abstract: Nocturia—waking up at night due to an urge to urinate—becomes more frequent with age and degrades one's quality of life. Treatments to mitigate nocturia exist, but the underlying pathophysiology of aging is poorly understood. The fruit bat *Carollia perspicillata* is an attractive alternative to rodents as an animal model for aging studies due to its greater longevity (more than 10 years in captivity) and similarity to humans, including brain structure, menstruation, single main uterine cavity, and prominent male external urethra. Collecting and analyzing urine spots on paper, the so-called void-spot assay, is a simple, non-invasive method to determine urination patterns of laboratory animals. Here we report on adapting the void-spot assay to the airborne lifestyle of bats. First, we developed a protocol for urine collection. Holding individual animals in separate cages for two hours captured a sufficient number of urination events while minimizing behavioral disruption. We also tested the effect of different paper types on the shape and size of urine spots and on the fluorescence spectrum of dried urine. The second step, photography of the collection sheets, required a high-resolution digital camera to image the urine spots in sufficient detail. To match the fluorescence spectrum of dried urine, we tested different excitation light sources and emission filters. Finally, we improved image processing by preserving the dynamic range of the raw image files; performing flat-field correction for uneven illumination; and testing segmentation with thresholding, random-forest-based pixel classification, and pretrained deep-learning models. We are now measuring the inter-rater reproducibility of our optimized void-spot assay and determining baseline urination volumes and frequencies of *Carollia*.

Disclosures: R. Kollmar: None. **A. Adetunji:** None. **M. Serri:** None. **N. Jones:** None. **J. Weiss:** None. **M. Stewart:** None. **R. Orman:** None.

Poster

PSTR387

Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: *In Vitro* Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: /

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: UK DRI-Edin0010
Dunhill Medical Trust Fund PhD Scholarship

Title: Using a targeted protein degradation technique to study synaptic compensation mechanisms countering synapse loss in Alzheimer's disease

Authors: *Y. CHANG[1,2], P. OPAZO[1,2];
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Abstract: Synapse loss is a key feature of many neurodegenerative diseases involving cognitive deficits, and is also the strongest correlate of cognitive decline in Alzheimer's disease (AD). In addition, the integrity of dendritic spines, the post-synaptic sites of excitatory synapses, have been shown to play a key role in cognitive resilience against AD. Given the critical role of synaptic loss in cognitive decline, we hypothesize that synaptic compensation and repair play a vital role in counteracting synapse loss and as a consequence, in delaying the onset of cognitive deficits. The aim of our study is to artificially induce spine loss in order to study the emergence of compensatory mechanisms over time using longitudinal imaging. To this end, we use 2-photon microscopy to image dendritic spines in an *in-vitro* model system: rat organotypic hippocampal slice cultures. To induce spine loss, we promote the degradation of drebrin, a post-synaptic F-actin binding protein that is abundant in spines, using the degradation-tag (dTAG) system. By imaging the same dendrite over time, we visualise structural changes in dendritic spines in both the spine loss phase and the following compensation phase. In our preliminary data, we show the optimization of this dTAG system to induce spine loss, and its ability to promote the emergence of synaptic compensation over days (e.g. the enlargement of surviving spines and regeneration of new spines). Following this, we plan to look into the transcriptional landscape at relevant time points to elucidate the mechanisms of spine compensation.

Disclosures: Y. Chang: None. **P. Opazo:** None.

Poster

PSTR387

Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: *In Vitro* Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.01/B93

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG084421
NIH AG068992

Title: Development of iPSC-derived Astrocytes for Disease Modeling

Authors: *N. BUTELET, A. MURCHISON, P. ZHOU, M. NICHOLSON, D. V. LESSARD, W. W. POON;
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Abstract: Emerging studies highlight the transcriptomic diversity of regionally-specified human astrocytes and the contribution of reactive astrocytes to neurological disease. Thus, there is a growing need for human astrocytes to model human disease and to be utilized in drug discovery efforts. While human primary sources of astrocytes are difficult to obtain, induced pluripotent stem cell (iPSC)-based technologies have enabled the development of protocols to generate hiPSC astrocytes. Many of the described methods for iPSC-derived astrocytes employ the use of serum, which leads to reactive astrocytes, limiting their use to study the role of astrocytes

contributing to neuroinflammatory mechanisms of disease. Additional methods involve organoid-based differentiation that requires extended culturing that is not amenable to commercial scaling. Alternative transcription factor-based methods yield astrocytes quickly, but these astrocytes often do not recapitulate all the functional astrocyte properties. Providing a solution to the above-mentioned methods, here we describe a robust and rapid, serum-free protocol for the generation of cryopreserved iPSC-derived astrocytes from different disease backgrounds. These iPSC-derived astrocytes consistently display functional properties similar to primary astrocytes and can be combined together with iPSC-derived neurons and microglia from the same genetic background yielding isogenic co-cultures facilitating the study of human astrocytes and their contribution to neurological and neurodegenerative disease pathogenic mechanisms.

Disclosures: N. Butelet: A. Employment/Salary (full or part-time):; NeuCyte, Inc. **A. Murchison:** A. Employment/Salary (full or part-time):; NeuCyte, Inc. **P. Zhou:** A. Employment/Salary (full or part-time):; NeuCyte, Inc. **M. Nicholson:** A. Employment/Salary (full or part-time):; NeuCyte, Inc. **D.V. Lessard:** A. Employment/Salary (full or part-time):; NeuCyte, Inc. **W.W. Poon:** A. Employment/Salary (full or part-time):; NeuCyte, Inc., UC Irvine.

Poster

PSTR387

Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: *In Vitro* Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.02/B94

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Perturbation of lipid membrane composition and protein spreading between neurons

Authors: *F. PALESE[1], V. KLEIN[1], G. OTTONELLO[2], C. ZURZOLO[1];
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Abstract: A common feature of NDs is the accumulation of misfolded proteins such as Amyloid Beta (AB) and alpha-Synuclein (Syn) in different types of proteinaceous aggregates, the formation of which follows a prion-like spreading mechanism. In this regard, multiple studies have investigated the ability of neuronal and non-neuronal cells to transfer AB and Syn through Tunneling Nanotubes (TNTs). However, the causes and mechanisms of amyloid proteins misfolding and accumulation are still poorly understood. Given the high interaction of both AB and Syn with membranes, studying the lipid composition of these structures appears to be pivotal to understand physiological function and misfolding mechanism of amyloidogenic proteins. In this regard, a peculiar class of glycerophospholipids, called N-acylethanolamines (NAPEs), which are enriched in the neuronal membranes after injurious stimuli, have been shown to regulate the binding and stabilization of proteins that interact with the inner leaflet.In this study

we investigate the role of NAPEs in AB/Syn cell processing, aggregation and spreading. We firstly generated neuronal cell lines in which NAPE content is increased, noticing that these cells are more capable of forming TNTs, compared to normal cells. We next challenged NAPE-enriched and control cells with exogenous pre-formed fibrils of Syn and AB. Interestingly, we detected higher levels of AB in NAPE-enriched cells compared to control ones, pointing to either an increased internalization, or reduced clearance capability. On the contrary Synuclein levels were not affected by NAPE accrual, indicating that NAPEs can specifically influence AB biology. Moreover, using a coculture system we observed increased spreading of AB oligomers from NAPE-enriched donors to naïve acceptor cells, but no difference in Synuclein challenged cocultures.Altogether these results indicate that the enrichment of NAPE membranes content - which is generally detected under pathological conditions in the brain - is associated with (i) TNT built-up, (ii) AB intracellular accumulation and (iii) AB inter-neuronal spreading. Further investigations are needed to clarify NAPEs role in response to AB toxicity in comparison to Synuclein processing which is unaffected.

Disclosures: F. palese: None. **V. Klein:** None. **C. Zurzolo:** None.

Poster

PSTR387

Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: *In Vitro* Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.03/B95

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NS128039

Title: Sexually dimorphic mGluR5-GIRK signaling pathway in control and oligomeric Aβ-exposed hippocampal neurons

Authors: *H. LUO, H. KHATTAB, E. MARRON, K. D. WICKMAN;
Pharmacol., Univ. of Minnesota, Minneapolis, MN

Abstract: Excitation/inhibition imbalance has been linked to synaptic and cognitive dysfunction in Alzheimer's Disease (AD). Oligomeric amyloid β (oAβ), a predominant pathogenic factor in AD, induces hyperexcitability by enhancing excitatory signaling [e.g., the activity of metabotropic glutamate receptor 5 (mGluR5)]. However, impact of oAβ on inhibitory signaling is less well-understood. Using electrophysiology, we recently identified the suppression of a crucial inhibitory influence in mouse hippocampal (HPC) neurons - signaling via G protein-gated inwardly-rectifying K^+ (GIRK/Kir3) channels - following *in vitro* and *in vivo* oAβ incubation, and in APP/PS1 mice. We also established a pathophysiological contribution of GIRK suppression to synapse loss and cognitive deficits induced by oAβ, as well as a therapeutic benefit of enhancing GIRK activity in an acute amyloidapathy model and aged APP/PS1 mice. Interestingly, the oAβ-induced GIRK suppression was seen in male but not female HPC across

all models. For example, GIRK current suppression preceded synapse loss and was observed in HPC cultures from male but not female mice [oAβ x sex: $F_{1,59}$=5.287, P=0.0250; n=15-17/group]. We identified that the male-only neuroadaptation was mediated by an oAβ-activated mGluR5-PLA2-GIRK pathway. Strikingly, when we pretreated female cultures with a selective estrogen receptor α (ERα) antagonist (MPP), the otherwise absent oAβ-induced GIRK suppression now revealed itself [MPP x oAβ: $F_{1,34}$=25.11, P<0.0001; n=6-13/group]. MPP treatment did not alter the extent of GIRK suppression by oAβ in male cultures [MPP x oAβ: $F_{1,17}$=0.03580, P=0.8522; n=5-6/group]. Moreover, the oAβ-triggered GIRK suppression in female neurons in the presence of MPP required mGluR5 activation [MPP x MTEP (oAβ+): $F_{1,32}$=15.23, P=0.0005; n=6-13/group]. It has been suggested that the membrane-bound ERα interacts with Group I mGluR in the HPC. This may point to a fundamental sex difference within mGluR5-PLA2-GIRK pathway that is independent of oAβ and linked to less abundant ERα-mGluR5 interaction due to sex-divergent ERα expression and localization. Indeed, a selective mGluR5 agonist, CHPG, triggered GIRK suppression in male but not female HPC neurons [CHPG x sex: $F_{1,31}$=7.570, P=0.0098; n=7-10/group]. Pre-treatment of female cultures with MPP unblocked the GIRK suppression induced by CHPG [MPP x CHPG: $F_{1,32}$=4.689, P=0.0379; n=7-12/group]. Collectively, these suggest sex-divergent mGluR5 signaling pathways in the HPC, and a possible presence of oAβ-induced GIRK suppression in ERα-deficient females, potentially contributing to female AD pathophysiology and the sex differences in AD.

Disclosures: H. Luo: None. **H. Khattab:** None. **E. Marron:** None. **K.D. Wickman:** None.

Poster

PSTR387

Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: *In Vitro* Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.04/B96

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Florida Department of Health, Ed and Ethel Moore Alzheimer's Disease Research, Grant #21A12

Title: Age and Alzheimer's disease-related alterations in spontaneous synaptic transmission in male and female mice

Authors: *M. CUESTAS-TORRES[1], K. ALVIÑA[2];
[1]of Neurosci., Univ. of Florida, Gainesville, FL; [2]Neurosci., Univ. of Florida Dept. of Neurosci., Gainesville, FL

Abstract: Age and Alzheimer's disease-related alterations in spontaneous synaptic transmission in male and female miceMarcela Cuestas Torres and Karina Alviña. Department of Neuroscience, University of Florida, Gainesville FL, 32607Abstract Aging is a gradual pathophysiological process characterized by impaired hippocampal function and

cognitive decline. Aging is also one of the leading risk factors for the development of neurodegenerative disorders like Alzheimer's disease (AD). Understanding the cellular and synaptic impact of aging process will facilitate the identification of targets for age-related neurodegenerative disease and the formulation of potential treatments that promote recovery of hippocampal synaptic function and might rescue cognitive function. However, the cellular mechanisms that underlie hippocampal synaptic dysfunction characteristic of both aging and AD are not fully understood. Therefore, to evaluate age and AD related alterations in the spontaneous synaptic transmission in the hippocampus of wild type and AD mouse models, we used whole-cell patch clamp electrophysiology in brain slices and monitored spontaneous excitatory synaptic currents (sEPSCs). Analysis of amplitude and frequency of sEPSCs has been widely used to evaluate the presynaptic and postsynaptic alterations separately. Therefore, we recorded from hippocampal CA1 pyramidal cells and compared amplitude and frequency of sEPSCs in adult (3-6 months old), aged (18-24 months old) and AD (18-24 months old, CRND8 mouse line over expressing human APP) mice, both male and female. We also used picrotoxin to block GABAA receptor mediated inhibition. We found that age and AD genotype resulted in increased sEPSCs amplitude in a sex-dependent manner, specifically, sEPSCs amplitude increased in male mice and decreased in female, which might be related to postsynaptic events. In addition, AD genotype was associated with reduced sEPSCs frequency in female mice only, suggesting presynaptic alterations. Moreover, exposure to picrotoxin resulted in reduction of both sEPSCs amplitude and frequency in females, indicating that AD genotype increases susceptibility to GABAergic transmission. Our findings highlight the presynaptic and postsynaptic effects of aging and AD in both males and females and contribute to dissecting cellular mechanisms underlying dysfunctional synaptic transmission during aging and AD neuropathology.

Disclosures: M. Cuestas-Torres: None. **K. Alviña:** None.

Poster

PSTR387

Mechanisms of Synaptic Dysfunction in Alzheimer’s Disease: *In Vitro* Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.05/B97

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA R21AG084041
Alzheimer's Association AARGD-22-932597

Title: Identification of gene regulators of morphoelectric properties in entorhinal cortex layer ii neurons

Authors: *A. MORELLO[1], Z. STANDEN[2], J. SHIN[4], M. RAPHAEL[5], A. FAYZULLAEV[2], J. I. LUEBKE[3], J.-P. ROUSSARIE[3];

[1]Boston Univ. Grad. Program In Anat. & Neurobio., Boston, MA; [3]Anat. & Neurobio., [2]Boston Univ., Boston, MA; [4]Los Angeles, CA, ; [5]Holt, MI.

Abstract: Neurofibrillary tangles first appear in layer IIa neurons of the entorhinal cortex (EC) in preclinical stages of Alzheimer's Disease (AD) following widespread accumulation of amyloid beta plaques. Stellate and fan cells, the principal neurons of this layer in medial and lateral EC, respectively, form the perforant pathway conveying multi-sensory input into the hippocampus. Pathological lesion formation in EC (plaques and tangles) are associated with changes in neuronal excitability and overall epileptiform activity in early stages of AD. In turn, these changes in neuronal activity likely contribute to downstream pathological features of AD. It is currently not known which genes are the main intrinsic regulators of stellate and fan cell activity and how amyloid beta accumulation may affect these regulators. To determine which genes are central for tuning electrophysiological properties of stellate and fan cells, and the mechanism by which AD pathology dysregulates these properties, we have designed a multimodal pipeline to comprehensively profile single EC layer II neurons in wild-type mice and mice with amyloid beta accumulation (APP^{SAA} KI). Here, we present a novel methodological approach in which patch-sequencing is coupled with multiplex in-situ hybridization and neuron reconstruction to collect electrophysiological, morphological, and transcriptional characteristics for individual stellate and fan cells registered in their precise anatomical location. By sampling from a relatively homogeneous population of neurons in the presence or absence of amyloid, we are able to identify genes responsible for electrophysiological and morphological variance across cells. We first show that detection of markers of layer IIa subdivisions by multiplex in-situ hybridization is possible on thick slices that underwent patch-sequencing. Preliminary data further indicates higher firing frequency ($p = 0.015$ at 180 pA depolarizing step) and greater hyperpolarizing "sag" amplitude ($p = 0.005$) in APP^{SAA} KI compared to WT (APP n=5, WT n =17). Moreover, through nucleus harvesting of individual stellate and fan cells, we used nuclear RNA to synthesize cDNA libraries which we are currently sequencing. Our study will uncover new master regulators of functional properties for the neurons most vulnerable in AD, yielding new mechanistic insight in the elusive preclinical stages of the disease.

Disclosures: A. Morello: None. **Z. Standen:** None. **A. Fayzullaev:** None. **J.I. Luebke:** None. **J. Roussarie:** None.

Poster

PSTR387

Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: *In Vitro* Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.06/B98

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant

Title: A role of APP-CTF accumulation in synapse formation and neuronal network activity in Alzheimer's disease mouse models

Authors: *M. O. IKHANE[1], C. DEYTS[2], V. RIEKES VIANA[1], L. PEREIRA CALIXTO DE OLIVEIRA[1], R. WANG[1], A. PARENT[1];
[1]Univ. of South Florida, TAMPA, FL; [2]Vanqua Bio, Chicago, IL

Abstract: The amyloid precursor protein (APP) plays a significant role in Alzheimer's disease (AD), as its abnormal processing by secretases leads to the accumulation of β-amyloid peptides (Aβ) in the brains of AD patients. Presenilin (PS1), as part of the γ-secretase complex, is involved in the cleavage of APP. The familial AD-linked PS1 variants have been associated with the abnormal cleavage of APP, leading to the accumulation of Aβ and APP C-terminal fragments (APP-CTF). Previous work from our lab demonstrated that APP-CTF accumulation promotes axodendritic outgrowth. The remaining question is whether APP-CTF regulates synaptic function through synapse formation and maturation alterations, which might contribute to the development of familial AD. Accordingly, we prepared hippocampal brain slices and generated primary cortical cultures from E18 mouse embryos using the familial AD-linked PS1M146V knock-in (PSIKI), the PS1 knockout (PS1KO), and the wildtype (Wt) littermates. Synapse density and spine formation were examined. We observed an increase in dendritic protrusions in neuronal cultures from PS1KO (n=15, 93% increase, $p<0.01$), PS1KI (n=26, 87% increase, $p<0.01$), and Wt neurons overexpressing APP-CTF (n=20, 57 % increase, $p<0.01$) as compared to Wt littermates. We also observed an enhancement of synapse density and spine formation in the hippocampus of 6-month-old PS1KI mice, an effect not observed in 2-week and 2-month-old mice. To investigate the influence of APP expression on the functional development of neurons, we also examined the spontaneous network-wide bursting activity in neuronal cultures using multi-electrode array (MEA) technology. We recorded electrical events from the MEA probes in 4, 7, 11, 14, and 18 days old cortical culture generation. MEA recordings revealed progressive increases in neuronal spiking and burst activity in older cultures across genotypes, indicative of the intrinsic development of synaptic connections as neurons mature structurally, leading to established network activity that alludes to their functional development. In conclusion, our findings support the idea that APP-CTF accumulation influences synapse and spine formation, suggesting a role of APP-CTF in synaptic development and function.

Disclosures: M.O. Ikhane: None. **C. Deyts:** None. **V. Riekes Viana:** None. **L. Pereira Calixto De Oliveira:** None. **R. Wang:** None. **A. Parent:** None.

Poster

PSTR387

Mechanisms of Synaptic Dysfunction in Alzheimer’s Disease: *In Vitro* Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.07/B99

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DBT - JUNIOR RESEARCH FELLOWSHIP
Pratiksha Trust Extra-Mural Support for Transformational Aging Brain Research (EMSTAR/22/078)

Title: Modified hippocampal synaptic plasticity in Alzheimer's Disease: the role of calcium

Authors: *S. SINHA[1], N. SINGH[2], S. NADKARNI[3];
[1]Indian Inst. of Sci. Educ. and Res., Pune, India; [2]Biol., IISER Pune, Pune, India; [3]IISER Pune, Pune, India

Abstract: Synaptic dysfunction marks early Alzheimer's Disease (AD) and is linked to cognitive decline. Endoplasmic reticulum (ER)-mediated calcium signaling, crucial for synaptic plasticity, is also impaired early in AD. One major AD risk factor is mutations in presenilins, which form calcium leak channels on the ER membrane. Mutated presenilins compromise the leak, resulting in modified ER calcium. The expression of Ryanodine receptors (RyRs) present on the ER membrane is also reported to be modified in AD synapses. This can further affect synaptic strength. Yet, the precise interplay between RyR-mediated calcium dynamics and synaptic dysfunction in AD remains unclear. To investigate the interaction between altered calcium signaling anomalies in plasticity in AD, we built a spatially and biophysically detailed computational model of a hippocampal CA3-CA1 synapse, integrating AD-related changes—reduced presenilin leak and altered RyR expression. Reducing presenilins' calcium leak overloaded the ER. Furthermore, AD-related changes in our model reduced the STP, as reported in experiments. Blocking ER calcium in control synapses replicated AD-like STP impairment, highlighting the contribution of ER calcium signaling to STP. In experiments, both overexpression and underexpression of RyRs, along with varied ER calcium levels, were reported. We tested each of these hypotheses in our computational model and found that overexpressed RyRs and ER calcium overload quantitatively replicate reduced synaptic strength seen in experiments, while RyR underexpression increases it. High ER calcium and RyR overexpression lead to rapid ER calcium efflux, depleting vesicles and reducing facilitation. To replicate experimental protocols and validate our results, we simulated postsynaptic membrane currents in response to neurotransmitter release from multiple presynaptic inputs. Plasticity readout from our postsynaptic CA1 model aligns with the CA3-AD model, validating the altered STP mechanism from our studies. Presynaptic STP sets neurotransmitter release rates for induction of long-term potentiation (LTP). We show how altered STP impacts LTP profiles in AD, offering insights into AD-related memory impairments.

Disclosures: S. Sinha: None. **N. Singh:** None. **S. Nadkarni:** None.

Poster

PSTR387

Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: *In Vitro* Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.08/B100

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: France 2030

Title: Brain-on-chip technology and high-content analysis system to improve and accelerate preclinical Alzheimer's disease drug research

Authors: *J. COLIN[1], A. ALLOUCHE[1], S. KRIDI[1], J. RONTARD[2], B. MAISONNEUVE[2], T. HONEGGER[2], N. VIOLLE[1];
[1]ETAP-Lab, Vandoeuvre-lès-Nancy, France; [2]NETRI, Lyon, France

Abstract: Alzheimer's disease (AD) remains a major unmet medical need despite 3 decades of investment in drug research. Drug failed at clinical stages for various reasons including a limited understanding of the etiology of AD and its corollary: a poor translational value of preclinical models. Indeed, the complexity of the human brain creates significant challenges for advancing neurological drug development. While animal models seem not to reproduce the human physiopathology of AD, current in vitro models do not resume human brain architecture and environment. Brain-on-chips (BoC) have emerged as a promising alternative, potentially overcoming some of these limitations. However, BoC also have some drawbacks, including poor interoperability with standardized tools dedicated to outcome measurement in cell culture such as imaging or electrophysiology systems.
Here, we present our last advances toward innovative BoC models of AD combining high-throughput screening methods with automated microscopy to improve and accelerate the search for new effective therapies.
First, we optimized cultivation of rodent neurons and human induced pluripotent stem cells (hiPSCs) derived neurons within chips (DuaLink Shift, NETRI, France) composed of 3 chambers connected by microchannels, but virtually isolated thanks to a fine microfluidic control. By isolating soma and synapses via microchannels, we reproduced the projection of neurons between distanced brain areas. Second, soluble oligomeric species of amyloid beta and Tau (respectively AβO and TauO, ETAP-Lab, France) were added into the different chambers at various concentrations and developmental stages to reproduce cerebral microenvironment of AD patients. The use of a high-content analysis system enabled rapid acquisition of complex images to assess cell viability, cell morphology as well as synaptic and neurite densities.
Our data 1) show that both rodent and hiPSCs-derived neurons can be reproductively cultivated in NETRI's chip to create BoC, 2) confirm that AβO and TauO significantly affect neurite outgrowth, synaptic loss, and neuronal viability in cell cultures and 3) demonstrate that these oligomers differentially affect neurons after a somatic or axonal exposure, underlying that compartmentalized models are of great interest for AD preclinical research.
In conclusion, the present results represent a new step in the development of relevant, automatized, and reproducible models of AD, potentially allowing the elucidation of the new pathological mechanisms and the screening for effective treatments.

Disclosures: J. Colin: A. Employment/Salary (full or part-time):; ETAP-Lab. **A. Allouche:** A. Employment/Salary (full or part-time):; ETAP-Lab. **S. Kridi:** A. Employment/Salary (full or part-time):; ETAP-Lab. **J. Rontard:** A. Employment/Salary (full or part-time):; Netri. **B. Maisonneuve:** A. Employment/Salary (full or part-time):; Netri. **T. Honegger:** A. Employment/Salary (full or part-time):; Netri. **N. Violle:** A. Employment/Salary (full or part-time):; ETAP-Lab.

Poster

PSTR387

Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: *In Vitro* Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.09/B101

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA R01 AG077103

Title: Synaptic and histopathological changes in septohippocampal circuitry within the Ts2 mouse model of Down syndrome and Alzheimer's disease

Authors: *R. RAMACHANDRA[1], E. K. WEBBER[2], H. PIDIKITI[3], K. IBRAHIM[3], M. J. ALLDRED[4], S. D. GINSBERG[5], G. E. STUTZMANN[6];
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Abstract: Individuals with Down syndrome (DS) exhibit intellectual and developmental disabilities, and by early midlife will develop Alzheimer's disease (AD) pathology along with the associated cognitive decline and dementia. The septohippocampal network, a hub for learning and memory, includes basal forebrain cholinergic neurons (BFCNS) which project to the hippocampus and progressively degenerate in the Ts2 mouse model of DS. We hypothesize that dysfunction in the septohippocampal network, a hallmark of disease progression in both AD and DS, may be attenuated by maternal choline supplementation (MCS) during ageing. To test this, local field potential recordings were conducted in acute hippocampal slices of Ts2 mice and control disomic (2N) littermates at 4 or 12 months of age at the CA1 Schaffer collateral. We assessed a range of synaptic phenotypes such as basal synaptic excitability, short term plasticity, and long-term potentiation (LTP), the cellular correlate of learning and memory encoding in the hippocampus. In conjunction with field recordings, we used immunohistochemistry to analyze changes in the expression pattern of synaptic vesicles and synaptic integrity, ChAT-expressing cholinergic neurons, and vacuolar ATpase subunits (V-ATPAse). This was also supported by transcriptomic data delineating genetic changes in calcium signaling and endosomal pathways mediating cell signaling and synaptic transmission in hippocampal neurons. Preliminary hippocampal slice data from 12-month mice show synaptic strength and plasticity are diminished in Ts2 mice compared to their 2N counterparts and younger Ts2 mice. The MCS offered some protection against these deficiencies in aged mice. Initial data indicated reduced expression of V-ATPase1 and synaptophysin in aging Ts2 mice as compared to 2N littermates and younger Ts2 mice. Transcriptomic analysis revealed significant changes to both calcium signaling pathways as well as markers of synaptic LTP and LTD, and that MCS rebalances these changes in Ts2

animals. Results indicate synaptic connectivity is negatively affected as Ts2 mice age at the molecular and physiological levels in hippocampus and BFCNs, and MCS ameliorates these deficiencies. Interrogation of septohippocampal connectivity deficits in this mouse model will help define mechanisms of degeneration that aid our understanding of pathological aging in DS and inform AD therapeutic intervention.

Disclosures: R. Ramachandra: None. **E.K. Webber:** None. **H. Pidikiti:** None. **K. Ibrahim:** None. **M.J. Alldred:** None. **S.D. Ginsberg:** None. **G.E. Stutzmann:** None.

Poster

PSTR387

Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: *In Vitro* Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.11/B102

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R21AG073610
NIH Grant R01MH119105

Title: Real-time imaging of synaptotoxic PRR7 secretion via exosomes

Authors: *D. NIEVES TORRES, D. GRAU, S. H. LEE;
Pharmacol. and Toxicology, Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Decline in excitatory synapse numbers is a hallmark of the early stages and progression of Alzheimer's Disease (AD), with deposition of amyloid beta (Aβ) oligomers only enhancing this synaptic loss. Proline-rich 7 (PRR7) is a postsynaptic neuronal protein found to induce the removal of excitatory synapses on hippocampal neurons, capable of eliminating synapses in local, neighboring neurons via its release on exosomes (Lee et al., Nat Commun). Previously, we showed that the release of PRR7 on exosomes was an activity-dependent process. Interestingly, we have found that soluble Aβ oligomers significantly increase the protein levels of PRR7 being released on neuronal exosomes through biochemical analyses. To study the mechanisms responsible for the specific and dynamic release of PRR7-containing exosomes, we have developed a novel exosome reporter system utilizing a pH-sensitive GFP tagged PRR7 (SEP-PRR7) for real time imaging in live cultured rat hippocampal neurons. Changes in overall fluorescence intensity as well as changes in peak somatic and dendritic fluorescence intensity ($\%\Delta F/F_0$) are measured over time to determine changes in release events of PRR7-containing exosomes. Neurons displayed basal low fluorescence events under normal growth conditions. However, NMDA treatment greatly enhanced the SEP-PRR7 fluorescence intensities, which becomes blocked upon the addition of NMDA receptor blocker APV. Excitingly, preliminary studies showed that the administration of Aβ oligomers also greatly and rapidly increases both the somatic and dendritic fluorescence intensities of SEP-PRR7 over minutes time scale. Interestingly, upon administration of NR2B-antagonist Ro 25-6981 maleate, the Aβ oligomers-

induced fluorescence intensities were decreased in these neurons, suggesting the involvement of NR2B-containing NMDA receptors in this process. Studies are currently underway to further uncover the molecular mechanisms involved in the exosomal secretion of PRR7. Overall, our studies aim to reveal novel mechanisms that trigger the release of PRR7-containing exosomes as a means of spreading of neurodegeneration utilizing a novel and innovative imaging technique.

Disclosures: D. Nieves Torres: None. **D. Grau:** None. **S.H. Lee:** None.

Poster

PSTR387

Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: *In Vitro* Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.12/B103

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG15379
NIH AG44486
NIH AG15379

Title: Dysregulation of the glutamate transporter GLT-1/EAAT2 by PS1 familial Alzheimer's disease mutations

Authors: *F. PERRIN[1], B. LUNDIN[2], M. SADEK[2], P. SINHA[2], O. BEREZOVSKA[2]; [1]Neurol., Massachusetts Gen. Hosp. Dept. of Neurol., Boston, MA; [2]Neurol., Massachusetts Gen. Hosp., Boston, MA

Abstract: Several studies have implicated aberrant default-mode network activity and glutamate dysregulation in early Alzheimer's Disease (AD) pathology (Bookheimer et al., 2000; Quiroz et al., 2010; Hascup et al., 2015). Our investigation builds upon these findings by elucidating the direct relationship between PS1/γ-secretase and the central nervous system glutamate transporter, GLT-1/EAAT2 (Zoltowska et al., 2018). In our previous work, we demonstrated the pivotal role of PS1 in enhancing GLT-1 cell surface delivery and multimerization under normal conditions. However, in sporadic AD brains, PS1/GLT-1 interaction is diminished and leads to GLT-1 aggregation, due to PS1 acting as a rogue chaperone (Perrin et al., 2023 [preprint]). Notably, individuals with familial Alzheimer's disease (fAD) PS1 mutations, particularly during childhood, exhibit increase epileptiform activity (Joutsa et al., 2017). Clinical data reveal an 87-fold increase in seizure incidence among early-onset fAD PS1/2 mutation carriers compared to the general population (Amatniek et al., 2006). Our study aims to dissect the impact of PS1 fAD mutations on the GLT-1/PS1 interaction. We focused on specific mutants located closed to the interaction site that we identified in our previous work (Perrin et al., 2024). We employed Fluorescence Lifetime Imaging Microscopy (FLIM) assays to demonstrate reduced interaction between GLT-1 and some specific PS1 mutants (P264L, G266S, L271V, V272A, E280A) in intact cells. Next, we used flow cytometry and observed a decrease in GLT-1 cell surface

expression associated with these fAD PS1 mutations. Native Page analysis revealed alterations in GLT-1 multimerization profiles, specifically reduced GLT-1 trimers in PS1 fAD mutants compared to PS1 wild-type. Our study sheds light on the intricate interplay between PS1 mutations, glutamate transport dysfunction, and neurological manifestations in Alzheimer's disease pathogenesis. These findings underscore the functional consequences of altered PS1/GLT-1 interaction, particularly in trafficking dynamics, potentially elucidating the strong correlation between fAD PS1 mutations and epileptiform activity.

Disclosures: F. Perrin: None. **B. Lundin:** None. **M. Sadek:** None. **P. Sinha:** None. **O. Berezovska:** None.

Poster

PSTR387

Mechanisms of Synaptic Dysfunction in Alzheimer's Disease: *In Vitro* Models

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.13/B104

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant STTR 1R41AG081123-01A1
NYS CATN2

Title: In Vitro Human Model of Senescent Cell Mechanisms of Neurodegeneration of Synaptic Connectivity and Neuroregeneration Through Senolytic Discovery

Authors: M. B. PAREDES-ESPINOSA[1], *J. PALUH[2];
[1]Nanoscale Sci. and Engin., Univ. at Albany, SUNY, CNSE, Albany, NY; [2]Univ. at Albany CNSE, Malta, NY

Abstract: Human stem cell neurotechnologies offer opportunities for establishing complex *in vitro* organoids and multi-system gastruloids that mimic normal developmental or pathology conditions for disease analysis and drug discovery in human-neural tissue based models. The Paluh lab is at the forefront in developing complex gastruloid models of the innervated enteric gut, intrinsic nervous system of the multichambered contractile heart, and alginate embedded neural ribbon 3D CNS multineuron neuronal circuitry architectures for synaptic connectivity applications relevant to the CNS brain or spine. Here we extend application of our neural ribbon 3D technology to evaluation of senescence on neuronal circuitry synaptic connectivity, applying electrophysiology (MEA), single cell RNA-Seq and hiPSC lines that represent complex demographics of age, sex and ethnicities, along with normal and disease sources. Our goal is to assist sxRNA Technologies (sxRNATech) to establish a senolytics toolkit for Alzheimer's Disease and Alzheimer's-Disease-Related Dementias (ADRD). The Senescence Associated Secretory Phenotype (SASP) is poorly understood in regard to its impact on gene regulation and functional synaptic connectivity of neurons, interneurons and neural support cells. 3D Neural ribbons offer multimodal analysis capabilities for biomarker tracking, whole genomic

transcriptomics, electrophysiology and target manipulation of senescent regulators and sxRNATech microRNA senolytics. By application of microRNA targeting tools (sxRNATech) we evaluate the ability to halt neuroregeneration and promote regeneration to restore synaptic connectivity. This brain on chip technology is expected to enable rapid analysis, diagnostics and senolytics discovery relevant to tracking and alleviating senescent stress on neuronal circuitry networks. The research is supported by NIH STTR and NYS CATN2 awards.

Disclosures: M.B. Paredes-Espinosa: None. **J. Paluh:** A. Employment/Salary (full or part-time):; SUMMER SALARY. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; PI ON STTR COLLABORATION.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.01/B105

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG061800
NIH P30AG10161
NIH P30AG72975
NIH R01AG15819
NIH R01AG17917
NIH U01AG46152
NIH U01AG61356

Title: Multiscale integration identifies synaptic proteins associated with human brain connectivity

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[1]Neurol., Univ. of Alabama at Birmingham, Birmingham, AL; [2]RUSH, Chicago, IL; [3]Neurol., UAB, Birmingham, AL; [4]Rush Alzheimer's Dis. Ctr., Rush Univ. Med. Ctr., Chicago, IL; [5]SUNY Upstate, Syracuse, NY

Abstract: Neuroimaging measurements are far-removed from their molecular underpinnings at synapses, and this disconnect hinders progress towards a basic understanding of brain connectivity. To bridge this gap, we analyzed a unique cohort of 98 individuals who provided neuroimaging and genetic data, contemporaneous with dendritic spine morphometric, proteomic, and gene expression data from the superior frontal and inferior temporal gyri. Through cellular

contextualization of the molecular data with dendritic spine morphology, we identified hundreds of proteins that explain inter-individual differences in functional connectivity and structural covariation. The found proteins are enriched for synapses, energy metabolism, and RNA processing. By integrating data at the genetic, molecular, subcellular, and tissue levels, we link specific biochemical changes at synapses to connectivity between brain regions. These results demonstrate the feasibility of integrating data from vastly different biophysical scales to provide a molecular understanding of human brain connectivity.

Disclosures: J. Herskowitz: None. **B. Ng:** None. **K.M. Greathouse:** None. **D.A. Bennett:** None. **C. Gaiteri:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.02/B106

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIH AG061800
NIH AG054719
NIH AG063755
NIH AG068024

Title: Neuritin (NRN1) provides synaptic resilience against tau in Alzheimer's Disease

Authors: *D. PUGH[1,2], A. WEBER[3], K. M. GREATHOUSE[3], D. A. BENNETT[4], N. T. SEYFRIED[5], J. H. HERSKOWITZ[3];
[1]UAB Neurosci. Grad. Programs, Birmingham, AL; [2]Department of Neurology, University of Alabama at Birmingham School of Medicine, Birmingham, AL; [3]Dept. of Neurol., Univ. of Alabama at Birmingham Sch. of Med., Birmingham, AL; [4]Rush Alzheimer's Dis. Ctr., Rush Univ. Med. Ctr., Chicago, IL; [5]Human Genet., Emory Univ., Atlanta, GA

Abstract: There is increasing recognition of a subpopulation of cognitively resilient individuals that live to advanced age with intact cognitive function despite high levels of Alzheimer's disease (AD) pathology. Neuritin (NRN1) was recently identified as a synaptic protein associated with resilience in a proteomic study conducted in the Religious Order and Rush Memory and Aging Project (ROSMAP, n = 109), which demonstrated that NRN1 can deter amyloid-beta (Aβ)-induced synaptic deficits. In order to support the promise of NRN1 as a rationale therapeutic target to promote resilience and delay dementia onset, it is imperative to assess whether NRN1 can provide neuroprotection against tau. Matched post-mortem brain tissue samples from Brodmann area 37 (BA37) occipital temporal gyrus from 128 ROSMAP cases were analyzed using multiplex tandem mass tag mass spectrometry (TMT-MS). Relative

protein abundance of NRN1 was plotted against cognitive performance, NFT density, and Braak staging. Western blotting and densitometry analysis was performed to assess relative protein quantification of NRN1 in postmortem control, progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD) brains. Multi-electrode array (MEA) analyses of rat primary neuronal cultures were performed. Single neuron electrophysiological activity was recorded and mean action potential frequency (Hz) was calculated as the ratio of the total number of spikes recorded and the duration of recording in seconds. Among ROSMAP resilient individuals, NRN1 protein abundance positively correlates with global cognition, episodic memory, semantic memory, and working memory. NRN1 protein abundance was significantly decreased in AD and inversely correlated with global neurofibrillary tangle burden and Braak staging. In contrast, NRN1 protein levels were equivalent among controls, PSP, and CBD brains, suggesting selective vulnerability in AD. MEA analyses revealed treatment of neuronal cultures with recombinant NRN1 protected against tau-induced synaptic deficits. Our findings provide compelling human evidence that links NRN1 protein abundance with cognitive performance and tau pathology in AD. We hypothesize that therapeutic targeting of NRN1 may enhance synaptic plasticity and protect synapses from the degenerating forces of Aβ and tau in AD.

Disclosures: D. Pugh: None. **A. Weber:** None. **K.M. Greathouse:** None. **D.A. Bennett:** None. **N.T. Seyfried:** None. **J.H. Herskowitz:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.03/B107

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIH AG061800
NIH AG054719
NIH AG063755
NIH AG068024

Title: Dendritic spine remodeling and synaptic tau levels in the entorhinal cortex of Alzheimer's disease

Authors: *J. EBERHARDT[1], K. M. GREATHOUSE[2], J. H. HERSKOWITZ[3];
[1]UAB, Birmingham, AL; [2]Neurol., UAB, Birmingham, AL; [3]Neurol., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Cognitive decline in Alzheimer's disease (AD) correlates well with the extent of tau pathology and synapse or dendritic spine loss. Thus, studying synaptic processes may aid in the identification of new therapeutic targets that limit cognitive decline. The entorhinal cortex (EC)

is an initial site of tau tangle formation, from which tau can propagate to other brain regions through synaptic connections. We developed a pipeline whereby dendritic spine density and morphology as well as synaptic tau measurements from the EC were incorporated into a correlation network to identify relationships between spine morphology and synaptic tau in AD patients. Golgi-stained dendrites and dendritic spines from postmortem human BA28 EC were imaged using high-resolution brightfield microscopy and digitally reconstructed in 3D for morphometric analysis. Synaptosome fractions were isolated from the same BA28 samples. Levels of synaptosome and insoluble phosphorylated tau were measured by ELISA. Dendritic spine density in the EC is reduced in AD, but not in cognitively normal individuals with AD pathology (CAD) cases. AD cases exhibit more synaptosomal pS396 tau than CAD cases. Next, we compared our findings in humans with rodent models of AD, including the humanized tau (htau) mice. Overall, our studies reveal notable relationships between levels of phosphorylated tau in the EC and dendritic spine morphology in both mice and humans.

Disclosures: J. Eberhardt: None. **K.M. Greathouse:** None. **J.H. Herskowitz:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.04/B108

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Cure Alzheimer's Fund
Alzheimer's Association; AARF-23-1150672

Title: Preservation of neurons and synapses in the entorhinal cortex identifies brains resilient to Alzheimer Disease neuropathological changes

Authors: *S. KUMAR[1], C. KLEIN[2], A. C. AMARAL[2], C. AGUERO[3], T. GOMEZ-ISLA[4]; [1]Neurol., Mass Gen. Hospital-Harvard Med. Sch., Boston, MA; [2]Neurol., Massachusetts Gen. Hosp., Boston, MA; [3]Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA; [4]Massachusetts Gen. Hosp., Boston, MA

Abstract: Background: Increasing evidence suggests that Alzheimer Disease neuropathological changes (ADNC) may develop up to two decades in advance of disease symptoms. The entorhinal cortex (EC) is one of the first and most vulnerable areas to neuronal loss in AD. We and others previously showed that not everyone who harbors ADNC will inevitably develop dementia during life. We have termed this phenomenon 'resilience' to ADNC. Here we have investigated the integrity of synapses and neurons in the EC of individuals who met criteria for high probability of AD at autopsy but never develop dementia. We hypothesized that the anatomical preservation of those elements in the setting of plaques and tangles can serve to

identify resilient brains **Methods:** A total 18 brains from the Massachusetts General Hospital ADRC Brain Bank representing the following three categories were included: 1) Demented with high probability of AD at autopsy (‘demented AD’); 2) Non-demented with probability of AD at autopsy (‘resilient’); 3) Non-demented with no or only negligible ADNC at autopsy (‘control’). Cases were matched for age and gender. Detailed quantitative assessments of plaque burden, number of tangles (intraneuronal and ghost), number of neurons, GFAP+ astrocytes, and CD-68 microglial cells were conducted on sections containing EC using the appropriate antibodies. Expansion microscopy (ExM), a technique that allows to obtain nanoscale resolution to visualize individual synaptic elements, was applied to quantify pre- and post-synaptic elements and mature synapses in these brains. **Results:** Demented AD and resilient cases had equivalent amyloid plaque loads and tau tangle counts in the EC. Demented AD cases had significantly higher numbers of GFAP+ astrocytes and CD68+ microglia, and significantly lower numbers of neurons and synapses (by about 70%) compared to resilient brains. **Conclusions:** These findings suggest that preservations of neurons and synapses in the EC, a brain area that presumably harbored ADNC for decades in both demented AD and resilient, identifies individuals who cope with robust plaque and tangle loads without developing dementia during life (‘resilient’). Additional studies to understand the mechanism/s underlying synaptic resilience are ongoing.

Disclosures: S. Kumar: None. **C. Klein:** None. **A.C. Amaral:** None. **C. Aguero:** None. **T. Gomez-isla:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer’s Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.05/B109

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association Discovery Grant ALZDISCOVERY-1052089

Title: Regional alterations in hippocampal integrins across tauopathy disease stage in htau mice

Authors: B. CSUBAK, M. A. SMITH, S. D. CRISH, ***C. M. DENGLER-CRISH**; Pharmaceut. Sci., Northeast Ohio Med. Univ., Rootstown, OH

Abstract: Integrins are essential bidirectional signaling proteins and cell adhesion molecules that critically regulate cellular processes. In the brain, integrins mediate glial function, synaptogenesis, blood-brain barrier integrity, and long-term potentiation. There is increasing evidence that integrin dysfunction may play a role in dementia pathogenesis. Pathological tau has been shown to bind αVβ integrins on astrocytes, activating intracellular signaling pathways that cause inflammation and neurotoxic damage. Our previous research demonstrated that pharmacological integrin modulation with the “neuroprotective” hormone irisin prevented

emergence of tauopathy and inflammation in brains of presymptomatic 4-month-old female htau mice (Bretland et al., 2021), a transgenic model that develops age-related tau overexpression, neuropathology and cognitive deficits. However, a follow-up study demonstrated that the same treatment given to fully symptomatic 11-month-old female htau mice exacerbated hippocampal neuropathology, upregulated αVβ integrin expression, and increased integrin-mediated inflammatory signaling. Here, we used multi-channel immunofluorescence to visualize αVβ integrin expression across hippocampal regions in fixed brain tissue from 11-month-old female htau mice treated with irisin (100 μg/kg i.p.) or saline vehicle. We found that irisin treatment differentially altered αVβ integrin subtype expression in synaptic layers of dentate gyrus, CA1 and CA3 hippocampal regions. These results provide the first spatially-resolved characterization of hippocampal integrin dysregulation in tauopathy model mice.

Disclosures: B. Csubak: None. **M.A. Smith:** None. **S.D. Crish:** None. **C.M. Dengler-Crish:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.06/B110

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: University of South Carolina School of Medicine Office of the Associate Dean of Research and Graduate Education to DJH
University of South Carolina Office of Undergraduate Research to TAM
NIH K01AG061263 to JAM
NIH P20GM109091 to JAM

Title: Targeting D-serine metabolism to reverse age-related cognitive and neurobiological deficits

Authors: *D. J. HOROVITZ, M. A. TIEMAN, J. A. MCQUAIL;
Univ. of South Carolina, Columbia, SC

Abstract: N-methyl-d-aspartate receptors (NMDARs) are crucial for learning and memory and have been implicated in age-related memory loss and Alzheimer's disease (AD) pathology. NMDARs contribute divergent effects on neuroplasticity or neurodegenerative processes and this functional diversity is attributed to subpopulations of receptors that can be distinguished based on subcellular localization, activation by endogenous co-agonist, and intracellular signaling pathways. Specifically, activation of synaptic NMDARs is associated with synaptic plasticity, learning and memory, and neuroprotection through ERK signaling, while activation of extrasynaptic NMDARs induces Ca^{2+}-mediated excitotoxicity and cell damage. D-Serine is the

essential co-agonist of synaptic NMDARs, so we hypothesized that increasing D-serine in the synaptic cleft may potentiate NMDAR signaling associated with neuroplasticity and reverse memory loss in aging. We screened aged, 22-month-old, male and female F344 rats for spatial learning deficits in a multi-day, place-learning version of the Morris water maze relative to performance of 4-month-old young adults. Following, rats were re-tested on a delayed match-to-place (DMTP) task in the water maze to confirm persistent cognitive impairment. Aged rats impaired on both tasks were then treated with various doses of a d-amino acid oxidase inhibitor (DAOI), to inhibit enzymatic degradation of endogenous D-serine, prior to retesting in the DMTP task. We show that treatment with DAOI produced a dose-dependent improvement in memory and increased ERK phosphorylation in the prefrontal cortex and hippocampus. These results indicate that prolonging D-serine availability is a promising therapeutic strategy to rescue memory in aging. Future studies will examine the effects of DAOI on D-serine levels in the aged brain and measure the effects of age and memory on DAO and serine racemase, the enzyme responsible for D-serine synthesis. Our long-term goal will be to establish the mechanisms by which DAOI treatment improves memory in aging and determine if cognitive rescue is also protective against AD-related neuropathology.

Disclosures: D.J. Horovitz: None. **M.A. Tieman:** None. **J.A. McQuail:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.07/B111

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant P20GM109091 to JAM
NIH Grant K01AG061263 to JAM

Title: Operant task paradigms for probing age-related task-switching costs in rats

Authors: H. A. DUFALA[1], A. ESLAMI[1], D. J. HOROVITZ[1], T. S. EICH[2], P. J. VENTO[1], ***J. A. MCQUAIL**[1];
[1]Univ. of South Carolina, Columbia, SC; [2]USC, Los Angeles, CA

Abstract: Human neuropsychological studies have consistently demonstrated age-related declines in specific cognitive processes that underlie the ability to switch between simple tasks. However, there is a scarcity of analogous experimental paradigms using animal models to investigate these phenomena. In this study, we introduce a dual-task paradigm integrating match-to-sample and non-match-to-sample operant tasks to assess task-switching 'costs' in male and female F344 rats of varying ages (4-10 months for younger rats, and 12+ months for older rats). In both tasks, rats were trained to sample a pre-selected lever, and after delays of up to 24

seconds, their memory for the sampled lever was tested during a choice phase when two levers were presented. In the single-task condition, rats were reinforced for selecting the same lever as the one presented in the sample phase ('match-to-sample'). In the dual-task condition, rats encountered varying response contingencies within each session, signaled by cue-lights in each trial, prompting them to either choose the sampled lever as before, or switch to a non-matching response strategy and choose the non-sampled lever ('non-match-to-sample'). Following assessment of single- and dual-task testing, rats were evaluated on operant tests of cognitive flexibility, using the same cues and response levers, that included learning an initial discrimination ('press lever under illuminated cue-light'), followed by a stimulus-response reversal ('press lever under non-illuminated cue-light'), and finally a set-shift task ('disregard cue-lights and press the lever on the right-side'). While older rats performed comparably to younger rats in the matching-only condition, they exhibited decreased accuracy when required to switch between the two rules in the dual-task condition, particularly evident on non-matching trials after longer delays. Subsequent tests ruled out impairments in explicit learning, reversal learning, or set-shifting in older rats, indicating specific deficits in task-switching abilities. Importantly, our findings parallel those observed in aging humans, suggesting the utility of our small-animal model in studying age-related task-switching effects. Future experiments will build upon this novel task to explore different types of costs (local switching vs. mixing costs) using structured block designs reverse-translated from neuropsychological methods in human cognition studies. These innovative approaches aim to elucidate the neurobiological mechanisms underlying increased task-switching costs in aging, potentially informing interventions to mitigate age-related cognitive decline.

Disclosures: H.A. Dufala: None. **A. Eslami:** None. **D.J. Horovitz:** None. **T.S. Eich:** None. **P.J. Vento:** None. **J.A. McQuail:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer’s Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.08/B112

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH T32 GM007267
Owens Family Foundation
The Miller Family

Title: Bacterial extracellular vesicles contain short chain fatty acids that might play a role in Alzheimer’s disease pathogenesis

Authors: *S. WACHAMO[1,2], A. THAKUR[1,2], M. BROADWAY[1,2], A. GAULTIER[1,2];
[1]Neurosci., [2]Univ. of Virginia, Charlottesville, VA

Abstract: AD is the most common form of dementia affecting 50 million people worldwide, with estimated global costs of $1 trillion annually. AD is characterized by a progressive decline in cognitive function associated with the deposition of amyloid beta, the formation of neurofibrillary tangles composed of hyperphosphorylated Tau protein, and neuroinflammation. This in turn results in the loss of synapses, neuronal dysfunction, and eventually neuronal death. It is still not well understood what triggers the pathological hallmarks of AD. However, emerging evidence implicates a crucial role of the microbiota in AD through microbially produced metabolites, but the precise mechanisms remain to be elucidated. Based on previous studies, we hypothesized that the gut microbiota regulates AD pathology through release of bacterial extracellular vesicles (BEVs) that contain microbial metabolites. To test this hypothesis, we isolated BEVs from the intestinal contents of WT and 5XFAD mice using ultracentrifugation followed by size exclusion chromatography. BEVs were characterized by BCA assay (total protein), ZetaView nanoparticle tracking analysis (size and concentration), ELISA or Western blot (BEV specific markers), Cryo-TEM (size and morphology), and omics analysis (cargo). Our preliminary data suggests gut microbiota dysbiosis in 5XFAD compared to WT mice. Targeted metabolomics of WT BEVs revealed the presence of short chain fatty acids (SCFAs), which have been shown to play an important role in the pathogenesis AD. Furthermore, our experiment that leveraged the Cre-LoxP system showed that BEVs can traffic to the brain. Ongoing and future experiments will assess the cargo of 5XFAD BEVs and compare those with WT BEVs, examine the functional role of BEVs in the brain and the immune system, and manipulate the contents of BEVs and investigate how this alteration modulates the pathogenesis of AD. Together, BEVs isolated from fecal samples or peripheral blood have enormous potential to serve as diagnostic tools due to their potentially altered cargo during brain disorders. Discovery of biomarkers in BEVs, like SCFAs, will offer simple, inexpensive, non-invasive, and easily available diagnostic tools for early detection and intervention.

Disclosures: S. Wachamo: None. **A. Thakur:** None. **M. Broadway:** None. **A. Gaultier:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer’s Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.09/B113

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: European Research Council Grant agreement No. #951294
University of Bordeaux’s IdEx “Investments for the Future” program/GPR BRAIN_2030

Title: The heterogeneous extracellular space properties of amyloid plaque shape nanoparticle navigation

Authors: J. ESTAÚN-PANZANO[1], Y. DEMBITSKAYA[2], I. CALARESU[3], Q. GRESIL[4], E. DOUDNIKOFF[5], T. LESTE LASSERRE[6], T. AMEDEE[7], ***V. PLANCHE**[5], L. COGNET[8], L. GROC[9], V. U. NÄGERL[10], E. BEZARD[11];
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Abstract: A hallmark of Alzheimer's disease (AD) is the accumulation of amyloid plaques, primarily composed of misfolded amyloid β (Aβ) peptides. Therapeutic strategies targeting these plaques, like the immunisation approaches, face the hurdle of penetrability. We employed a complementary set of nanoscopic imaging techniques to investigate the extracellular space (ECS) alterations in a mouse AD model. Two-photon shadow imaging *in vivo* revealed cortical amyloid plaques and their surroundings, highlighting a dense corona of cells and a penetrable core. Quantum dot tracking unravelled that ECS rheological parameters are heterogeneous in and around plaques, with an increased diffusivity within plaques and low nanoparticle density in the core. Using another type of nanoparticle, single-walled carbon nanotube, we confirmed these altered local diffusion properties in the cortex of AD mice. We found an altered ECM, notably disrupted within the amyloid plaque, providing a rationale for the altered molecular dynamics in AD brain tissue and shedding new light on strategies to develop effective Aβ plaques-penetrating therapies.

Disclosures: J. Estaún-Panzano: None. **Y. Dembitskaya:** None. **I. Calaresu:** None. **Q. Gresil:** None. **E. Doudnikoff:** None. **T. Leste Lasserre:** None. **T. Amedee:** None. **V. Planche:** None. **L. Cognet:** None. **L. Groc:** None. **V.U. Nägerl:** None. **E. Bezard:** A. Employment/Salary (full or part-time):; Motac. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac, TREEFROG Therapeutics, SE Therapeutics.

Poster

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Circuit and Synaptic Alterations in Alzheimer’s Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.10/B114

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Single cell transcriptome analysis identified a unique neutrophil type associated with Alzheimer's Disease

Authors: *X. ZHANG[1], G. HE[2];
[1]Shanghai Mental Hlth. Ctr., Shanghai, China; [2]Shanghai Mental Hlth. Ctr., Shanghai City, China

Abstract: Neutrophils are the primary mediators of the innate host defense against pathogens. While their involvement in the pathogenesis of central nervous system (CNS) diseases such as multiple sclerosis (MS) and stroke is well-documented, their role and mechanisms in Alzheimer's disease (AD) progression remain poorly investigated. Neutrophils were conventionally considered as homogeneous and transcriptionally inactive cells. However, single-cell RNA sequencing (scRNA-seq) provides an unbiased approach to explore their heterogeneity. Here, we investigated the molecular characteristics of neutrophils purified from the peripheral blood of APP/PS1 mice using scRNA-seq. Our analysis revealed two distinct neutrophil subsets enriched in APP/PS1 mice. Gene ontology (GO) analysis unveiled that differentially expressed genes (DEGs) in these AD-associated neutrophils were enriched in pathways related to chemotaxis and protein translation, suggestive of an activated neutrophil state. Subsequent experiments using flow cytometry and immunohistochemical staining confirmed the infiltration of these AD-associated neutrophils into the brain of APP/PS1 mice. These findings shed light on the potential of these neutrophils as biomarkers for clinical diagnosis and as targets for therapeutic intervention.

Disclosures: X. Zhang: None. **G. He:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.11/B115

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Templeton Medical Research Foundation

Title: Sex-related differences in optic nerve glia of the 3xTg mouse model of Alzheimer's disease

Authors: *M. TERZIC[1], C. M. DENGLER-CRISH[2], S. D. CRISH[3];
[1]Northeast Ohio Med. Univ., Uniontown, OH; [2]Pharmaceut. Sci., Northeast Ohio Med. Univ., Rootstown, OH; [3]Pharmaceut. Sci., NEOMED, Rootstown, OH

Abstract: Alzheimer's disease (AD) disproportionately affects women, who make up 2/3 of all diagnoses and often carry greater pathological burdens than men. Determining the mechanisms underlying sex-specific differences in disease onset and progression could identify new, desperately needed therapeutic targets. Deficits in visual system function, along with retinal accumulation of amyloid-beta and hyperphosphorylated tau, have been shown to occur before onset of cognitive deficits in AD, and are associated with inflammatory damage to the retina and optic nerve. Additionally, we recently reported sex differences in these visual system disturbances in the 3xtg-AD mouse. To begin investigating mechanistic sources of these sex-related vulnerabilities, we performed whole transcriptome analysis of optic nerve microglia and astrocytes in 12-month-old male and female 3xtg mice, an age where they exhibit advanced, pervasive pathology. To accomplish this, 6 µm frozen cross-sections of fixed optic nerves from 3xtg mice were taken and mounted on charged slides. Sections were immunostained for Iba1 (microglia) and GFAP (astrocytes) to identify glial cells and incubated with NanoString Technologies Mouse Whole Transcriptome Atlas of short, complementary RNA probes conjugated to unique, photocleavable oligonucleotide "bar codes" (19,962 genes). Regions of interest (ROI) were automatically segmented by fluorescence across the entire nerve: Iba1-positive nerve area and GFAP-positive nerve area. Each ROI was illuminated with UV light to release oligo bar codes, which were collected and then quantified with an nCounter platform. Our analyses revealed 80 microglial and 65 astrocytic pathway genes that were differentially expressed by sex. Top hits included pathways involved in Aβ processing, inositol processing, integrin signaling, blood-brain barrier function, and other neuropathological cascades. In summary, glial transcriptomes of whole nerve sections affected by AD pathology may provide a unique and powerful opportunity to identify novel, sex-specific mechanistic targets involved in AD progression.

Disclosures: M. Terzic: None. **C.M. Dengler-Crish:** None. **S.D. Crish:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.12/B116

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Discovery of Novel Ophthalmic Imaging Biomarkers for Neurodegenerative Disease

Authors: *S. CICALESE[1], R. DROLET[2];
[1]Neurosci., Merck, Lansdale, PA; [2]Neurosci., Merck, West Point, PA

Abstract: Stephanie Cicalese[1], Xiaoli Ping[2], Christopher Nunes[2], Weiwei Luo[3], Xiaolan Shen[2], Jacob Marcus[1], Sean Smith[1], Jason Uslaner[1], Robert Drolet[11]MRL Neuroscience, [2] MRL Lab

Animal Resources, [3] MRL Singapore Imaging Biomarkers

Background: The retina is increasingly investigated as a site of Alzheimer's Disease (AD) manifestation, with amyloid β (Aβ) plaques being found in the retina of AD and mild cognitively impaired (MCI) patients, however functional changes in the retina are poorly understood. Retinal imaging techniques can be utilized for neurodegenerative biomarkers since the retina is the only central nervous system organ available for this kind of tracking in a low cost and non-invasive manner. Further development of these techniques may provide invaluable tools for pre-clinical AD diagnosis, monitoring and drug efficacy trials.

Method: Retinal imaging techniques were using to compare the retina of wild-type (WT) or AD transgenic mice containing the APP: Swedish and Indiana mutation expressed under the prion promoter (TgCRND8) at 20 and 30 weeks of age. Optical coherence tomography (OCT) for retinal thickness assessment, Fundus Autofluorescence Imaging (FAF) for aggregate visualization, and electroretinogram (ERG) for retinal neuronal electrical activity were performed. Aβ ELISA was also performed on retina and cortex to evaluate amyloid burden correlation.

Results: Mean retinal insoluble Aβ 1-42 was significantly elevated in TgCRND8 mice relative to WT at 23 weeks (35 vs 4.2 pg/mL, $P<0.05$) and 42 weeks (77.9 vs 7.5 pg/mL, $P<0.05$), and correlated with insoluble cortical brain Aβ 1-42 (Pearson's $r=0.847$, $P=0.001$). Total retinal thickness was significantly higher in TgCRND8 mice relative to WT (233 μm vs 225 μm, $P<0.01$). Specifically, the retinal nerve fiber layer (RNFL) was thicker in TgCRND8 mice (28.5 vs 22.9 μm, $P<0.01$). FAF imaging revealed significant autofluorescent aggregates in TgCRND8 mice relative to WT in the RNFL (94.3 vs 59.8 spots, $P<0.01$) and outer plexiform layer (OPL) (87.8 vs 49.3 spots, $P<0.01$). Lastly ERG revealed reduced b wave amplitude in TgCRND8 mice relative to WT (197.45 vs 457.9 μV, $P<0.001$) and photopic negative response (PhNR) wave amplitude (8.2 μm vs 25.7 μV, $P<0.001$). These values are indicative of dysfunctional bipolar and retinal ganglion cell electrical activity.

Conclusions: TgCRND8 mice have functional changes in retina thickness, FAF lipofuscin deposition, and ERG, which may be useful biomarkers for drug development, efficacy trials and AD neurodegenerative monitoring.

Disclosures: S. Cicalese: None. **R. Drolet:** None.

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PSTR388

Circuit and Synaptic Alterations in Alzheimer’s Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.13/B117

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA Grant AG063903
FAPESP Grant 2021/10925-8
Pat & Shirley Ryan Family Research Acceleration Fund

Title: A novel conformation sensitive antibody fragment used for isolation and biochemical characterization of neurotoxic oligomers of the β-amyloid peptide

Authors: *R. M. DE CAMPOS[1,2], N. AYON[3], J. JERISHA[1], E. CLINE[1], N. PINHEIRO[4], M. F. CARRARO[4], V. B. JUSKA[3], N. L. KELLEHER[5], A. S. SEBOLLELA[4], W. L. KLEIN[1]; [1]Neurobio., Northwestern Univ., EVANSTON, IL; [2]Biochemistry and Immunology, University of São Paulo, Ribeirão Preto, Brazil; [3]Chem., Northwestern Univ., Evanston, IL; [4]Biochem. and Immunol., Univ. of São Paulo, Ribeirão Preto, Brazil; [5]Mol. Biosci., Northwestern Univ., EVANSTON, IL

Abstract: Alzheimer's disease (AD), the most prominent cause of dementia worldwide, is characterized by brain accumulation of soluble oligomers of β-amyloid peptide (AβO) and neuronal tangles formed by hyperphosphorylated Tau. Evidence suggests AD pathogenesis begins many years before any dementia symptoms, with mounting evidence implicating AβOs as critical players in disease triggering, raising the need to elucidate their biochemical identity. Amongst the antibodies selected against AβOs, single chain fragment variable (scFv) NUsc1 demonstrated robust selectivity neutralizing AβO-induced toxicity, both *in-vitro* and *in-vivo*, targeting primarily species over 50kDa. Therefore, NUsc1 is a prime candidate for isolating and characterizing AD-relevant toxic soluble AβOs. NUsc1 shows selective binding to a subset of AβOs in primary cultured neurons. Also, cell viability analysis demonstrates that NUsc1 effectively neutralizes AβO-induced cell death in differentiated SH-SY5Y cells. The NUsc1-AβO complex was successfully isolated from synthetic oligomer preparations and brain-derived AD mouse extracts using a non-denaturing immunoprecipitation. A novel technique measured the molecular interaction between NUsc1 and its targeted AβO species, the biolayer interferometry (BLI). NUsc1 presents a nanomolar affinity binding to these species, indicating stable and selective binding between the scFv antibody and its targeted neurotoxic AβOs. In addition, Thioflavin-T (ThT) probing and transmission electron microscopy (TEM) show that NUsc1 inhibits the *in-vitro* formation of β-amyloid oligomers and fibrils. The sizing of AβO species isolated by NUsc1, performed by preliminary techniques as centrifugal filtration and size exclusion chromatography (SEC), and confirmed by more detailed morphological and mass characterization using atomic force microscopy (AFM) and individual ion mass spectrometry analysis (I^2MS), indicates NUsc1 preferentially binds to globular AβO species with a diameter of approximately 2.4nm and a mass range between 50-250KDa. The isolation and characterization of human brain-derived AβOs are currently ongoing. Those findings confirm NUsc1 selectivity against neurotoxic high molecular weight AβOs, contributing to determining the biochemical identity of AD-associated toxic aggregates and revealing scFv antibody NUsc1 as a promising tool for diagnostic and therapeutic approaches.

Disclosures: R.M. De campos: None. **N. Ayon:** None. **J. Jerisha:** None. **E. Cline:** None. **N. Pinheiro:** None. **M.F. Carraro:** None. **V.B. Juska:** None. **N.L. Kelleher:** None. **A.S. Sebollela:** None. **W.L. Klein:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.14/B118

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Circulating Branched Chain Amino Acids (BCAAs) Are Linked To Alzheimer's Disease Related Cognitive Deficits

Authors: *Z. HAQUE, A. C. SHIN;
Nutritional Sci., Texas Technol. Univ., Lubbock, TX

Abstract: Alzheimer's disease (AD) is a progressive neurological disorder causing irreversible cognitive decline, usually in older adults. Type 2 diabetes (T2D) is a strong risk factor for AD that shares common pathophysiological features including amyloid and/or tau deposits in the brain and metabolic perturbation which is interestingly shown to preceed AD symptoms. Emerging evidence suggests that circulating branched-chain amino acids (BCAAs) are associated with T2D. While excess BCAAs are shown to be harmful to neurons, whether aging affects BCAA metabolism that can further exacerbate the pathology of AD is poorly understood. Hence, we hypothesized that high circulating BCAAs are associated with metabolic dysregulation as well as AD progression. To test this, we conducted behavioral tests, glucose tolerance test (GTT), insulin tolerance test (ITT), BCAA assay, and western blot (WB) in 8 and 12-month-old male wild-type (WT) and transgenic APP/PS1 mouse model of AD. There was no difference in insulin sensitivity and cognitive function in the WT and AD mice of 8 months except for impaired glucose tolerance in 8-month AD mice. The 12-month APP/PS1 mice displayed significant impaired glucose tolerance, insulin sensitivity, and elevated plasma BCAAs as well as cognitive decline compared to the WTs. Interestingly, plasma BCAAs and impaired glycemic control were also strongly correlated with cognitive decline in 12-month APP/PS1 mice. However, protein analysis in the liver showed no significant difference in BCAA catabolism of WT and AD mice of both 8 and 12-month groups. Collectively, this study reveals a positive association between circulating BCAAs, metabolic perturbation, and cognitive decline in AD along with aging. Further investigation in different developmental stages of the disease, different tissues, and female mice may help to assess the potential of BCAAs as a predictive or diagnostic biomarker of AD.

Disclosures: Z. Haque: None. **A.C. Shin:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.15/B119

Topic: C.02. Alzheimer's Disease and Other Dementias

NIH Grant R01-AG077692
AARF-22-972333

Title: Seizure susceptibility and memory deficits in prodromal $APP^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$mice

Authors: *K. HOAG, A. J. BARBOUR, E. LANCASTER, D. M. TALOS, F. E. JENSEN; Neurol., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Neuronal hyperexcitability and memory deficits have been observed in prodromal Alzheimer's Disease (AD) patients and in mouse models of AD. Although reported incidences of seizures in people with AD varies between studies, frequencies as high as 60% of AD patients comorbid with epileptiform activity have been reported. Our lab has shown that in the Five times familial AD (5XFAD) model there is an excitatory:inhibitory (E:I) imbalance that emerges early in the disease course, including increased seizure susceptibility, which was associated with memory deficits. Here we sought to determine whether increased seizure susceptibility and memory deficits, suggestive of an E/I imbalance, are also present in prodromal Amyloid Precursor Protein knock-in ($APP^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$) mice which express physiological APP, as opposed to APP overexpression in 5XFAD mice. $APP^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ and age-matched WT control mice at four months of age underwent either contextual fear conditioning (CFC) to test memory or an established pentylenetetrazol (PTZ) kindling (or control, saline) protocol to induce seizures. We found that less PTZ administrations were required for $APP^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice to develop tonic-clonic seizures than their WT counterparts ($p=0.0295$) demonstrating increased seizure susceptibility suggestive of E:I imbalance in $APP^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice. A different group of $APP^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ and WT mice underwent memory recall tests at 1 day and 28 days post CFC to test recent and remote memory, respectively. We found that $APP^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice at the remote timepoint displayed memory deficits ($p<0.05$, $n=8\text{-}9$), but had normal memory at the recent timepoint ($n=8\text{-}9$), indicating deficits in cortical-mediated memory in prodromal $APP^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$mice. Overall, we demonstrated memory deficits and neuronal hyperexcitability in prodromal $APP^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice. These results further characterize $APP^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice and add to mounting evidence of E:I imbalance at a prodromal timepoint of AD, thus indicating seizure activity and E:I imbalance as therapeutic targets.

Disclosures: K. Hoag: None. **A.J. Barbour:** None. **D.M. Talos:** None. **F.E. Jensen:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.16/B120

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01AA025718
Fondecyt-1221080
PhD ANID fellowship-21202521

Title: Early reduction of Long-term depression in the nucleus Accumbens is accompanied by increase in food reward behavior in a transgenic Alzheimer's disease mouse model

Authors: *N. RIFFO-LEPE, L. ARMIJO WEINGART, J. GONZÁLEZ-SANMIGUEL, P. SAAVEDRA SIEYES, G. RAMOS FLORES, H. ZAMBRANO, D. HAMMERSLEY, E. FERNÁNDEZ-PÉREZ, L. G. AGUAYO;
Physiol. Dept., Univ. of Concepción, Concepción, Chile

Abstract: Alzheimer's disease (AD) is a progressive neurological disorder that leads to dementia in an increasingly aging population. The classical paradigm of AD argues that the presence of extracellular amyloid plaques in the brain would initiate the disease, causing cognitive impairment such as memory loss. However, these symptoms correspond to advances stages of AD, while non-cognitive symptoms such as apathy and compulsive behaviors are early reported in patients, associated with dysfunction of the mesolimbic system, where the nucleus Accumbens (nAc) is a critical integration hub. Recently, we reported intraneuronal accumulation of amyloid-beta protein (iAβ) in the nAc of APPswe/PS1E9d mice (2xTg) with AD at early stages, associated with structural and synaptic alterations. However, it remains unknown how iAβ in the medium spiny neurons (MSN) of the nAc would impair neural plasticity and reward-related behaviors. To address this gap, we used electrophysiology technique to analyze if early accumulation of iAβ affects synaptic plasticity of the nAc. We applied high-frequency stimulation (HFS) to induce long-term depression (LTD) in the nAc of 3- and 6-month-old WT and 2xTg mice. We observed no significant changes in LTD percentage at 3-month-old, but a reduction at 6-month-old from 47 ± 8% in WT to 11 ± 6% in 2xTg mice. Interestingly, LTD reduction was accompanied with a significant AMPA/NMDA ratio decrease. Additionally, fluorometric analysis of GCamP6s-associated Ca2+ activity in brain sections from 6-months-old WT and 2xTg mice in presence of CNQX, an AMPA-receptor antagonist, showed higher inhibition in the nAc of 2xTg mice, suggesting an increase in calcium-permeable AMPA receptors (CP-AMPAR). This result was substantiated through electrophysiological recordings in brain slices, showing higher inward rectification at positive voltages along with increased inhibition of excitatory currents in presence of CP-AMPAR antagonist NASPM in the nAc of 6-month-old 2xTg mice, suggesting a postsynaptic mechanism for the reduced LTD. Finally, to evaluate how these early synaptic changes would be reflected in reward-related behaviors alterations, we used a conditional place preference paradigm, showing a significant increase in conditioning and consumption of chocolate in 6-month-old 2xTg mice. Overall, these findings indicate that accumbal MSNs are an early brain target of amyloid pathology, showing synaptic disfunction along with reward processing behavior impairment, suggesting that AD pathology might initiate in mesolimbic areas contributing to non-psychiatric symptoms, such as apathy and loss of motivation.

Disclosures: N. Riffo-Lepe: None. **L. Armijo Weingart:** None. **J. González-Sanmiguel:** None. **P. Saavedra Sieyes:** None. **G. Ramos Flores:** None. **H. Zambrano:** None. **D. Hammersley:** None. **E. Fernández-Pérez:** None. **L.G. Aguayo:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.17/B121

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Arizona Alzheimer's Consortium
Arizona State University

Title: Alzheimer's disease and fine motor degeneration: investigating changes in fine motor accuracy and coordination using a novel rodent reaching task

Authors: *A. MELICK[1], D. LUKACIK[2], H. A. BIMONTE-NELSON[3], S. Y. SCHAEFER[4], S. BEEMAN[3], J. L. VERPEUT[5];
[1]Psychology, Arizona State Univ., Tempe, AZ; [2]Arizona State Univ., Scottsdale, AZ; [3]Arizona State Univ., Tempe, AZ; [4]Biomed. Engin., Arizona State Univ., Tempe, AZ; [5]Psychology Dept., Arizona State Univ., Tempe, AZ

Abstract: Alzheimer's disease (AD) is the most common dementia, with a projected 7 million Americans diagnosed by 2025 (Rajan et al., 2021). Symptoms, like memory and executive function deficits, are hard to discern from normal aging and seen at late stages, reducing the efficacy of treatment. In 2023, seniors spent $345 billion on medical care, with $87 billion in out-of-pocket costs (Alzheimer's Association, 2023). Thus, affordable and accessible methods for early disease detection are crucial. Of note, motor impairments arise in AD preclinical stages (Buchman & Bennett, 2011). The Quick Behavioral Exam to Advance Neuropsychological Screening (qBeans) is an upper-extremity motor task that predicts functional decline over 1 year, hippocampal atrophy, and amyloid with 88% sensitivity in those with Mild Cognitive Impairment (Schaefer et al., 2020; Schaefer et al., 2022). It uses accessible materials and procedures, and is an economical alternative to current diagnostics. With that said, the exact mechanisms and early neural changes qBEANS detects remain elusive. To investigate early biomarker and microstructural shifts, we created a preclinical fine motor task analogous to qBeans and tested behavior in typical Fisher LSD rats (n = 6) and TGF344-AD rats (n = 12), a preclinical model of AD. Adult rats were required to reach for a sugar pellet from 1 of 3 increasingly difficult bowls for 10 minutes with 3 trials per bowl over 9 days. Each grasp was rated as a success, drop, or failure. The average rate (%) of performance was found to be positively correlated with increasing failures as bowl difficulty increased ($R^2 = 0.75$) and

negatively correlated with decreasing successes ($R^2 = 0.6$). While success performance was not significantly different across bowls, there was a significant difference in total reaches per day ($p = 0.009$). The number of failures per day increased from bowl 1 (mean 3.87 SD 2.23) to bowl 2 (mean 7.8 SD 8.90), and from bowl 2 to bowl 3 (mean 11.2 SD 5.95), meaning we created a rodent qBEANS task that requires multiple strategies and stages with increasing difficulty. Reach poses and kinematics will be quantified using the machine learning software, SLEAP Estimates Animal Poses (SLEAP), to elucidate changes in reach strategy, latencies, and speed of task completion. The development of this task to study motor dysfunction is useful for a plethora of neurodegenerative disorders and may bridge the gap between the presence of pathology and neural dysfunction in early motor degeneration prior to an observable decline in cognition.

Disclosures: A. Melick: None. **D. Lukacik:** None. **H.A. Bimonte-Nelson:** None. **S.Y. Schaefer:** None. **S. Beeman:** None. **J.L. Verpeut:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.18/B122

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIA R00AG078400
NIA 1K99AG078400-01

Title: Effects of ketone diester supplementation on fear extinction impairments in the TgF344AD rat model of Alzheimer's disease across the lifespan

Authors: *M. A. MCCUISTON[1], C. M. HERNANDEZ, III[2], J. P. CARCAMO DAL ZOTTO[3], P. YOHANNES[1], S. SKJEFTE[1], A. BANERJEE[4];
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Abstract: Fear extinction impairments appear in normative aging in conjunction with impaired executive functions. There is a greater prevalence of fear-based disorders in older populations and in those with Alzheimer's disease (AD). The basal lateral amygdala (BLA) is a part of central medial lobe structures that is involved in fear-based circuitry. We have previously shown extinction impairments in TgF344AD (AD) rats across the lifespan that are associated with BLA hyperexcitability and inflammation. Ketogenic diets have been shown to reduce epileptiform activity and inflammation. To determine if the extinction memory impairments can be ameliorated by leveraging a supplement that mimics the anti-epileptiform and anti-inflammatory properties of a ketogenic diet, we supplemented young and aged WT and TgAD rats with a

subchronic dose of a ketone diester (or saline) prior to fear memory acquisition and extinction. We hypothesize that a daily dose of ketone diesters can rescue fear extinction deficits through targeting mechanisms related to hyperexcitability and/or neuroinflammation in AD rats.

Disclosures: M.A. McCuiston: None. **C.M. Hernandez:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.19/B123

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA Grant RF1 AG064859
NIA Grant F32 AG058456

Title: Alterations in synaptic plasticity, cognitive function, and neuroinflammation in 5xFAD mice following a mild cortical infarct

Authors: *H. N. FRAZIER[1], M. J. COLEMAN[1], S. J. MESSMER[2], H. C. MUZYK[1], D. J. BRAUN[1], V. A. DAVIS[1], C. S. BAILEY[1], J. M. ROBERTS[2], L. J. VAN ELDIK[1];
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Abstract: Vascular dysfunction is one of the most common comorbidities reported in Alzheimer's disease (AD) patients and is thought to exacerbate neurodegenerative processes such as inflammation and cognitive decline. Previously, we found that diet-induced cerebral small vessel disease (hyperhomocysteinemia) in amyloidogenic mice was associated with worsened cognitive performance and impaired synaptic plasticity. However, this model is complicated by concurrent peripheral dysfunction, which limits its ability to report on vascular changes specific to the CNS. For the present study, we therefore induced mild cortical infarcts in 5xFAD mice using a tandem CCA/distal MCA occlusion, as this represents a less severe and more CNS-relevant model of vascular injury. Briefly, male and female WT and 5xFAD mice (8-9 months old) received a 60 min occlusion localized to the anterior region of a single hemisphere. After 7 days, mice underwent behavioral assessment using the spontaneous open field activity, rotarod, and frailty index tests. Mice were then euthanized for tissue harvest and preparation of acute brain slices for electrophysiological comparisons of injured and non-injured hemispheres. Plasma samples were also obtained at 3 different timepoints for biochemical analysis of relevant AD biomarkers and pro-inflammatory cytokines. Results from behavioral assessments indicated that male mice of both genotypes scored higher on measures of frailty compared to females. Spontaneous open field activity results showed that female mice traveled further and faster on average, but no differences in % time spent in the center zone were detected. Interestingly, extracellular field recordings taken in hippocampal area CA1 revealed

significantly lower fiber volley amplitudes in injured hemispheres compared to non-injured, suggesting the infarct may have led to functional deficits in adjacent hippocampal neurons despite being localized to the cortex. Sex and genotype effects were also detected on measures of basal synaptic strength (EPSP amplitude and slope) as well as measures of hyperexcitability. Overall, this work indicates that mild vascular injuries in the cortex of 5xFAD mice can alter synaptic function in adjacent hippocampal neurons and suggests the presence of sex- and genotype-dependent responses to vascular injury in this model.

Disclosures: H.N. Frazier: None. **M.J. Coleman:** None. **S.J. Messmer:** None. **H.C. Muzyk:** None. **D.J. Braun:** None. **V.A. Davis:** None. **C.S. Bailey:** None. **J.M. Roberts:** None. **L.J. Van Eldik:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.20/B124

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DoD Grant AZ1701452 G.E.S

Title: Traumatic brain injury and chronic stress, comorbid contributors to pathogenic signaling mechanisms of Alzheimer's disease

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Abstract: Traumatic brain injury (TBI) is a significant risk factor for developing Alzheimer's disease (AD). In addition, chronic stress (CS) is increasingly recognized as a potent contributor to accelerated dementia. These conditions (AD, TBI, CS) share several comorbidities, such as deficits to memory and behavioral regulation. We hypothesize that these shared qualities stem from progressive and chronic alterations to Ca^{2+} homeostasis, resulting in downstream pathological cell signaling, impaired synaptic function, and compounding maladaptive changes to neuronal physiology. The interaction between TBI and chronic stress and its influence on AD pathophysiology is understudied. We address this knowledge gap by employing acute brain slice electrophysiology, 2-photon Ca^{2+} imaging, and behavioral analysis to answer questions from a combined single cell, network, and organismal perspective in our male and female 3xTg-AD and age matched non-transgenic (NonTg) control mouse models. These mice receive mild TBI or

SHAM treatment after group housing control or social isolation conditions for one month, the latter used to elicit a chronic stress response. 30 days post-TBI, mice undergo behavioral assessments to test for spatial recognition and memory alongside anxiety-linked phenotypes. Brains are then extracted for slice electrophysiology including local-field potential recoding in the CA1 Schaffer collaterals of the hippocampus, whole cell patch clamp recording, and 2-photon Ca^{2+} imaging. Our current data indicate that NonTg-TBI mice show sustained markers of neuronal pathophysiology similar to 3xTg-SHAM mice, such as increased endoplasmic reticulum Ca^{2+} release and frequency of spontaneous excitatory post synaptic currents. This suggests that TBI induces chronic cell signaling deficits consistent with an AD-like neuronal phenotype. At a network level, NonTg-TBI mice appear to recover hallmarks of synaptic plasticity such as basal synaptic strength, paired pulse facilitation, and long-term potentiation (LTP) by 30 days post impact. In contrast, 3xTg-AD mice do not show recovery of synaptic plasticity 30 days post-TBI. Instead, they retain deficits to LTP, the cellular correlate of learning and memory. Interestingly, though single-cell deficits persist 30 days post injury, NonTg-TBI mice may maintain synaptic plasticity via compensatory mechanisms. To date, our data showing intracellular Ca^{2+} handling deficits post-TBI and their downstream synaptic consequences support the hypothesis that risk factors modulating Ca^{2+} homeostasis impact neuronal vulnerability and progression to AD.

Disclosures: E.K. Webber: None. **D.F. Steinbrenner:** None. **N.M. Barrington:** None. **G.E. Stutzmann:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer’s Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.21/B125

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: DoD Grant AZ1701452GES

Title: Acute and chronic effects of mild traumatic brain injury and their contribution to Alzheimer's disease

Authors: *N. M. BARRINGTON[1], D. A. PETERSON[2], J. WHALLEY[3], G. E. STUTZMANN[4];
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Abstract: Traumatic brain injury (TBI) is estimated to impact 2.8 million people annually, with 75% of those injuries classified as mild TBI or concussion. Prior head injury is the #2 risk factor for sporadic Alzheimer's disease (AD), second only to age. Many of the pathologic hallmarks of TBI overlap with AD, including beta amyloid aggregates, phosphorylation of tau, activation of microglia and astrocytes resulting in inflammation, and alterations in gene-level pathways contributing to neurodegeneration. Our objective is to identify common histopathologic and differentially expressed gene pathways during the acute and chronic stages following mild TBI to pinpoint common drivers of alterations found in both TBI and AD. Following single or repeat mild controlled cortical impacts (CCI) in the 3xTg-AD mouse model (3xTg) and age/sex matched non-transgenic control mice (NonTg) at 6-7 months of age, animals were euthanized at 7 or 30 days following the final impact and brain tissues extracted for immunohistochemical or single cell RNA sequencing analysis. Tissues were stained using phospho-tau (serine 262), amyloid (4g8), and microglia (Iba1) markers and imaged on a Leica SP8 confocal microscope to assess the progression of pathology and inflammation. An additional group of animals will be subjected to repeat mild CCI, after which single cell RNA sequencing will be conducted, and transcripts compared to known gene libraries to identify TBI-mediated gene drivers that contribute to progression toward AD. Immunohistochemical analysis thus far reveals sustained and increased phospho-tau burden at the 30-day timepoint post-TBI in both the 3xTg and NonTg mouse strains relative to their respective sham controls. Similar patterns were observed with amyloid and Iba1 staining, with the greatest increases in Iba1 density found in the cortex in comparison to hippocampal regions. Interestingly, the TBI-NonTg mice had similar patterns of histopathology as the sham-3xTg mice. To date, our data suggests mild TBI results in a pathological and inflammatory response similar to that seen in AD animals, leading to a compounding affect when AD animals undergo TBI. Transcriptomic analyses will help elucidate whether these are similar processes occurring in parallel, or whether TBI and AD together lead to unique transcriptomic and cellular alterations that result in accelerated AD progression in at-risk individuals that experience head injury.

Disclosures: N.M. Barrington: None. **D.A. Peterson:** None. **J. Whalley:** None. **G.E. Stutzmann:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.22/B126

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: Chambers-Grundy Endowment

Title: Tau PET imaging in Alzheimer's Disease: Investigating the mapping of plasma biomarkers pTau181, NfL, and GFAP onto PET patterns

Authors: *L. PASIA[1,2], D. MATTHEWS[3], M. MUGOSA[2], A. RITTER[4,5], J. CUMMINGS[2], D. BARIA[1], H. TANG[1], N. VINSDATA[1], J. W. KINNEY[2];
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline and memory loss. Current understanding of AD pathology highlights the importance of developing reliable biomarkers for early diagnosis and disease monitoring. Plasma-based biomarkers, such as tau phosphorylated at threonine 181 (pTau181), neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP), have shown promise in reflecting AD pathology. Tau positron emission tomography (PET) imaging is a well-established method for assessing tau pathology in the brain, but it is costly and not widely available. Identifying non-invasive blood-based biomarkers that correlate with tau PET measures could provide a more accessible and cost-effective approach for AD diagnosis and monitoring. This study leveraged data from a clinical trial investigating the effects of Rasagiline, a selective monoamine oxidase B (MAO-B) inhibitor in patients with mild-to-moderate AD. While the primary focus of the trial was to assess the potential therapeutic benefits of Rasagiline, the collected samples provided a unique opportunity to evaluate the association between blood-based biomarkers and tau PET imaging at baseline, prior to Rasagiline administration. We analyzed baseline data from a cohort of 46 patients with mild-to-moderate AD who participated in the Rasagiline clinical trial. The association between plasma concentrations of NfL, pTau181, GFAP, and tau PET imaging was evaluated. These biomarkers were chosen based on their association with the Amyloid/Tau/Neurodegeneration (ATN) framework, which characterizes the pathological hallmarks of AD. Our results demonstrate a strong association between tau PET levels and plasma concentrations of pTau181 and GFAP. This finding highlights the potential utility of these biomarkers as non-invasive tools for assessing tau pathology in AD. Further research is needed to confirm these results in larger cohorts to explore the use of NfL, pTau181, and GFAP as complementary biomarkers to tau PET imaging in clinical settings. Validation of these findings could lead to development of more accessible and cost-effective approaches for AD diagnosis and monitoring.

Disclosures: L. Pasia: None. **D. Matthews:** 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.; Radiological Society of North America PET Tau Profile Work- ing Group, PET Amyloid Profile Working Group. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ADM Diagnostics, Inc.. F. Consulting Fees (e.g., advisory boards); lzheimer's Drug Development Foundation. **M. Mugosa:** None. **A. Ritter:** F. Consulting Fees (e.g., advisory boards); Corium Pharmaceuticals, Lundbeck/Otsuka. **J. Cummings:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Artery, Vaxxinity, Behrens, Alzheon, MedAvante-Prophase, and Acu-men. F. Consulting Fees

(e.g., advisory boards); cadia, Actinogen, Acumen, AlphaCognition, ALZpath, Aprinoia, AriBio, Artery, Biogen, Biohaven, BioVie, BioXcel, Bristol-Myers Squib, Cassava, Cerecin, Diadem, Eisai, GAP Foundation, GemVax, Janssen, Jocasta, Karuna, Lighthouse, Lilly, Lundbeck, LSP/eqt, Mangrove Therapeutics, Merck, NervGen, New Amsterdam, Novo Nordisk, Oligomerix, ONO, Optoceutics, Otsuka, Oxford Brain Diagnostics, Prothena, eMYND, Roche, Sage Therapeu- tics, Signant Health, Simcere, sinaptica, Suven, TrueBinding, Vaxxinity, and Wren. **D. Baria:** None. **H. Tang:** None. **N. Vinsdata:** None. **J.W. Kinney:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.23/B127

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: COBRE Biobank GR17422

Title: Evaluating human plasma biomarkers for Alzheimer's disease: phosphorylated tau-217, amyloid-beta 1-40, and amyloid-beta 1-42

Authors: *M. MUGOSA[1,2], L. PASIA[4], J. W. KINNEY[5,3], A. RITTER[6,7,8], Z. YANG[9]; [1]Pam Quirk Brain Hlth. and Biomarker Lab., UNLV, Las Vegas, NV; [2]Dept. of Brain Hlth., [3]Pam Quirk Brain Hlth. and Biomarker Lab., Univ. of Nevada, Las Vegas, Las Vegas, NV; [4]Univ. of Nevada, Reno, Reno, NV; [5]Dept. of Brain Hlth., Univ. of Nevada Las Vegas, Henderson, NV; [6]; [7]Hoag's Pickup Family Neurosciences Inst. US, Newport, CA; [8]Cleveland Clin. Lou Ruvo Ctr. for Brain Hlth. US, Las Vegas, NV; [9]Cleveland Clin., Las Vegas, NV.

Abstract: Evaluating Human Plasma Biomarkers for Alzheimer's Disease: Phosphorylated Tau-217, Amyloid-beta 1-40, and Amyloid-beta 1-42
MayaRae Mugosa1Lorenzo Gabriel Pasia, M.A.3Aaron Ritter, M.D2Jefferson Kinney, Ph.D1
1Dept. of Brain Health, University of Nevada, Las Vegas 2Cleveland Clinic Luo Ruvo Center for Brain Health 3School of Medicine, University of Nevada, Reno
AbstractAlzheimer's disease (AD) is a progressive neurodegenerative disease affecting memory and cognitive processes, making it increasingly difficult for individuals to perform daily tasks as the disease progresses. Early diagnosis is crucial for optimal treatment and support. Current diagnostic methods for AD include positron emission tomography (PET), computed tomography (CT), magnetic resonance imaging (MRI), evaluation of cerebrospinal fluid (CSF) and other invasive and expensive tools. Blood biomarkers provide more cost-effective and scalable assessment and disease progression. We have previously evaluated the association of glial fibrillary acidic protein (GFAP), phosphorylated tau-181 (ptTau181), and neurofilament light protein (NfL) with amyloid PET values in the Center for Neurodegeneration and Translational

Neuroscience well characterized clinical cohort (Kinney, 2023). Using chemiluminescent enzyme immunoassay (CLEIA) technology on the Lumipulse G1200 platform, we evaluated concentrations in human plasma for three proteins associated with the Amyloid/Tau/Neurodegeneration (ATN) framework. Amyloid-beta 1-40 (ab1-40), amyloid beta 1-42 (ab1-42), and phosphorylated tau-217 (pTau217) plasma concentrations were analyzed in relation to brain amyloid status, as determined by 18F-AV-45 PET imaging. We investigated differences between disease states and their connection to amyloid status to identify potential biomarkers or significant correlations. Our preliminary results suggest a strong association between pTau217 levels and amyloid status, and promising associations with clinical measures.
Yang, Z., Sreenivasan, K., Toledano Strom, E. N., Osse, A. M., Pasia, L. G., Cosme, C. G., Mugosa, M., Chevalier, E. L., Ritter, A., Miller, J. B., Cordes, D., Cummings, J. L., & Kinney, J. W. (2023). Clinical and biological relevance of glial fibrillary acidic protein in alzheimer's disease. Alzheimer's Research & Therapy, 15(1). https://doi.org/10.1186/s13195-023-01340-4

Disclosures: M. Mugosa: None. **L. Pasia:** None. **J.W. Kinney:** None. **A. Ritter:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.24/B128

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA R01AG067420
NIA R01DC014296
NIA P30AG062421
NIH S10OD020039
Simons Foundation 811255
NIA K00AG068432

Title: Precision Measurement of Longitudinal Brain Change Within Individuals Enabled by Cluster Scanning

Authors: *M. ELLIOTT[1], J. A. NIELSEN[2], L. HANFORD[1], A. HAMADEH[3], T. HILBERT[4], B. C. DICKERSON[5], B. HYMAN[5], R. MAIR[1], M. ELDAIEF[6], R. L. BUCKNER[1,7,8]; [1]Dept. of Psychology, Ctr. for Brain Sci., Harvard Univ., Cambridge, MA; [2]Psychology, Brigham Young Univ., Provo, UT; [3]Baylor Col. of Med., Houston, TX; [4]Siemens, Lausanne, Switzerland; [5]Dept. of Neurol., Massachusetts Gen. Hosp., Charlestown, MA; [6]Neurol. and Psychiatry, Massachusetts Gen. Hosp., Charlestown, MA; [7]Department of Psychiatry,

Massachussetts General Hospital, Charlestown, MA; [8]Athinoula A. Martinos Center for Biomedical Imaging, Massachussetts General Hospital, Charlestown, MA

Abstract: Measurement error limits the statistical power to detect group differences and ultimately our ability to monitor longitudinal brain change within individuals (e.g., hippocampal volume). Higher error requires larger, longer, and more expensive clinical trials slowing the ability to find effective interventions. Recent advances in scan acceleration with compressed sensing (CS) enable fast structural scans (~1 min) that achieve morphometric errors that are close to the errors in longer traditional scans (Elliott et al., 2023a). As acceleration allows multiple scans to be acquired in rapid succession, it becomes possible to pool estimates to increase measurement precision, a strategy known as "cluster scanning" (Nielsen et al., 2019; Elliott et al., 2023b). Here we explored trajectories of brain morphometric change using cluster scanning in a longitudinal study of 60 individuals (14 younger adults, 23 cognitively unimpaired older adults, 9 adults with frontotemporal dementia, and 14 adults with mild cognitive impairment or Alzheimer's Dementia). Participants were intensively sampled with 12-16 CS T_1-weighted scans (Siemens research application sequence) on a 3T Siemens MAGNETOM Prisma at each visit and completed 2-6 visits within ~1 year. Morphometric errors from a single CS scan (1 min 12 secs) were, on average, 12% larger than a standard ADNI structural scan (5 min 12 secs). Pooled estimates from eight clustered CS acquisitions led to errors that were 51% smaller than a standard structural scan. Analyses of longitudinal trajectories of hippocampal volume and cortical thickness revealed that cluster scanning enabled the detection of brain change in most individuals in less than a year. Furthermore, idiosyncratic trajectories were apparent including accelerated trajectories in cases of rapid cognitive decline, accelerated hippocampal atrophy after cancer treatment, and trajectories of brain aging in cognitively unimpaired older adults that suggested both resilience to typical brain aging and decline before clinical diagnosis. Cluster scanning reveals trajectories of brain aging and neurodegeneration previously obscured in measurement error and group averaging. Our results suggest a path to a deeper understanding of individual differences in brain aging and neurodegeneration as well as efficient measurement of change in individuals and small groups.

Disclosures: M. Elliott: None. **J.A. Nielsen:** None. **L. Hanford:** None. **A. Hamadeh:** None. **T. Hilbert:** A. Employment/Salary (full or part-time):; Employed at Siemens Healthineers. **B.C. Dickerson:** None. **B. Hyman:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; research grants from the National Institutes of Health, Cure Alzheimer's Fund, Tau Consortium, and the JPB Foundation – and sponsored research agreements from Abbvie, BMS, and Biogen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dewpoint, Novartis, and lattus. F. Consulting Fees (e.g., advisory boards); AbbVie, Ambagon, Aprinoia Therapeutics, Arvinas, Avrobio, AstraZenica, Biogen, BMS, Cure Alz Fund, Cell Signaling, Dewpoint, Latus, Novartis, Sofinnova, Vigil, Violet, Voyager, WaveBreak. **R. Mair:** None. **M. Eldaief:** None. **R.L. Buckner:** F. Consulting Fees (e.g., advisory boards); Consultant for Pfizer, Roche, and Cognito.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.25/Web Only

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Biomarker identification in cognitive impaired ageing population

Authors: *V. SINGH[1], V. MISHRA[2];
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[2]Neurol., Inst. of Med. Sci., Varanasi, India

Abstract: ***AbstractVineeta Singh(Presenting author, Ph.D)* and Vijaya Nath Mishra (Senior author, MD, DM Neurology)*****Affiliation: *Department of Geriatrics, IMS, BHU, Varanasi-221005 **Department of Neurology, IMS, BHU, Varanasi-221005**
Alzheimer's disease (AD) represents a progressive neurodegenerative disorder characterized by cognitive decline, with mild cognitive impairment (MCI) constituting an intermediary stage between normal aging and dementia. This multimodal study aimed to identify potential biomarkers for MCI diagnosis in a North Indian population cohort. Following screening of 2000 elderly individuals, 10 MCI cases and 10 age/gender-matched healthy controls underwent neuropsychological assessment (Hindi Mental State Examination, Montreal Cognitive Assessment), diffusion tensor imaging (DTI), and blood-based biochemical analyses. Compared to controls, MCI subjects exhibited reduced cognitive performance scores, altered levels of homocysteine, thyroid hormones, vitamins B12 and D, and DTI evidence of white matter degeneration in left parietal lobe, right fornix, corpus callosum genu, and bilateral forceps minor regions. Plasma 1H NMR metabolomic profiling coupled with multivariate/non-parametric statistical analyses revealed 8 differentially expressed metabolites in MCI, with elevated lactate, N-acetylaspartate, histidine and reduced formate, choline, alanine, creatinine, and glucose levels. In silico analyses implicated choline in MCI/AD pathogenesis. Differential gel electrophoresis and MALDI-MS/MS proteomics identified 3 dysregulated plasma proteins in MCI (1 downregulated, 2 upregulated). Bioinformatic pathway/network analyses associated these candidates with complement activation, coagulation, platelet function, and AD pathways, highlighting apolipoprotein E and transthyretin as key interactors, with transthyretin representing a potential MCI plasma biomarker signature. This integrated multimodal approach combining neuropsychological, neuroimaging, biochemical, metabolomic and proteomic profiling identified promising biomarker panels for MCI diagnosis.

Disclosures: V. Singh: A. Employment/Salary (full or part-time):; Part time, Department of Geriatrics, IMS, BHU. 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.; Dr. SS Chakarborti. **V. Mishra:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.26/B129

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Department of Biotechnology, Ministry of Science and Technology, Government of India) (Award No. BT/HRD/01/05/2012)

Title: Impact of isoflurane on glutathione: oxidative stress mapping platform for longitudinal in vitro MR spectroscopic study

Authors: *P. MANDAL[1,2], Y. ARORA[1], A. SAMKARIA[1], J. MAROON[3], V. FODALE[4]; [1]Natl. Brain Res. Ctr., Gurgaon, India; [2]Neurosurgery, UPMC, Pittsburgh, PA; [3]Neurosurg., UPMC, Pittsburgh, PA; [4]Dept. of Adult and Childhood Human Pathology, Univ. of Messina, Messina, Italy, Messina, Italy

Abstract: PURPOSE: Glutathione (GSH) is a master antioxidant protecting against oxidative damage, and significant depletion of GSH in the hippocampus is considered as early diagnostic biomarker of Alzheimer's disease (AD). Inhaled anesthetics are an integral part of general anesthesia protocol. protocol. Comparative analysis between isoflurane and propofol groups demonstrated a significant reduction of serum GSH peroxidase level after surgery in the isoflurane group, but not in the propofol group. The objective is to develop a platform involving magnetic resonance spectroscopy (MRS) for detecting GSH as well as isoflurane in vitro model.
METHODS: MRS data was obtained utilizing a 3T MR scanner (Prisma, Siemens) equipped with a 64-channel ^{1}H head coil. The acquisition for GSH detection was done using the MEGA-PRESS sequence, with the parameters: ON = 4.40 ppm, OFF = 5.00 ppm, TE = 120 ms, TR = 2500 ms, voxel size = 25 × 25 × 25 mm, and average= 32. Isoflurane was detected using ^{19}F MRS studies involving a dual tune ($^{19}F/^{1}H$) head coil. GSH data was processed using KALPANA package and ^{19}F data was processed using Siemens package.
RESULTS: The GSH peak area (in phantom) showed a slow decline over time. On the contrary, GSH peak area is depleted much faster in the presence of isoflurane. This novel experimental scheme based on in vitro studies, can be a potential clinical application as similar setup can be applied in clinical conditions.
CONCLUSION: Noninvasive MRS scheme presents a valuable approach for monitoring brain oxidative stress from GSH levels in pre- and post-surgery patients longitudinally. Similar experimental scheme can also be used to monitor any residual anesthetics in the brain after surgery through ^{19}F MRS spectroscopy.
Correspondence: pravat.mandal@gmail.com

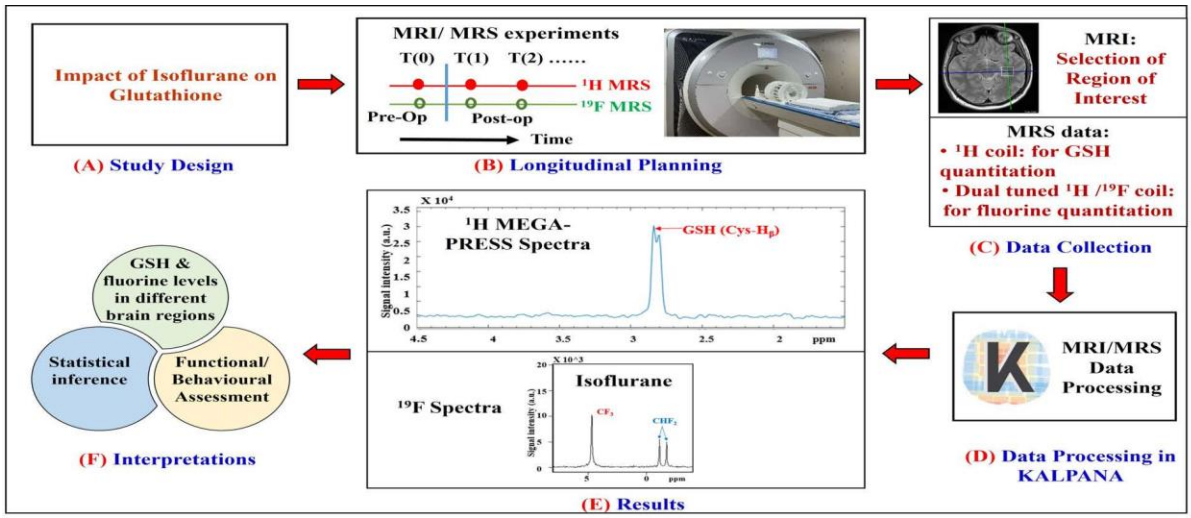

Figure 1
Proposed platform for longitudinal monitoring of GSH and fluorine due to inhaled anesthetic. The scheme involves formulation of (A) study design to address the impact of a particular anesthetic on brain cognition by measuring GSH and fluorine content in brain regions (B) longitudinal planning of observations in MR scanner for screened participants at various time points (pre and post operation observations) (C) Data Collection: MRI scanner equipped with suitable coil (^{1}H and dual tuned ^{1}H /^{19}F) for MRI/MRS data collection from human participants. (D) The raw data can be analyzed in an appropriate toolbox (in this study KALPANA is utilized for MRS data processing, step (E)) GSH and Fluorine spectra obtained from KALPANA. (F). Using these results, important interpretations can be made.

Disclosures: P. Mandal: None. **Y. Arora:** None. **A. Samkaria:** None. **J. Maroon:** None. **V. Fodale:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.27/B130

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01 AG039684
NIH Grant R56 AG052576
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NIH Grant P30 AG072958
NIH Grant F32 AG084167

Title: Standard and high-resolution diffusion imaging of cortical disconnection in Alzheimer's disease

Authors: *J. L. MERENSTEIN, J. ZHAO, A. W. SONG, D. J. MADDEN;
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Abstract: In addition to the accumulation of neuropathologies (i.e., amyloid beta, neurofibrillary tangles), Alzheimer's disease (AD) has been associated with the degradation of white matter structural pathways that connect distributed brain regions - an observation termed "cortical disconnection". Characterizing the extent of white matter tissue degradation is clinically important as this process occurs prior to the accumulation of AD-related neuropathologies. However, previous studies of AD-related differences in white matter structural connectivity have primarily used standard resolution diffusion-weighted imaging (DWI) protocols. These protocols cannot adequately resolve white matter connections in fine-grained regions with crossing fibers or high curvature and may therefore have missed more subtle white matter tissue degradation in adults with AD. To address this limitation, this study compared measures of white matter structural connectivity derived from a clinically feasible high-resolution multiplexed sensitivity encoding (MUSE) DWI protocol (1 mm isotropic voxels; 1μ;;l volume) or a standard resolution DWI protocol (1.5 mm isotropic voxels; 3.375 μ;;l volume), using a sample of 22 adults with AD and 22 demographically matched healthy controls. White matter structural connectivity was assessed using the graph theoretical measure of system segregation, which measures the degree to which individual brain networks are distinct from one another. As expected, results indicated that, for both DWI protocols, structural system segregation across the whole brain was significantly higher for adults with AD relative to healthy controls. However, the high-resolution DWI protocol achieved higher sensitivity to AD-related differences in structural connectivity than the standard resolution DWI protocol, consistent with findings from prior studies of rodents and healthy younger adults. This increased sensitivity was particularly evident for the visual network and the limbic network, the latter of which is highly vulnerable to the effects of AD-related neuropathologies. Together, these findings suggest that this clinically feasible, high-resolution DWI methodology may detect more subtle AD-related differences in white matter connectivity than standard resolution DWI protocols.

Disclosures: J.L. Merenstein: None. **J. Zhao:** None. **A.W. Song:** None. **D.J. Madden:** None.

Poster

PSTR388

Circuit and Synaptic Alterations in Alzheimer's Disease Progression: New Insights and Biomarkers

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.28/B131

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant RF1 AG051504

Title: Blood-based gene and co-expression network levels are associated with AD/MCI diagnosis and cognitive phenotypes and preserved in the brain

Authors: *X. CHEN[1], X. WANG[2], J. S. REDDY[3], Z. QUICKSALL[3], T. NGUYEN[10], D. REYES[4], J. GRAFF-RADFORD[5], C. R. JACK, Jr.[5], V. J. LOWE[4], D. S. KNOPMAN[5], R. PETERSEN[6], K. KANTARCI[4], K. NHO[11], M. ALLEN[7], M. CARRASQUILLO[8], A. J. SAYKIN[12], N. ERTEKIN TANER[9];
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Abstract: *Background* Alzheimer's disease (AD) patients have decline in cognition and brain pathology detectable by neuroimaging and/or cerebrospinal fluid biomarkers. However, molecular disease mechanisms are complex and heterogeneous. Developing cost-effective blood-based biomarkers reflecting brain molecular perturbations in AD is crucial. We identified blood-based gene and co-expression network level changes associated with AD/mild cognitive impairment (MCI) diagnosis and AD-related phenotypes. *Methods*We performed differentially expressed gene (DEG) and weighted gene co-expression network analysis, followed by meta-analysis, using blood transcriptome data of participants from the Mayo Clinic Study of Aging and the Alzheimer's Disease Neuroimaging Initiative. The neuroimaging phenotypes include microhemorrhages, infarcts, amyloid burden, hippocampal volume, and white matter hyperintensities. The cognitive phenotypes include subtest and composite scores for memory, language, visuospatial, and executive function. We constructed brain co-expression network using AMP-AD brain transcriptome data of 6 regions and performed preservation analysis between blood and brain networks.
*Results*We identified 6 modules(M) significantly associated with diagnosis or cognition (FDR <0.05). M1, M10, and M21 positively associate with memory, M1 and M10 associate with language and M21 with visuospatial function. M1 is enriched in DEGs associated with language while M10 and M12 in executive function. M5 negatively associates with logical memory delayed recall scores (LMDR), memory, executive, and language and is enriched in DEGs for these phenotypes. M8 negatively associates with memory. M13 positively associates with LMDR. M5 is up-regulated while M10 is down-regulated in AD/MCI patients. Cell-type enrichment analysis showed M5 is enriched in basophils, monocytes, and neutrophils; M8 in monocytes and neutrophils; M12 in natural kills cells; M21 in B cells (FDR <0.05). Gene ontology terms enriched in these modules indicated broad consistency with their cell types. Among those blood modules, modules M1, 5, 8, and 10 were preserved in the brain network.
*Conclusions*We identified modules significantly associated with AD/MCI or cognition using blood transcriptome data, and four were preserved in the brain networks. These findings nominate blood transcriptome changes and their enriched biological processes as potential pathomechanisms in AD/MCI development and cognitive decline. We aim to investigate these transcripts as potential biomarkers and therapeutic targets through additional replication and experimental validation studies.

Disclosures: X. Chen: None. **X. Wang:** None. **J.S. Reddy:** None. **Z. Quicksall:** None. **T. Nguyen:** None. **D. Reyes:** None. **C.R. Jack:** None. **V.J. Lowe:** None. **D.S. Knopman:** None. **R. Petersen:** None. **K. Kantarci:** None. **K. Nho:** None. **M. allen:** None. **M. Carrasquillo:** None. **A.J. Saykin:** None. **N. Ertekin Taner:** None.

Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.01/B132

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Brain delivery and efficacy of a BBB shuttle-enhanced aducanumab biosimilar in a mouse model of Alzheimer's disease.

Authors: *M. RAMOS VEGA[1], S. B. VERGO[4], Y. GALLERO-SALAS[2], E. ALEXIOU[1], F. WICHERN[5], J. HECKSHER-SØRENSEN[6], H. HANSEN[3];
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Abstract: Background & Aim: Accumulation of amyloid β (Aβ) in the brain is a neuropathological hallmark of Alzheimer's disease (AD). Recently approved Aβ-directed antibodies such as aducanumab have demonstrated modest efficacy in AD, which may potentially be explained by poor blood-brain barrier (BBB) penetration. Transferrin receptor (TfR) mediated brain delivery of therapeutic monoclonal antibodies across the BBB is a promising concept in drug development for CNS disorders. **Methods:** APP/PS1 transgenic mice received a single administration of an aducanumab biosimilar (10 mg/kg), aducanumab biosimilar fused with an anti-TfR molecule as a BBB-shuttle (TfR-aducanumab, 10 mg/kg) or control hIgG (10 mg/kg). Other APP/PS1 mice were treated once weekly for 12 weeks with aducanumab biosimilar (2 mg/kg or 10 mg/kg), TfR-aducanumab (10 mg/kg) or control hIgG (10 mg/kg). One hemisphere was co-stained with antibodies against human IgG (drug distribution) and Aβ, while the other whole-hemisphere was stained with Congo red. All samples were cleared and scanned on a light-sheet fluorescence microscope. A custom image analysis algorithm was developed for automated anatomical mapping and quantification of drug distribution and Aβ plaques in a total of 840 brain regions using a custom mouse brain atlas. **Results:** Aβ-directed therapeutic antibodies, but not control hIgG, accumulated in brain areas with high Aβ plaque load, notably the cortex . A more homogeneous and deeper brain penetration was achieved using TfR-aducanumab (2 mg/kg) compared to aducanumab (10 mg/kg). Both TfR-aducanumab and aducanumab reduced plaque load in a discrete set of brain regions. **Conclusions:** This study supports the applicability of a TfR-based shuttle to improve CNS delivery and efficacy of therapeutic antibodies in AD.

Disclosures: M. Ramos Vega: A. Employment/Salary (full or part-time):; Gubra. **S.B. Vergo:** A. Employment/Salary (full or part-time):; Lundbeck. **Y. Gallero-Salas:** A. Employment/Salary (full or part-time):; Gubra. **E. Alexiou:** A. Employment/Salary (full or part-time):; Gubra. **F.**

Wichern: A. Employment/Salary (full or part-time):; Lundbeck. **J. Hecksher-Sørensen:** A. Employment/Salary (full or part-time):; Gubra. **H. Hansen:** A. Employment/Salary (full or part-time):; Gubra.

Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.02/B133

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Ministry of Health & Welfare (HI22C0467)
Ministry of Culture, Sports, and Tourism (R2022020030)

Title: Therapeutic Impacts of Transcranial Direct Current Stimulation on Sleep and Amyloidopathy in a Mouse Model of Alzheimer's Disease

Authors: *S. YU, M. ZAHEER, M. PARK, T. KIM;
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Abstract: Background: Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique that modulates neuronal activity through low-intensity electrical currents. Alzheimer's disease (AD) is a brain disorder associated with a decline in overall cognitive functions, such as memory and attention. Sleep disturbances, commonly observed in AD, are bi-directionally associated with the disease severity. Recently, reactive astrocytosis in response to amyloidopathy in the brain has been suggested as a pathogenic mechanism for memory dysfunction and sleep disturbances. In this context, the therapeutic potential of tDCS in reducing amyloid-beta and improving cognitive functions has been increasingly reported. However, the specific mechanisms of tDCS in AD remain unclear. Here, we hypothesized that tDCS could restore sleep disturbances by reducing reactive astrocytosis, and enhance amyloid-beta clearance via activated glymphatic system. **Methods:** EEG/EMG surgery, along with the installation of tDCS electrodes, was performed on all experimental mice. EEG electrodes were placed on the frontal and parietal areas, while tDCS electrodes were placed on the contralateral frontal and cerebellum areas. Twenty-minute tDCS with 200 μA was administered for two weeks. Electroencephalography was recorded to quantify sleep and 40-Hz auditory steady-state response (ASSR) before and after the tDCS treatment. Amyloid beta and astrocytic GABA were assessed by immunohistochemistry (IHC). **Results:** Our preliminary data showed that two-week tDCS increased NREM and REM sleep duration in 5xFAD mice. These therapeutic effects were not observed in the tDCS sham group. In addition, the count and area of amyloid plaques in both the hippocampus and cortex were reduced in the tDCS group compared to those of the sham group. Furthermore, in the tDCS stimulation group, there was an increase in 40-Hz ASSR-induced gamma power compared to the baseline. Quantification of astrocytic GABA is pending.

Conclusion: These findings suggest that tDCS may alleviate sleep disturbances in AD, contributing to the enhanced amyloid-beta clearance, potentially via the glymphatic pathway. Further investigation of the beneficial changes in reactive astrocytosis and the underlying molecular mechanism is warranted.

Disclosures: S. Yu: None. **M. Zaheer:** None. **M. Park:** None. **T. Kim:** None.

Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.03/B134

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Machine learning based analysis pipeline to assess spatial memory deficits in Tg2576 mice

Authors: T. BRAGGE[1], T. HEIKKINEN[1], J. ROKKA[1], P. POUTIAINEN[2], *J. RYTKÖNEN[3], **S. BÄCK**[4];
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Abstract: Alzheimer's disease (AD) is a devastating neurodegenerative disease with hallmark symptoms on memory loss and protein plaques in the brain. Preclinical research with behavioral assays remains the most common readout for cognitive assessment of animals. However, memory testing can be time consuming and prone for observer bias. Novel machine learning approaches to image and video analysis tools have opened new possibilities to the analysis of behavioral data analysis with its objective detection, overall speed, and improved quality of data. DeepLabCut (DLC) is a software package for markerless 2D and 3D pose estimation based on transfer learning with deep neural networks (Lauer et al. 2022, Nat Methods 19, 496-504). In this study we evaluated the spatial memory and learning (Barnes maze) of tg2576 mouse line, using video recording and automated analysis pipeline. Furthermore, the amyloid beta burden in the brain of mice was evaluated with PET imaging.
At the age of 18-19 months, 15 transgenic B6;SJL-Tg(APPSWE)2576Kha, Taconic model #1349 (Tg2576) and 8 wild type (WT) mice underwent Barnes maze test. It is a two-phased test in which spatial learning and memory are assessed in the first phase (acquisition phase), while the second phase (reversal learning phase) is for the evaluation of cognitive flexibility. Automated analysis pipeline was implemented for Barnes maze after DLC based tracking of video recordings of animal movement on the testing arena. A cohort of the mice (7 tg2576, 5 WT) were imaged with small animal PET scanner (BioPET, Sedecal) for amyloid burden using [^{18}F]-Flutemetamol. The animals were dosed intravenously with 10-12 MBq of [^{18}F]-

Flutemetamol and a 15 min static PET scan was taken 30 minutes post dosing and the radioactivity concentration was analyzed in different brain regions after co-registering the PET image to mouse brain atlas (PMOD v3.7).
A genotype difference was observed in Barnes maze. The Tg2576 mice took more time to learn the location of escape box compared to WT counterparts. Interestingly PET imaging did not show significant differences in different brain regions in the tracer uptake between Tg2576 and WT mice (multiple unpaired t-test, $p<0.05$).
As a summary, we have established novel analysis pipeline for analysis of Barnes maze behavioral data. Machine learning does not just streamline and enhance the repeatability of scientific studies, but it also allows us to scale up the amount of data that we can process in an efficient manner.

Disclosures: T. Bragge: A. Employment/Salary (full or part-time):; Charles River Discovery. **T. Heikkinen:** A. Employment/Salary (full or part-time):; Charles River Discovery. **J. Rokka:** A. Employment/Salary (full or part-time):; Charles River Discovery. **P. Poutiainen:** None. **J. Rytkönen:** A. Employment/Salary (full or part-time):; Charles River Discovery. **S. Bäck:** A. Employment/Salary (full or part-time):; Charles River Discovery.

Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.04/B135

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH U24 MH133236
NIH R01 AG082127

Title: Endothelial Cell Targeting Enhancer-AAV Vectors

Authors: *E. VELAZQUEZ[1], O. DEY[1], W. CAO[1], H. ZHANG[3], Q. YE[2], T. C. HOLMES[4], B. REN[5], X. XU[6];
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Abstract: Endothelial cells in the brain are critical structural components necessary for proper functioning of the blood-brain barrier (BBB). Vascular abnormalities occur very early before the onset of pathological symptoms in common neurological disorders including Alzheimer's disease (AD). Thus endothelial cells are likely potential targets for treatment for neurodegenerative diseases early in the course of disease progression. Key tools for such novel therapeutic approaches required the means to genetically target brain endothelial cells. However,

most viral vectors that are designed for delivering genetic payloads to the brain do not effectively target endothelial cells. We have developed a set of new enhancer AAV vectors that specifically target brain endothelial cells; the vector design utilized ATAC-seq data to identify potential endothelial-cell (EC) enhancer sequences, and was packaged with the AAV-PhP.eB capsid, which has been shown to facilitate viral entry across the BBB. Extensive ex vivo and in vivo characterization of EC-enhancer AAVs expressing fluorescent markers or / and Cre in wild type, Ai9 reporter and AD mouse brains show that these vectors allow for high transduction selectivity of the endothelial cells. This suggests that AAV vectors that target endothelial cells can be used in the future for gene therapy for AD or other neurological disorders.

Disclosures: E. Velazquez: None. **O. Dey:** None. **W. Cao:** None. **H. Zhang:** None. **Q. Ye:** None. **T.C. Holmes:** None. **B. Ren:** None. **X. Xu:** None.

Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.05/B136

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Combination studies between sigma-1 receptor agonist and TSPO ligand in a pharmacological mouse model of Alzheimer's disease

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Abstract: Alzheimer's Disease (AD) is the most common cause of dementia in the elderly population, estimated to represent 60%-80% of dementia cases. The pathology is characterized by the extracellular accumulation of aggregating amyloid-β (Aβ) proteins forming senile plaques, intracellular neurofibrillary tangles composed of abnormally phosphorylated tau protein, and a massive neuroinflammation. In AD patients, oxidative damage occurs before the detection of Aβ accumulation, suggesting an early role of oxidative stress in the disease. TSPO (Translocator Protein) is involved in Ca2+ signaling cascades, steroidogenesis, and cell survival and death, thereby helping maintain homeostasis in the cell. TSPO is activated by high Ca2+ and ROS generation that occurs an increased rate in AD patients. Interestingly, anti-inflammatory and neuroprotective effects of TSPO ligands were observed in several clinical studies such as anxiety, depression, traumatic brain injury and Alzheimer's disease. The sigma-1 receptor (S1R) is a membrane-associated protein expressed in neurons and glia, highly enriched in mitochondria-associated endoplasmic reticulum (ER) membranes (MAMs). S1R interacts with different partners to regulate cellular responses, including ER stress, mitochondrial physiology and Ca2+ fluxes. S1R can be activated/inactivated by small molecules, and accumulating preclinical data suggest that S1R agonists are protectants in neurodegenerative diseases.

In this study, the potentialities of S1R drug-based combinations with Etifoxin (EFX, TSPO agonist) was analysed in a pharmacological mouse model of AD (Aβ25-35 intracerebroventricular injection). Aβ25-35-treated mice were administered with a S1R agonist (PRE-084) and/or TSPO agonist (EFX), 20 min before Aβ25-35. Mice were then tested for spatial short-term memory on day 8 and non-spatial long-term memory on days 9-10, using the spontaneous alternation or passive avoidance tests, respectively.
The efficacy of combinations using maximal non-active or minimal active doses of S1R agonist or TSPO agonist was analyzed using calculations of the combination index, based on simple isobologram representation. Data showed that most of the TSPO agonist-based combinations led to synergistic protection against Aβ25-35-induced learning deficits, for both long- and short-term memory responses. These study showed that drug combinations based on TSPO agonist and S1R agonist may lead to highly effective and synergistic protection in AD.

Disclosures: L. Crouzier: None. **T. Moujellil--Legagneur:** None. **T. Maurice:** None.

Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.06/B137

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Kine-pd-28, a new cytokine-derived peptide, improves synaptic dysfunction and behavioral impairment in mouse models of alzheimer's disease.

Authors: *H.-L. CHA[1], J. KANG[2], H. LEE[2], I. RYU[2], S. PARK[2], C. GU[2];
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Abstract: Dementia is one of disastrous neurodegenerative diseases. Especially, Alzheimer's disease (AD) is the most common disease that is characterized by the pathological deposition of abnormal amyloid beta (Aß) peptide or hyperphosphorylated tau. Despite the piling studies on AD for approximately 1 century since the diagnosis criteria were established, AD-targeted drug development remains still in requirement because of the unelucidated etiology from molecular mechanism to functional neural dysfunction such as severe memory loss and cognitive impairment. KINE-PD-28, a nonameric peptide, is derived from cytokine. Here, we suggest KINE-PD-28 as the new candidate drug that not only could reduce the pathological accumulation of Aß through but also could improve synaptic dysfunction and behavioral impairment. As the candidate drug to ameliorate cognitive symptom, we examined a set of behavioral tests using 5xFAD mutant mice that were injected subcutaneously with KINE-PD-28. We obtained improved behavioral results from the Elevated Plus Maze test, modified Y Maze test, and object recognition tests including the spatial context information. We also found the recovered LTP

amplitude of the drug-injected group through *in-vivo* LTP experiments significantly. As the putative drug for disease-modifying therapy (DMT), we also found a reduction of Aß when KINE-PD-28 was injected intravenously with 5xFAD at 4-month-old age for 4 weeks, 8 times in total. Additionally, we also found that microglia as a putative mechanistic target of KINE-PD-28 through *in vitro* assay using microglia cell line, BV-2 cells. KINE-PD-28 showed an anti-inflammatory effect in the LPS-induced inflammatory condition. Furthermore, the candidate drug also affects the phagocytotic activity of microglial cells to clear the treated Aß *in vitro*. Altogether, we suggest that KINE-PD-28 could be the potential First-in-Class drug as the DMT as well as symptom-reducing therapy, which is urgently required to satisfy the unmet needs of the current clinical field.

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Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.07/B138

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: PID2022-139016OA-I00
2021 LLavor 0086
2023 Producte 0092
2023 Llavor 005
2021FISDU 00182

Title: Discovery and Design of Novel Small-Molecule inhibitor of G9a for CNS conditions

Authors: *A. BELLVER SANCHIS[1], A. IRISARRI[2], M. RIBALTA[2], B. PÉREZ[3], A. MARTÍNEZ[4], J. BREA[4], S. VÁZQUEZ[5], C. ESCOLANO[5], C. G. FERRE[6];
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Abstract: Alzheimer's disease (AD) poses a significant global health crisis, affecting millions worldwide with no effective treatments available. Therefore, it is important to develop therapies to alleviate or delay AD symptoms and improve patients' quality of life. While its etiology remains elusive, epigenetic dysregulation, particularly involving G9a-mediated histone methylation, has emerged as a potential therapeutic target. G9a is responsible for methylating H3K9, which leads to transcription repression, and evidence suggests that it is one of the crucial epigenetic alterations involved in learning and memory formation. This study explores the

development of selective G9a inhibitors for AD treatment. Utilizing structure-based virtual screening, a novel compound (LB-15) was identified, exhibiting potent G9a inhibition *in vitro* and ameliorating cognitive impairment and amyloid-beta (Aβ) aggregation in a *C. elegans* AD model. Supported by competitive grants, a comprehensive optimization effort synthesized over 100 derivatives of LB-15, culminating in its selection as the lead compound, being the most selective against G9a. Moreover, other interesting characteristics are that they do not inhibit GLP (another histone/lysine methyltransferase) and exhibit a high BBB permeability. *In vivo* studies using the SAMP8 mouse model demonstrated promising results, validating LB-15's potential for AD treatment. Interestingly, LB-15 treatment in SAMP8 mice rescued cognitive decline and was measured via NORT and OLT. In addition, the density of dendritic spines and the length of dendritic branches were evaluated, showing an increase in the treated group. Of note, treatment with LB-15 reduced H3K9me2 levels in SAMP8 mice. We also obtained the PK/TK and DMPK profiles, hERG safety, and cytotoxicity. This research highlights the therapeutic potential of G9a inhibition (LB-15) and the journey toward developing effective AD therapeutics.

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Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.08/B139

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: TCU SERC Grant

Title: Comparing the effects of macronutrient-matched typical American andMediterranean diets on Alzheimer's-related markers including metabolic dysfunction

Authors: *M. BERTRAND[1], L. P. GUNDERSON[2], G. W. BOEHM[2], M. J. CHUMLEY[1]; [1]Biol., Texas Christian Univ., Fort Worth, TX; [2]Psychology, Texas Christian Univ., Fort Worth, TX

Abstract: Metabolic disorders, including obesity, insulin resistance, and dyslipidemia, are risk factors for late-onset Alzheimer's disease (AD). Further, consumption of a poor diet, such as a typical American diet (TAD) composed of saturated fats and simple carbohydrates, promotes the development of both metabolic disorders and AD. In contrast, adherence to a plant-based Mediterranean diet (MD) rich in unsaturated fats has been found to reduce disease risk. Previous animal research has explored the relationship between nutrition and AD risk. However, most studies investigate extremely high-fat diets, consisting of 40-60% kcal from fat, which do not represent the composition of a human TAD or MD (typically 35% kcal fat). Additionally, prior studies often investigate individual nutritional components rather than incorporating a comprehensive diet model. To address these limitations, our lab developed two comprehensive, macronutrient-matched TAD and MD that more closely mimic human diets in the U.S. and Mediterranean, respectively. Previous studies in our lab found that six months of TAD consumption resulted in cognitive deficits, increased inflammation, elevated soluble amyloid beta levels, and excess hepatic lipid deposition, in comparison to the MD. Our current study looked to further characterize metabolic diseases under this diet model, specifically investigating obesity, insulin resistance, and dyslipidemia given their association with increased AD risk. Male and female C57BL/6J mice consumed either the TAD or MD from the age of 4 to 7 months. We conducted behavioral tests, including open field, elevated zero, and object-location memory task, to assess spatial memory and anxiety-like behavior. Insulin resistance was measured using the voluntary oral glucose tolerance test and insulin tolerance test. After three months on diet, we analyzed serum concentrations of cholesterol and triglycerides, inflammatory cytokines, and metabolic hormones. We also collected cortical brain tissue to quantify the levels of soluble amyloid beta and conducted a histological analysis of the liver and epididymal white adipose tissue. This study provides a direct comparison between a comprehensive TAD and MD, specifically relating to AD and metabolic disease risk.

Disclosures: M. Bertrand: None. **L.P. Gunderson:** None. **G.W. Boehm:** None. **M.J. Chumley:** None.

Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Jeanna Kempner Fellowship
5T32AG067952-03
The Don and Nancy Mafrige Professor in Neurodegenerative Disease Endowment
Mitchell Center for Neurodegenerative Diseases

Title: Spatiotemporal assessment of glutamatergic synaptic neurotransmission in 3xTg-AD following repeated low dose small molecule attenuation of a key lipolytic enzyme

Authors: *J. CURRIE[1], M. MALLIPUDI[2], S. BUDHWANI[1], C. NATARAJAN[3], S. SREENIVASA MURTHY[3], K. GARZA[1], B. KRISHNAN[4];
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Abstract: Study of synaptic integrity using conventional electrophysiology is a gold standard for quantitative assessment of neurodegeneration. Fluorescence assisted single synapse long-term potentiation (FASS-LTP) offers a high throughput method to assess the synaptic integrity of neurotransmission in and among brain regions as a measure of pharmacological efficacy in translational models. We report an optimized version of FASS-LTP by adapting the activation step to improve synaptosomal response despite the quality of frozen tissues and or postmortem interval delays. We utilized FASS-LTP on the frontal cortex (FC), parietal cortex (PC), midbrain (MB), and cerebellum (CB) of 6- and 12-month old 3xTg-AD mice. We injected sibling cohorts of 2-3 mice with VU0155069 (VU01, a small molecule inhibitor of phospholipase D isoform 1, 1mg/kg, intraperitoneally) or vehicle (0.9% saline) for 15 weeks, every two days, and repeated FASS-LTP assays in triplicate. We used flow cytometry to evaluate pre- and postsynaptic colocalization of fluorescence labeled antibodies. Western blots were run to corroborate colocalization of antibodies. Previously our group described repeated VU01 dose to prevent progression of hippocampal synaptic dysfunction by promoting resilience to dendritic spine dystrophy. Our results indicate a varied response to VU01 and glutamatergic rescue depending on the brain region and progression of neurodegenerative state. In the 6-month FC and PC, we see a 231% and 144% increase, respectively, in response to VU01 treatment when compared to saline treated groups. In the 12-month FC and PC, we see a decline in transmission as shown by the 92% increase and 53% decrease, respectively. The 6-month MB and CB demonstrate a 46% decrease and 11% increase, respectively, to VU01 treatment when compared to saline treated groups. In the 12-month cohort, the MB response increases to -7% and the CB response increases to 14%. Our adapted FASS-LTP method offers a robust analysis of synaptosomes isolated from frozen tissue samples, expressing greater sensitivity. This accessible approach proves valuable for analyzing animal and human synaptic models in physiological and disease states. Further, FASS-LTP can be used to explore processes related to GABAergic, serotonergic, and other neurotransmitters to better understand neurotransmission in various neurological disorders. The mixed response to VU01 suggests the current dosage, length used, and method of administration may have varying efficacy in each age group. Future considerations may involve adjusting the dosage of VU01, altering the duration of exposure to the inhibitor, and changing the route of administration.

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Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.10/B141

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R44AG071040

Title: Normalization of suppressed protein phosphatase 2A (PP2A) activity as a therapeutic approach to Alzheimer's Disease

Authors: *M. OHLMEYER[1], **R. NICHOLLS**[2], O. ARANCIO[3];
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Abstract: SFN Abstract
Normalization of suppressed protein phosphatase 2A (PP2A) activity as a therapeutic approach to Alzheimer's Disease
Russell Nicholls[1], H. Zhang[1], O. Arancio[1], Johanna Ohlmeyer[2], Michael Ohlmeyer[2*]
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Disclosure: **OTTAVIO ARANCIO:** Founding Member of Neurokine Therapeutics**; HONG ZHANG:** NoneRussell Nicholls: Paid consultant to Atux Iskay.
Multiple lines of evidence suggest a role for PP2A in Alzheimer's Disease (AD). And, consistent with this role, genetic and pharmacological manipulation of PP2A activity has been found to reduce AD-related pathology and impairments in cell and animal models. Thus, small molecule activation of PP2A represents a potential new therapeutic modality for AD treatment. Through a combination of medicinal-chemistry techniques and electrophysiology, we investigated the potential beneficial effect of novel small molecule PP2A activating compounds by examining their ability to protect against impairments in long-term potentiation (LTP) (a type of synaptic plasticity thought to underlie memory formation) caused by exposure to soluble oligomers of amyloid-beta (Aβ) and tau. Consistent with our previously published data showing that prototype versions of these compounds reduced AD-related impairments in a hyperhomocysteinemic rat model of AD, we found that these next generation compounds also protected against AD-related impairments in *ex-vivo* murine hippocampal slice models of LTP. Next generation PP2A activating comounds, ATUX-6156 and ATUX-5800, are currently under development by Atux Iskay as novel therapeutics for AD, and potentially other PP2A linked neurodegenerative

conditions such as Parkinson's Disease and Frontotemporal Dementia.ATUX-6156 protects against tau-induced LTP impairments. The magnitude of LTP in the tau + ATUX-6156 groups decreases with decreasing ATUX-6156 concentration consistent with a linear dose-response relationship over 0.1 - 0.0001 micromolar ATUX-6156 for neuroprotective effects.

Disclosures: M. Ohlmeyer: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Atux Iskay Group LLC. **R. Nicholls:** None. **O. Arancio:** None.

Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.11/Web Only

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Jeanne B. Kempner Scholarship
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The Don and Nancy Mafrige Professor in Neurodegenerative Disease Endowment
Mitchell Center for Neurodegenerative Diseases

Title: Co-localization of pre-synaptic PSD95 and post-synaptic Nrx1bproteins with Phospholipase D1 in 3xTg-AD 12-month aged mice: A study of its association in neurodegeneration.

Authors: *S. MOHANTY[1], S. BUDHWANI[2], S. SREENIVASA MURTHY[3], B. KRISHNAN[4], H. ONUORAH[1];
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Abstract: Alzheimer's disease (AD) is the 5th leading cause of death in the United States. Synaptic dysfunction occurs earlier in AD patients and leads to progressive cognitive decline. Current therapeutics for AD offer some symptomatic relief while also having off-target effects. Therefore, there is a critical need to understand the mechanism for pathogenesis of AD for the development of novel therapeutic targets. Our lab has found an anomalistic increase in phospholipase D isoform 1 (PLD1), that breakdown phospholipids in AD postmortem brain samples, compared to control subjects, signifying its role in neurodegeneration. Previously, we have identified the PLD1 inhibitor (VU0155069), to show improvements in synaptic deficits, learning and memory tasks in an age-dependent manner in 3xTg-AD mice compared to saline

treatment. Previous studies have found PLD1 plays a mechanistic role in cytoskeleton modulation through cofilin phosphorylation, resulting in higher levels of G-actin and eventual disruption of the cytoskeleton and synapse. **In this study**, we performed pre-synaptic marker PSD95 and post-synaptic marker Nrx1β levels using immunofluorescence (IF) and imaging after administration of VU0155069 inhibitor in the CA1, CA3 and DG region of the hippocampus, to test the hypothesis preventing aberrantly elevated PLD1 is sufficient to prevent progression of cognitive decline in neurodegenerative states by promoting synaptic resilience. PLD1 attenuation was achieved in 3xTg-AD model, by repeated treatment with low dose of PLD1 inhibitor (VU0155069), in 6 -and 12-month aged mice. Aged-matched siblings or control 3xTg-AD mice were treated with 0.9% saline. Brains were extracted, and 350 μm sagittal sections were obtained and subjected to IF with primary antibodies (PLD1, PSD95 and Nrx1β) and secondary antibodies (Goat anti-rabbit and Goat anti-mouse). Imaging was conducted using sectioning on the Keyence BZ-X800 fluorescent microscope. Five images for CA1, CA3 and DG regions of the hippocampus were taken for each tissue slice for a minimum of three animals. Images were analyzed using ImageJ where PLD1, PSD95, Nrx1β and integrated densities were obtained. We observed a reduced co-localization of Nrx1β with PLD1 in the inhibitor treated group compared to the saline treated. Additionally, there was reduced co-localization of PSD95 with PLD1 after repeated treatment with VU01 inhibitor. Our observations of 6- and 12-months validate our published observations of preserved dendritic spines and prove that reduced co-localization of PLD1 with the pre-synaptic and post-synaptic markers may be a key event in contributing to the synaptic resilience observed.

Disclosures: S. Mohanty: None. **S. Budhwani:** None. **S. Sreenivasa murthy:** None. **B. Krishnan:** None. **H. Onuorah:** None.

Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.12/B142

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Neuroscience Program, the Office of Research and Graduate Studies, College of Medicine, the John G. Kulhavi Professorship in Neuroscience, and the E. Malcolm Field and Gary Leo Dunbar Chair in Neuroscience at Central Michigan University.

Title: Effects of Liraglutide on STZ-Treated Aged C57 Mice

Authors: *G. TAVI[1,2,3], D. DOYLE[10,2,3,4], M. M. KINNEY[5,2,3], D. STORY[6,2,3], B. SRINAGESHWAR[7,2,3,4], J. ROSSIGNOL[8,2,3], G. L. DUNBAR[11,2,3,9];
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Abstract: Recent studies have shown that the anti-diabetic drug, liraglutide (LIR), can reduce age-related deficits in rodent models of Alzheimer's disease (AD) and age-related cognitive decline. The aim of the present study was to see if LIR treatments could reduce deficits in aged C57 mice that were given intracerebroventricular injections of the potent toxin, streptozotocin (STZ), which is used to model sporadic AD. STZ is a toxin derived from the bacteria *Streptomyces achromogenes* that can produce vascular dysfunction within mammalian models, likely through inducing endothelial dysfunction. In our study, aged (17-19-month-old) C57 mice received either bilateral intracerebroventricular injections of STZ or citrate buffer solution (CBS) vehicle and were subsequently treated with either LIR or Hank's buffered saline solution, intraperitoneally, once a day for 36 days. Mice were evaluated on three behavioral tests: passive avoidance, open-field, and novel object recognition tasks. Following behavior assessments, various organs, including the brain, pancreas, heart, spleen, liver, and kidneys were extracted, weighed, and processed for Western blot analyses to assess inflammatory markers, including TNF-alpha, SOD-1, and IL-6. Our findings suggest that LIR had only modest effects on the behavioral performance of the mice and this correlated with the changes observed in the biochemical profiles of the mice. These results suggest that the modest effects of LIR in the aged and STZ-treated C57 mice indicate that earlier interventions with LIR treatment might be needed in order to optimize its therapeutic effects.

Disclosures: G. Tavi: None. **D. Doyle:** None. **M.M. Kinney:** None. **D. Story:** None. **B. Srinageshwar:** None. **J. Rossignol:** None. **G.L. Dunbar:** None.

Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.13/C1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01 AG061111
NIH Grant R01 AG057187
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Title: Nicorandil: A potential therapeutic for Granulin/GRN-deficiency related disorders (FTLD and LATE).

Authors: *D. NIEDOWICZ[1], P. PRAJAPATI[2], W.-X. WANG[2], Y. ZHONG[3], S. FISTER[3], C. B. ROGERS[4], K. E. SAATMAN[5], P. T. NELSON[2];
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Abstract: Age-related neurodegenerative diseases constitute an enormous public health challenge. With the partial success of Abeta immunotherapies, increased attention is focusing on common non-Alzheimer's pathologies that contribute to the dementia clinical syndrome. Repurposing of commercially-available drugs provides one potential avenue for the rapid approval of therapeutics (and recruitment of drugs that are relatively safe given extensive clinical experience). Here, we investigated nicorandil- a K^+ATP channel agonist, nitric oxide donor, and vasodilator that is commonly used in Europe and Asia to treat congestive heart failure and angina in the elderly. The K^+ATP channel is an important regulatory protein in brain cells, such as astrocytes, neurons, and pericytes, providing a "metabolic sensor" to couple energy needs with excitatory states. In particular, we are investigating a role for nicorandil in the treatment of TDP-43-associated dementias, such as frontotemporal dementia (FTD) and limbic-predominant age-related TDP-43 encephalopathy (LATE). Both FTD (and its underlying pathology of frontotemporal lobar degeneration [FTLD]) and LATE have been linked with variation in the human *GRN* (Granulin) gene. LATE is the more common of these conditions, affecting more than $1/4^{th}$ of persons beyond age 80. In the present study, we used a mouse model - the *Grn* knockout (*Grn*-KO) mouse (Yin et al, 2010)- which has been widely used to model FTD. Middle-aged *Grn*-KO mice were treated with nicorandil (15 mg/kg/day in drinking water) and gene expression, cognition, and neuropathology were subsequently compared with untreated controls. Consistent with published reports, *Grn*-KO mice displayed markedly reduced survival, increased astrocytosis, and impaired cognition in multiple domains, in comparison to age-matched wild-type mice. *Grn*-KO mice treated with nicorandil had substantially reduced mortality, particularly among the females. In addition, nicorandil treatment decreased expression of pro-inflammatory genes, such as RelA and RelB and improved Morris Water Maze performance. Our work provides a preliminary indication that nicorandil has the potential to be an effective therapeutic for neurodegenerative diseases associated with *GRN* genetic deficiency.

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Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.14/C2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01-MH134119

Title: Allosteric agonism of B_2AR by amyloid beta leads to Alzheimer's Disease-related phenotypes via GRK-mediated signaling cascades

Authors: *J. DE CHABOT, Y. XIANG;
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Abstract: Alzheimer's Disease (AD) is the most common form of dementia with hallmarks, including accumulation of amyloid beta (Aß) peptide, intracellular neurofibrillary tangles, and chronic neuroinflammation. Aß antibodies have been approved by the FDA for treatment of AD, yet with limited success in a subset of patients and some adverse side-effects. Aß oligomers are also present in the healthy brain and can act on a variety of signaling pathways. Some research has suggested that Aß plays an important negative feedback role in synaptic homeostasis and function. This could explain why therapies aimed at removing it completely have resulted in toxic side-effects. Targeting specific downstream effectors of Aß could more specifically block disruptive cellular pathology while avoiding the negative consequences of total elimination of the peptide. The adrenergic system has been investigated in the context of AD since adrenergic receptors and Locus Coeruleus neurons are the first to show degeneration in the disease. Norepinephrine is a known neuromodulator of learning and memory. Specifically, Beta-2 adrenergic receptors (B2AR), which are widely expressed in the brain, have been attributed a role in learning, memory formation and consolidation. For instance, recently published research from our laboratory revealed that G-protein receptor kinase (GRK) phosphorylation followed by internalization of B2AR in neurons can lead to removal of a specific phosphodiesterase isoform (PDE4D5) from the nucleus, consequently increasing gene expression for memory formation and consolidation.Intriguingly, B2AR agonism has been shown to increase Aß production and accumulation in the brain via internalization of Amyloid Precursor Protein (APP) and gamma secretase. In addition, previous research from our laboratory found that Aß can directly bind and agonize B2AR at an allosteric site on the receptor. Similarly to catecholamines, agonism of B2AR by Aß can lead to phosphorylation and internalization of the receptor. However, how these two ligands can lead to distinct signaling pathways remains unclear. We hypothesize that Aß binding to B2AR can disrupt physiological nuclear signaling mediated by catecholamines, thereby decreasing gene expression and impairing memory consolidation. We also hypothesize that Aß agonism of B2AR leads to increased processing of APP and production of Aß, creating a positive feedback loop where Aß leads to more Aß production. Preliminary data suggest a GRK-phosphorylated-B2AR mediated increase in Aß load and neuroinflammatory markers in the brain of adult mice.

Disclosures: J. de Chabot: None. **Y. Xiang:** None.

Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.15/C3

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Theta and gamma fornix-deep brain stimulation (fx-DBS) improves recognition and spatial memory driving behavioral changes in a 5xFAD model of Alzheimer's Disease (AD)

Authors: *N. C. ZEPEDA[1], M. BECERRA[2], S. MEDVIDOVIC[1], M. BERGOSH[4], W. CHOI[5], Z. SMITH[2], T. WEI[2], E. AMJADI[6], M. DAYAN[5], E. WATKINS[6], E. SONG[1], M. PEREZ[7], I. CHEN[2], C. GARNER[2], K. LIU[5], M. S. BIENKOWSKI[3], D. J. LEE[1];
[1]Neurolog. Surgery, [2]USC, Los Angeles, CA; [3]USC, Los Angeles, CA, ; [4]Neurolog. Surgery, USC Keck Sch. of Med., Los Angeles, CA; [5]; [6]Neurolog. Surgery, [7]KSOM Univ. of Southern California, Los Angeles, CA

Abstract: Alzheimer’s Disease (AD) is characterized by amyloid beta (AB) protein deposition, phosphorylated tau protein tangles, neuronal and synaptic loss, neurotransmission deficits, and gross cerebral atrophy.1 This neurodegenerative disease is associated with decreased functional connectivity and associated memory and cognitive impairment.1,2,3 As such, neuromodulation is currently being pursued as a treatment modality for such circuitopathy. Specifically, the fornix has been postulated as a target for deep brain stimulation (fx-DBS), as it is essential in regulating memory.4

Investigational fx-DBS treatment for patients with mild AD have resulted in promising, but mixed cognitive results.1,4 Standard DBS uses high frequency (gamma: 130-180 Hz) stimulation; however, there is significant evidence that low frequency (theta: 5-12 Hz) oscillations are important for mechanisms of learning and memory. Here, we evaluate the potential benefits of theta vs. gamma frequency fx-DBS on recognition and spatial memory in a 5xFAD mouse model of AD.

5xFAD/wild-type (WT) male mice were assessed using Novel Object Recognition (NOR), Elevated Plus Maze (EPM) and Barnes Maze (BM) cognitive tasks in three different age groups (16-18wks, 38-40wks, and 53-55wks) under either theta, gamma or no stimulation compared to WT. fx-DBS groups received treatment for 10 days at 8hrs/day.

For NOR, there was no difference (p=0.1978) between WT and 5xFAD (no stim/theta/gamma) groups at the 16-18wk timepoint. In this model, cognitive deficits begin to develop at later time points. There was improvement in both theta and gamma fx-DBS treatment groups at 38-40wks (p=0.037) and 53-55wks (p=0.048). However, there was no difference between theta and gamma fx-DBS treatments at 38-40wks (p=0.980) or at 53-55wks (p=0.105).

For EPM, at the 16-18wk timepoint, the WT animals spent less time in the open arm compared to 5xFAD no stim (p=0.0389), theta (p=0.0076), or gamma (p=0.0053). However, there were no differences in 5xFAD no stimulation, theta or gamma groups compared to WT at 38-40wks (p=0.396) or 53-55wks (p=0.175).

There was no statistical significance between latencies across groups at 16-18wks for BM(p=0.3097). In contrast there were decreased latencies in the 5xFAD fx-DBS groups at 38-40wks (p=0.001) and 53-55wks (p=0.031) compared to 5xFAD no stimulation, suggesting a rescue in spatial memory.

Our 5xFAD model demonstrates cognitive deficits compared to WT controls. Both theta and gamma fx-DBS improve cognitive deficits in the older age groups of the 5xFAD model.

Disclosures: N.C. Zepeda: None. **S. Medvidovic:** None. **M. Bergosh:** None. **E. Amjadi:** None. **E. Watkins:** None. **E. Song:** None. **D.J. Lee:** None.

Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.16/C4

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Fad^{3t}: A Novel Transgenic Mouse Model for Accelerating Alzheimer's Disease Research and Drug Development

Authors: Z. LAI[1], S. SUN[1], Z. YU[1], M. XIE[1], D. JIA[1], J. WU[2], B. WILKINSON[2], ***R. FENG**[1]; [1]GemPharmatech, Nanjing, China; [2]GemPharmatech, San Diego, CA

Abstract: Alzheimer's disease (AD) presents a formidable challenge for researchers and drug developers. Traditional preclinical AD mouse models often exhibit protracted disease progression, which makes discovery and drug development time-consuming and costly. To address this challenge, we generated the FAD^{3T} (APP/PS1/Tau) transgenic mouse model, which encompasses three mutations that mirror pivotal genetic factors implicated in AD pathogenesis. Remarkably, this model exhibited phenotypic parallels to AD patients and revealed an early onset of a crucial pathogenic marker in FAD^{3T} mice. Elevated levels of phosphorylated Tau (Thr181) are detected at 1 month of age. Subsequent observations included Aβ deposition and elevated levels of Phospho-Tau (Thr217, Thr231) at 2 months, followed by elevated levels of Phospho-Tau (Ser396, Ser202, Thr205) at 4 months, all progressively intensifying with age. Notably, cognitive deficits resembling spatial learning and memory impairments manifested at earlier stages in these mice. Our findings underscore the valuable utility of the FAD^{3T} model in recapitulating the intricate etiology of AD. More importantly, in comparison to traditional AD mouse models, FAD^{3T} exhibited accelerated disease onset and progression, offering a rapid platform conducive to expediting drug testing cycles, particularly for therapies targeting disease progression. In conclusion, this innovative mouse model represents a transformative tool poised to enhance our comprehension of AD pathophysiology and facilitate the expedited development of therapeutic strategies.

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Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.17/C5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG071859-01A1

Title: RARE-LacZ mice as a model to study retinoic acid signaling at cellular resolution in Alzheimer's disease

Authors: *A. HINDLE[1], A. BAKER[1], S. SMITH[1], J. STRICKLAND[1], M. HERNANDEZ[1], J. MEDINA[2], N. TANNER[1], J. J. LAWRENCE[1];
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Abstract: The role of amyloid-beta and its upstream causes in Alzheimer's disease (AD) pathogenesis are unclear, prompting investigation into potential non-amyloidogenic pathways. We previously found evidence that the activity of retinoic acid (RA)—a metabolite of vitamin A (VA)—is impaired in human AD, and it has been reported that RA upregulates α-secretase, favoring the non-amyloidogenic pathway. We hypothesize that dietary VA levels affect hippocampal RA signaling and disease progression in AD mice. To study this, our goal was to cross AD mice with RARE-LacZ mice, a reporter model possessing multiple LacZ genes controlled by RA response elements (RAREs) on a CD1 background. We first crossed RARE-LacZ mice with C57BL/6J, -NJ, and CD1 mice (wildtype background strains of J20, hAβ-KI, and RARE-LacZ mice, respectively) to study learning on non-AD backgrounds. Water T maze (WTM) was used to examine simple discrimination (SD) and reversal learning at 4 months of age. Strain did not affect latency to platform, demonstrating equivalent and intact learning on all backgrounds (ns, repeated measures (RM) ANOVA). Hippocampal LacZ localized to a subset of mature doublecortin-negative, calbindin-positive dentate granule cells, extending into dendrites as well as axons (mossy fiber terminals) in CA3. We then crossed RARE-LacZ and J20 AD mice and weaned pups onto purified diets with standard (4 IU/g) or supplemented (20 IU/g) VA levels. $J20^{+/-}$ genotype significantly affected latency to platform during SD and reversal ($n=22$, $p<0.05$, RM ANOVA) at 4 months of age, although VA level did not significantly affect behavior. Immunostaining revealed no significant differences in dentate gyrus LacZ signal due to genotype or treatment, however LacZ signal was completely silenced in 2/5 WT and 3/6 $J20^{+/-}$ mice on control diet, and in 2/6 WT and 0/6 $J20^{+/-}$ on VA supplemented diet. Lastly, we weaned RARE-LacZxJ20$^{+/-}$ mice onto standard or VA-deficient (0.4 IU/g) diets. VA deficiency did not significantly affect aggregate WTM performance across all trials ($n=14$, ns, RM ANOVA), however we found a significant interaction between genotype and dietary VA level during reversal ($p<0.05$, RM ANOVA) and a significant difference between cohorts on latency to platform during the first reversal trial ($p<0.05$, one-way ANOVA). In conclusion, RARE-lacZxJ20 mice have useful characteristics for studies on RA signaling in AD, including strong

LacZ staining in RA-dependent granule cells as well as the emergence of behavioral effects due to genotype and dietary VA level by 4 months of age. Future studies on VA deficiency will clarify whether dietary VA levels impact LacZ expression level or incidence of silencing.

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Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.18/C6

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Environmental and social enrichment mitigate AD deficits in mouse models

Authors: *A. YOUNG, A. WILKINS, J. M. FLINN;
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Abstract: Lack of environmental and social enrichment negatively impacts cognition and behavior. Whether these deficits can be mitigated by enriched housing in mice remains an open question. We used (1) rTg4510 hTau and (2) non-carrier (-/-) strain mice (n=32) to investigate the potential therapeutic effect of enriched group housing. The cohort was divided into two conditions: (1) Block Party (with 1-3 members of the same sex) (n=16) and (2) standard housing (n=16). Behavioral tests including Morris Water Waze (MWM), Open Field ("OF"), Elevated Zero Maze (EZM), and biochemical analyses including western blot were run for all conditions. OF, and EZM were analyzed using a two-way ANOVA. MWM was analyzed using repeated measures ANOVA. A two-way ANOVA was used to analyze the western blot data. The OF test yielded a significant result for genotype and housing conditions ($p<.01$) for distance-traveled in center, time spent in center, time spent in the surround, number of bouts in center field, and number of bouts in surrounding field. Significant interaction results were found for MWM. A significant interaction effect was found between genotypes and housing on "latency to find the platform." Non-carrier (-/-) Block Party mice took less time to find the platform compared to non-carrier (-/-) standard housing mice. Meanwhile, non-carrier (-/-) Block Party mice took less time to find the platform compared to Alzheimer's Disease ("AD") Block Party mice. Western blots were conducted to analyze GFAP and BDNF expression. Significant differences were found between genotypes for GFAP expression ($p<.01$); AD mice had higher levels of GFAP. An interaction of BDNF expression was found in non-carrier (-/-) mice in the Block Party condition ($p=.03$). These Block Party non-carrier (-/-) mice expressed higher levels of BDNF, but Block Party had no effect in AD mice. These results indicate that environmental and social enrichment may mitigate neurological deficits, particularly increased anxiety, and memory degradation, caused by AD symptoms.

Disclosures: A. Young: None. **A. Wilkins:** None. **J.M. Flinn:** None.

Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.19/C7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: American Heart Association Grant 946666
NIH Grant 1R16NS134540-01]
NIH Grant 1R03AG081865-01

Title: Targeting the renin-angiotensin system for the treatment of cerebral amyloid angiopathy

Authors: N. NOTO[1], A. HERNANDEZ[2], Y. M. RESTREPO[2], C. VOGEL[2], J. A. BONETTI[3], S. MOEN[2], V. PULIDO-CORREA[4], R. C. SPETH[5], ***L. S. ROBISON**[6];
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Abstract: BACKGROUND: Cerebral amyloid angiopathy (CAA) is a cerebrovascular disease characterized by amyloid protein accumulation in the cerebral vasculature. CAA is highly comorbid with Alzheimer's disease, and on its own, is associated with an increased risk of stroke and vascular cognitive impairment and dementia (VCID). There is currently no treatment available for CAA. Epidemiological studies suggest that certain renin-angiotensin system (RAS)-targeting antihypertensive medications are associated with a decreased risk of dementia. This study assesses whether two FDA-approved RAS-targeting drugs: telmisartan [a moderately brain-penetrant angiotensin receptor blocker (ARB)], and lisinopril [a brain-penetrant angiotensin-converting enzyme (ACE) inhibitor], can be repurposed for the treatment of CAA. METHODS: At ~3 months of age, male and female Tg-SwDI mice began treatment with subpressor doses of either telmisartan (1 mg/kg/day) or lisinopril (15 mg/kg/day) dissolved in their drinking water or received plain drinking water only. Age- and sex-matched C57BL/6J mice receiving plain drinking water were used as wild-type controls. Mice had their blood pressure measured 2 and 4 months after the start of treatment. Following 4 months of treatment, mice underwent behavior testing prior to euthanasia at ~8 months of age. RESULTS: Voluntary oral consumption delivered doses similar to the target doses for both drugs. At the doses used, neither drug treatment significantly reduced blood pressure in Tg-SwDI mice. Our findings thus far suggest potential therapeutic benefits against CAA-induced cognitive-behavioral impairments in Tg-SwDI mice, which can vary based upon biological sex and drug class, with lisinopril

showing more promising results. CONCLUSIONS: Ongoing analyses and postmortem experiments are being completed to investigate the potential benefits of telmisartan and lisinopril to mitigate neuropathological and cognitive impairments in Tg-SwDI mice. If the findings support our hypothesis, these widely available RAS-targeting drugs could be repurposed to prevent and/or treat CAA, reducing the burden of neurodegenerative diseases that currently lack effective treatments.

Disclosures: N. Noto: None. **A. Hernandez:** None. **Y.M. Restrepo:** None. **C. Vogel:** None. **J.A. Bonetti:** None. **S. Moen:** None. **V. Pulido-Correa:** None. **R.C. Speth:** None. **L.S. Robison:** None.

Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.20/C8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG073826-01A1

Title: Tolerability of vitamin A and histone deacetylase inhibitors in Alzheimer's disease animal models

Authors: *S. SMITH[1], A. HINDLE[1], J. STRICKLAND[1], A. BAKER[1], I. N. GUZMAN[1], S. SINGH[2], C. BOSE[1], J. J. LAWRENCE[1];
[1]Pharmacol. and Neurosci., Texas Technol. Univ. Hlth. Sci. Ctr., Lubbock, TX; [2]Intrnl. Med., Texas Technol. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: In Alzheimer's disease (AD), histone deacetylase (HDAC)/acetylase balance is disrupted, suggesting impaired transcriptional control. Moreover, there is evidence that depletion of all-trans-retinoic acid (ATRA), a vitamin A (VA)-derived transcription factor, contributes to loss of transcription control. As an antioxidant, depletion of ATRA may also cause oxidative stress (OS), triggering Nrf2-mediated antioxidant defenses. Here, we investigated roles of VA, histone acetylation, Nrf2, and OS *in vitro*. Mouse HT22 cells were treated with the HDAC inhibitor vorinostat (VOR; 0-3.0 μM). Acetyl-histone H3 levels, relative to total H3 levels, were increased. HT22 cells were then pretreated with H_2O_2 followed by treatment with vorinostat and/or ATRA and analysis by MTT and lipid peroxidation assays. VOR and ATRA treatment caused no significant cytotoxicity up to 2.5 and 20 μM, respectively. H_2O_2 alone (25-50 μM) caused ~20-35% cell death ($p<0.0001$). ATRA (5 μM), in combination with VOR (0.5 μM), protected against H_2O_2-induced cytotoxicity. In *in vivo* studies, we set out to establish a tolerable dietary VOR dose that promoted HDAC inhibition in AD mouse brain. Humanized Aβ knock-in (hAβ-KI) AD mice were fed 0.18 or 0.36 mg VOR/gram purified diet for 2 weeks. Mice significantly increased in weight on the 0.18 mg/g diet but not on the 0.36 mg/g diet. Both VOR

diets increased brain acetyl-histone H3 levels and inhibited brain HDAC activity ($p< 0.05$). Interestingly, both doses of VOR significantly reduced H_2O_2 levels and increased the total antioxidant capacity ($p<0.05$). Also, mice fed the 0.18 mg/g VOR diet exhibited a significant increase in ATP level relative to controls ($p<0.05$). Mice were then fed the 0.18 mg/g diet over 2 months to test the tolerability of longer treatments. Mouse weights did not change significantly relative to starting weights, and weights also did not differ from mice on control diet. In conclusion, our *in vitro* studies demonstrate that VOR is an antioxidant at low doses. Moreover, we establish a tolerable and effective VOR diet *in vivo* for long term AD prevention studies. As a potential combinatorial strategy, ATRA and VOR may act synergistically as to reduce OS and HDAC activity, thereby counteracting AD progression.

Disclosures: S. Smith: None. **A. Hindle:** None. **J. Strickland:** None. **A. Baker:** None. **I.N. Guzman:** None. **S. singh:** None. **C. bose:** None. **J.J. Lawrence:** None.

Poster

PSTR389

Mouse Model Strategies for Alzheimer's Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.21/C9

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: A healthy brain needs vitamin-t

Authors: *F. MCGLONE;
Life Sci., Manchester Metropolitan Univ., Manchester, United Kingdom

Abstract: Chronic stress is the cause of many adverse changes in the brain and has also been found to be an antecedent of mood changes associated with cognitive impairment. Affiliative tactile interactions are known to buffer social mammals against neurobiological and behavioural effects of stress, and within human populations the recent COVID pandemic, where for the first time in human evolution affective touch interactions were significantly restricted, we saw the adverse consequences on people's stress levels and subsequent emotional state. But what's the mechanism? Here a case will be made for the role of a population of cutaneous low threshold thermo-mechanosensitive cfibres called c-low threshold mechanoreceptors (CLTM) as a neurobiological substrate responsible for regulating resilience to stress. CLTMs are velocity tuned, preferentially encoding gentle, dynamic touch and project to emotion processing brain areas. Due to the delayed nature of their input, they can't serve any useful discriminative role and are implicated in the affective and emotional aspects of touch. Children deprived of nurturing touch suffer life-long adverse neurodevelopmental consequences. The CLTM is now being understood to provide a singular vital protective function, from the nurturing touch of the mother to its absence is loneliness. And now we have evidence of their role in dementia from recent animal studies. A case will be made here for what we are describing, by analogy with the vital

role vitamins play in our physical health, for affective touch and their neuronal substrate the CLTM as ‘Vitamin-T’ to play a role in our emotional health.

Disclosures: F. McGlone: None.

Poster

PSTR389

Mouse Model Strategies for Alzheimer’s Disease III

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.22/C10

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Glial and microglial phenotypic changes after treatment with Lecanemab : vitro and vivo evidences

Authors: *N. CALLIZOT[1], A. HENRIQUES[2];
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Abstract: Lecanemab is a humanized monoclonal antibody (Ab) that recognizes protofibrils/oligomers and that prevents Aβ deposition. Lecanemab is the first US approved Ab for Alzheimer’s disease (AD). Its mode of action especially on inflammatory cells is still vague and needs to be deeply investigated. We have studied the long lasting Lecanemab’s effects in vitro in microglial cells and astrocytes after a chronic Aβ stress as well as its effects in aged mouse stereotaxically exposed to Aβ1-42 (as in vivo model of AD) . Primary rat cortical neurons, co-cultured with microglia and astroglial cells were used. After 11 days of culture, cells were injured with Aβ for 120 hours. Lecanemab was applied once one hour before Aβ injury or re-applied every 2 days. After fixation, microglia and astroglia phenotype were analyzed. For both cell types, pro and anti-inflammatory markers were assessed. Aged mice were stereotaxically infused with Aβ oligomeric fraction in CA1 hippocampal area. Animals were chronically treated with Lecanemab up to 4 weeks after the lesion. Cognition investigations and histological analysis on brains was performed, inflammation markers were investigated. Application of Aβ induced a proliferation of astrocytes and microglial cells. In addition, a significant increase in M1 microglia was observed (TREM2/OX-41 and Iba1) 72h after Aβ application followed with an increase of phagocytosis phenotype. A strong reduction of CD206 (M2 markers) was also detected. For astrocytes, a moderate astrogliosis was observed. Interestingly, Lecanemab treatment was able to increase number of S100A (+) cells (A2 population), associated with a moderate increase of GFAP area. In AD aged mice, cognition performances were improved and were associated with a significant reduction of brain amyloidic load in treated animals. Hippocampal section showed an increase of microglial activation (M1 and M2), and large astrogliosis (C3 positive cells). Lecanemab was able to mitagate Aβ1-42-induced toxicity and modulated the Aβ1-42-induced neuroinflammatory response. These

important and intriguing changes were observed both in vitro and in vivo. This study attempted to decipher the complex and unclear mode of action of Lecanemab

Disclosures: N. Callizot: A. Employment/Salary (full or part-time):; Neuro-Sys. **A. Henriques:** A. Employment/Salary (full or part-time):; Neuro-Sys.

Poster

PSTR390

Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.01/C11

Topic: C.03. Parkinson's Disease

Support: William N. and Bernice E. Bumpus Foundation Innovation Award (WBBF CU22-0241)

Title: Alpha-synuclein regulates the neuronal lipidome at the Mitochondria-Associated ER Membranes (MAM)

Authors: *P. A. BARBUTI[1], C. GUARDIA-LAGUARTA[1], Z. CHATILA[1], B. SANTOS[2], N. HATTORI[3], U. DETTMER[4], V. MENON[1], A. F. TEICH[5], R. KRÜGER[2], E. AREA-GOMEZ[1,6], S. E. PRZEDBORSKI[1];
[1]Columbia Univ., New York, NY; [2]Univ. of Luxembourg, Belval, Luxembourg; [3]Juntendo Univ. Sch. of Med., Bunkyo, Japan; [4]Harvard, Boston, MA; [5]Columbia Med. Ctr., New York, NY; [6]eCenter for Biol. Res. (CIB), - Margarita Salas, CSIC, Madrid, Spain

Abstract: In synucleinopathies such as Parkinson's disease (PD) and multiple system atrophy (MSA), alpha-synuclein (αSyn) plays a crucial role in the pathogenesis of disease. Furthermore, there is mounting evidence to suggest lipid dyshomeostasis is also associated with these diseases. In this study, we analyzed the lipid composition of post-mortem human samples, including the substantia nigra pars compacta (SNc) of PD donors and striatum of MSA donors, identifying lipid species to discriminate PD cases from MSA and control samples. Our analyses also found that the PD nigral samples displayed higher levels of phosphatidylcholine (PtdCho) and phosphatidylserine (PtdSer) species bound to long chain and unsaturated fatty acids. Since PD is associated with increased expression of αSyn via multiplications of the αSyn-encoding SNCA gene, we generated patient-derived, induced pluripotent stem cell (iPSC)-derived dopaminergic neurons carrying different dosages of αSyn from: no αSyn (SNCA-Knockout) to normal (SNCA-wild-type) to 1.5x endogenous level (SNCA-Duplication). Largely recapitulating the same analyses on the PD brain, we found a striking association between αSyn dosage and PtdSer. Since previous work had found that αSyn localizes to the mitochondria-associated endoplasmic reticulum (ER) membranes (MAM), we performed subcellular fractionation experiments using our simplified iPSC-derived neuronal model to investigate the effect of αSyn dosage at MAM, finding that αSyn level regulates PtdSer synthesis by feedback-control regulation of PtdSer

synthase 2 (PSS2). Our results reveal αSyn-driven changes in MAM activity alter the neuronal lipidome, and increased alpha-synuclein expression impairs the regulation of PtdSer in cellular membranes from affected cells. Our study offers mechanistic insight linking αSyn pathology to dysregulation of the neuronal lipidome as seminal factors in synucleinopathies, which have pathogenic implications and can be traced back to alterations at MAM. Approaches rescuing MAM should thus be considered in the development of much needed new therapeutic strategies for the treatment of these dreadful disorders.

Disclosures: P.A. Barbuti: None. **C. Guardia-Laguarta:** None. **Z. Chatila:** None. **B. Santos:** None. **N. Hattori:** None. **U. Dettmer:** None. **V. Menon:** None. **A.F. Teich:** None. **R. Krüger:** None. **E. Area-Gomez:** None. **S.E. Przedborski:** None.

Poster

PSTR390

Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.02/C12

Topic: C.03. Parkinson's Disease

Support: Noble Family Innovation Fund
UCLA CTSI Grant Number UL1TR001881
NIH/NINDS R01-NS128964

Title: Identify novel molecular mechanisms that regulate pathological alpha-synuclein transmission in neurodegenerative diseases by mathematic modeling

Authors: *C. PENG;
Neurol., UCLA, Los Angeles, CA

Abstract: Transmission of pathological α-synuclein (α-Syn) is a key mechanism for the progression of a group of neurodegenerative diseases collectively known as α-synucleinopathies. Previous studies have suggested that pathological α-Syn mainly transmits along the neuronal network. However, several key questions remain unanswered: (1) Does each connection in the mesoscale connectome contribute equally to pathological α-Syn transmission? Or are there sub-networks that predominantly affect the spreading of pathological α-Syn? (2) What genes or cell types determine the selective vulnerability of different brain regions to pathological α-Syn transmission? (3) What are the relative contributions of anterograde and retrograde transmission to the spreading of pathological α-Syn, and the genes that modulate the directionality-biased spreading processes? Here, we addressed these key questions with novel mathematical models. Strikingly, the spreading of pathological α-Syn is predominantly determined by the key subnetworks composed of only 2% of the connections in the connectome. We further explored the genes that are responsible for the selective vulnerability of different brain regions to transmission to distinguish the genes that play roles in presynaptic from those in postsynaptic

regions. By analyzing the cell type expression patterns of selective vulnerable genes, we found that the risk genes were significantly enriched in microglial cells of presynaptic regions and neurons of postsynaptic regions, indicating that both neurons and glial cells could modulate the transmission of pathological α-Syn, and different cell types play distinct roles in determining the selective vulnerability at presynaptic versus postsynaptic sites. Gene regulatory network analysis was then conducted to identify 'key drivers' of genes responsible for selective vulnerability and overlapping with Parkinson's disease risk genes. Overall, by identifying and discriminating between key gene mediators of transmission operating at presynaptic and postsynaptic regions, our study has demonstrated for the first time that these are functionally distinct processes.

Disclosures: C. Peng: None.

Poster

PSTR390

Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.03/

Topic: C.03. Parkinson's Disease

Support: CIHR PJT180582

Title: Differential toxicity induced by alpha-synuclein conformational strains in primary neurons

Authors: F. SAMUEL[1], R. SO[1], P. E. FRASER[2], J. WATTS[3], ***A. TANDON**[4];
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Abstract: Progressive accumulation of intracellular alpha-synuclein (asyn) aggregates is observed PD, DLB, and MSA. These synucleinopathies can be partly replicated in rodents following intracerebral seeding with recombinant asyn pre-formed fibrils (PFF) or human synucleinopathy-derived brain homogenates. In addition, distinct asyn PFF with unique conformational properties spread pathology differentially in various mouse brain regions, raising the possibility that distinct clinicopathological features of human synucleinopathies may be explained by differential toxicity to asyn conformers. Conformer properties are also sustained and refined by serial passaging in mice. We hypothesize that the mechanism for selective targeting of specific neuronal populations may be identified by measuring PFF internalization, trafficking, and mixing with cellular asyn. Here, we use primary neuron cultures to assess the susceptibility or resistance to distinct asyn conformers.
Hippocampal neurons isolated at E17 were exposed for 7-14 days to 7.5 ug/ml brain homogenate from A53T asyn transgenic (TgM83) mice inoculated with different asyn conformers, including PFF generated by adjusting NaCl concentration during aggregation ('no salt' (NS) and 'salt' (S) fibrils), human MSA brain homogenate, or spontaneously ill (SI) TgM83 brain homogenate.

S PFF- and MSA-derived homogenates induced pronounced Ser129 phosphorylated-asyn (pS129) puncta, whereas NS PFF- and SI-derived homogenates generated lower levels of pathology. These differences were not due to the amount of misfolded asyn or pS129 in the inoculum. Cell lysates contained sufficient asyn seed to faithfully propagate parent conformer pathology. We observed a loss of lysosome integrity in correlation with asyn pathology and aberrant trafficking of presynaptic markers, suggesting that asyn conformers disrupt proteostasis and induce synaptic reorganization.
These neuronal cultures offer a simple biological assay to assess mechanisms governing seeding capacity of asyn fibrils.

Disclosures: F. Samuel: None. **R. So:** None. **P.E. Fraser:** None. **J. Watts:** None. **A. Tandon:** None.

Poster

PSTR390

Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.04/C13

Topic: C.03. Parkinson's Disease

Title: Blocking the aerobic energy source in neurons via SGLTs delays α-synuclein transmission

Authors: *T. AHMED;
Neuropharm., Sch. of Med., Dept. of Brain Sci., Suwon, Korea, Republic of

Abstract: Progressive loss of dopaminergic neurons in the substantia nigra (SN) pars compacta (SNpc) and the formation of cytoplasmic inclusions containing misfolded α-synuclein (α-syn), Lewy bodies (LBs) are two major neuropathological hallmarks of Parkinson Disease (PD), which is the second most common neurodegenerative disease. PD and Diabetic mellitus (DM) share potential contributing factors and have overlapping pathology. Studies have unveiled a crucial connection between PD and DM. DM is characterized by impaired glucose metabolism and subsequent hyperglycemia. The elevated risk of developing cognitive abnormalities in individuals with impaired glucose metabolism has been well-documented. Hyperglycemia, a common pathogenesis in T2DM, has been shown to contribute to the onset of α-syn pathology in neurons and oligodendrocytes. Phosphorylated α-syn inclusions have been found in pancreatic β cells of T2DM subjects, indicating the existence of PD-related peripheral pathology in DM. But the complicated effect of glucose transporter on PD induced dopaminergic degeneration is still not well understood. Here, we demonstrate that α-syn fibrils induce active glucose energy transporter SGLTs activation in A53T alpha-synuclein overexpressing cells, which increases the uptake of α-syn into neurons. Furthermore, the inhibition of SGLTs inhibits the α-syn uptake and the formation of Lewy body-like inclusions. These results demonstrated a new therapeutic approach for PD.

Disclosures: T. Ahmed: None.

Poster

PSTR390

Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.05/C14

Topic: C.03. Parkinson's Disease

Support: Parkinson Canada Grant
Krembil Foundation Grant

Title: A benchmark instrumentation analysis of the alpha-synuclein seed amplification assay

Authors: *S. E. DI GREGORIO[1,3,2], S. ZAMPAR[2,3], G. GRIMMER[2,3], I. MARTINEZ-VALBUENA[2,3], J. WATTS[3,4], G. KOVACS[2,3,4], M. INGELSSON[2,3,5,4];
[2]Krembil Brain Inst., [1]Univ. Hlth. Network, Toronto, ON, Canada; [3]Tanz Ctr. for Res. in Neurodegenerative Dis., [4]Dept. of Lab. Med. & Pathobiology, [5]Dept. of Med., Univ. of Toronto, Toronto, ON, Canada

Abstract: Alpha-synuclein (α-syn) accumulates in the brain of patients with Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA). Evidence suggests that α-syn can adopt different conformations with various seeding and propagation properties. By the seed amplification assay (SAA) we can assess the influence of such seeds on the conversion of synthetic α-syn monomers into fibrils, which can be detected by thioflavin T. Recently, SAA has been utilized on brain, cerebrospinal fluid, and skin samples to diagnose and differentiate between the α-synucleinopathies. Due to the highly sensitive nature of the assay, several experimental conditions, such as temperature, pH, salt concentration, shaking frequency and intensity, as well as seed and template qualities, need to be carefully examined and defined. Here, we report significant differences in the α-syn seeding reactions depending on which plate reader instrument is employed.
A set of four DLB/PD and four MSA cases that had previously been evaluated by SAA were selected for the study. Two cases with progressive supranuclear palsy and a subject lacking neurodegenerative pathologies on neuropathological examination were included as negative controls. Samples of PBS soluble brain homogenates extracted from the frontal cortex and diluted to a concentration of 5 µg total protein were analyzed. To evaluate the effect of the different instruments on seeding and end product characteristics, two identical plates were set up and run in the Synergy H1 (Agilent BioTek) and FLUOstar (BMG Labtech) plate readers. For the duration of the assay, the instruments incubated (37°C) and cyclically disrupted (double-orbital, frequency of 400 rpm, for 1 minute, every 15 minutes) the seed-containing brain homogenates and synthetic α-syn monomer (recombinant N-His_6 K23Q α-syn) to form intermediate and fibrillar species capable of aggregation. Fluorescence from ThT was measured

over identical fixed intervals (15 min) to monitor seeding. With the FLUOstar we could observe, across all cases, an earlier initiation and faster rate of seeding compared to the same samples incubated in the Synergy H1. Differences in the reading function were evaluated by swapping the plates and comparing end-point measurements. It was found that the relative differences between the samples were conserved when read on both instruments, indicating that the observed differences are not due to differences in the optics. Enzymatic conformational stability assays and transmission electron microscopy are currently being employed to assess potential structural differences between the end products generated by the two instruments.

Disclosures: S.E. Di Gregorio: None. **S. Zampar:** None. **G. Grimmer:** None. **I. Martinez-Valbuena:** None. **J. Watts:** None. **G. Kovacs:** None. **M. Ingelsson:** F. Consulting Fees (e.g., advisory boards); BioArctic AB.

Poster

PSTR390

Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.06/C15

Topic: C.03. Parkinson’s Disease

Support: MJFF-021089
NIH NS112010

Title: Exploring Diagnostic Potential of Alpha-Synuclein Seeding Activity in Saliva and Serum of Living Parkinson's Disease Patients

Authors: *Z. WANG[1], T. L. GILLILAND[2], M. GERASIMENKO[2], S. G. CHEN[3], Q. KONG[4]; [1]Pathology, Case Western Reserve Univ., CLEVELAND, OH; [2]Case Western Reserve Univ., Cleveland, OH; [3]Univ. of Alabama at Birmingham, Birmingham, AL; [4]Dept. of Pathology, Case Western Reserve Univ., Cleveland, OH

Abstract: Exploring Diagnostic Potential of Alpha-Synuclein Seeding Activity in Saliva and Serum of Living Parkinson's Disease Patients Author: Zerui Wang[1], Tricia Gilliland[1], Maria Gerasimenko[1], Manuel V. Camacho[1], Shu Chen[2], Steve Gunzler[3], Qingzhong Kong[1,3]1. Department of Pathology, Case Western Reserve University2. Department of Pathology, University of Alabama at Birmingham3. Department of Neurology, University Hospitals Cleveland Medical center**Abstract** Synucleinopathies, a group of neurodegenerative disorders characterized by the prion-like propagation of misfolded alpha-synuclein proteins, encompass conditions such as Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA). The search for reliable and sensitive diagnostic biomarkers in peripheral tissues or body fluids that accurately reflect the spread and accumulation of misfolded alpha-synuclein is ongoing. In this study, we explored the seeding activities of alpha-synuclein in non-invasive body fluids—specifically, serum and saliva—from living patients. Using an

ultrasensitive real-time quaking-induced conversion (RT-QuIC) assay with immunoprecipitation-enhanced detection, we analyzed samples from probable PD (n=68), possible PD (n=17), and healthy controls (n=42). Our results revealed a sensitivity of 80% and specificity of 88.1% in serum, and a sensitivity of 81.63% and specificity of 84.62% in saliva. In a subset cohort of 38 probable PD, 10 possible PD, and 26 controls with both serum and saliva samples, we found that combining the RT-QuIC data of the two sample types significantly improved accuracy. This result suggests that the combined alpha-synuclein RT-QuIC analysis of serum and saliva has significant potential as a minimally invasive diagnostic tool for detecting PD at clinical and even preclinical stages.**Key words**: alpha-synuclein, seeding activity, synucleinopathies, RT-QuIC

Disclosures: Z. Wang: None. **T.L. Gilliland:** None. **M. Gerasimenko:** None. **S.G. Chen:** None. **Q. Kong:** None.

Poster

PSTR390

Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.07/C16

Topic: C.03. Parkinson’s Disease

Support: Michael J. Fox Foundation for Parkinson’s Research, MJFF-021089
NIH, U01 NS112010

Title: Characterization of end-product of alpha-synuclein RT-QuIC from different synucleinopathies.

Authors: *M. GERASIMENKO, T. GILLILAND, W. ZOU, Z. WANG;
Case Western Reserve Univ., Cleveland, OH

Abstract: Alpha-synucleinopathies represent a cluster of neurodegenerative disorders, including Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA) that present challenges to accurate diagnosis through conventional clinical detection methods. The underlying mechanism involves misfolding alpha-synuclein (aSyn) protein, forming aggregates in various neural elements, such as neurons, nerve fibers, and glial cells. These aggregated forms of aSyn possess the ability to self-propagate and spread across interconnected brain regions and the peripheral nervous system. Techniques like real-time quaking-induced conversion (RT-QuIC) and protein misfolding cyclic amplification (PMCA) are based on propagation features to detect synucleinopathies in biological specimens. Recent studies indicate that aSyn fibrils exhibit diverse structural forms or strains influenced by factors like brain region and cell type. In this study, we examined the end products (EPs) of RT-QuIC reactions using brain autopsy samples from PD patients and skin autopsy specimens from individuals with PD, DLB, and MSA as seeds to generate EPs. The α-synuclein aggregates displayed significant differences in synucleinopathy-seeded samples. Although the kinetic curves

of RT-QuIC facilitated the differentiation of synucleinopathies from control samples, discriminating between PD, MSA, and DLB proved challenging. Upon further characterization of RT-QuIC EPs, our findings revealed that PD and non-PD control brain and skin seeding EPs contained densely molecular aSyn aggregates, with PD aggregates exhibiting resistance to proteinase K digestion by western blot. EPs from DLB skin seeding exhibited properties similar to PD in terms of proteinase K digestion resistance, sucrose gradient, and guanidine gradient outcomes. While EPs from MSA skin seeding presented a stark contrast, displaying noticeable deviations from PD and DLB. MSA EPs demonstrated heightened sensitivity to proteinase K digestion, suggesting that MSA aSyn oligomers possess different characteristics compared to PD and DLB. These properties were further validated by sucrose gradient and guanidine gradient titration. Additionally, electron micrographs showed distinctions in fibril structures formed in EPs derived from brain versus skin seeding, with spherical-like oligomers detected in control seeding cases. These results offer valuable insights into the molecular characteristics of distinct synucleinopathies and suggest potential strategies for enhancing diagnostic precision for synucleinopathies.

Disclosures: M. Gerasimenko: None. **T. Gilliland:** None. **W. zou:** None. **Z. Wang:** None.

Poster

PSTR390

Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.08/C17

Topic: C.03. Parkinson's Disease

Support: IIT Gandhinagar

Title: Profiling of Aggregation-Prone Motifs in Alpha-Synuclein and implication for Targeted Therapeutic Development

Authors: *S. SHAH[1], S. GUPTA[2];
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Abstract: Profiling of Aggregation-Prone Motifs in Alpha-Synuclein andImplication for Targeted Therapeutic Development Sumedha Shah[1], Sharad Gupta[2*1,2]Department of Biological Sciences and EngineeringIndian Institute of Technology Gandhinagar, Gujarat, India*sharad@iitgn.ac.inDisclosures: Sumedha Shah: None, Sharad Gupta: NoneAbstractLewy bodies and neurites formed due to excessive accumulation of α-synuclein are thehallmarks of Parkinson's disease. Along with PD, α-synuclein aggregates have beenprominently observed in Lewy Body Dementia (LBD), Multiple System Atrophy (MSA), andin a subset of Alzheimer's Disease (AD) patients. The structures of the fibrillar region acrossdifferent synucleinopathies suggest the modification in each conformation is governed by thepathological condition. The

structural heterogeneity between oligomers and fibrillar forms ofaggregates derived from patient samples is still elusive. The cryo-EM structures of post-mortem brain-derived samples of PD, LBS, and MSA patients have revealed the polymorphicnature of well-arranged fibrillar forms with overlapping stretches. The discordance in mainaggregation forming segments remains unsolved even with numerous studies employingcomputational tools and experimental investigations of selected short peptides.Our study aims to identify the sticky peptide regions which can act as nucleation points. Wehave synthesized a series of offsetting 15-mer peptides covering the entire stretches fromrecently reported cryo-EM structures of fibrils isolated from patients with all majorsynucleinopathies. By utilizing a ThT-based aggregation assay, we have thoroughly profiledaggregation propensity and zeroed in on two aggregation hot spots, which could be thenucleation centres. The congregation patterns were experimentally validated by in-vitro assayssuch as Thioflavin T assay and Congo red binding assay as well as microscopic methods suchas CLSM, SEM, and AFM. We have also discovered that the aggregation propensities arehighly modulated by the flanking regions and post-translational modifications particularlyfocusing on acetylation and carbamylation that render the lysine residue chargeless.We are further utilizing the offsetting peptide library as a target detection tool for potentialtherapeutic development. The mildly aggregation-prone sequences appear to be of high interestas they can be utilized as standalone inhibitors or a potential target site for degraderdevelopment.

Disclosures: S. Shah: None. **S. Gupta:** None.

Poster

PSTR390

Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.09/C18

Topic: C.03. Parkinson’s Disease

Title: The therapeutic potentials of ceramide synthesis inhibition on alpha-synuclein pathology and motor function in parkinson's disease models

Authors: *E. LEE, J. LEE, C.-M. OH;
GIST, Gwangju, Korea, Republic of

Abstract: <META NAME="author" CONTENT="이은경">Parkinson's disease (PD) ranks as the second most common neurodegenerative disorder, prominently marked by motor function impairment and alpha-synuclein (a-syn) aggregation. Research increasingly underscores ceramides—a class of sphingolipids—as crucial to PD's pathogenesis, evidenced by their significant accumulation in the postmortem PD brains and altered plasma levels. Despite existing data from cellular and small animal models, there remains a substantial gap in understanding the in vivo effects of ceramide synthesis inhibition, especially using mouse models. This study aims

to fill this gap by evaluating the effects of ceramide synthesis inhibition on a-syn pathology and motor functions across several PD models.Caenorhabditis elegans expressing human alpha-synuclein-YFP (*NL5901*) were treated with myriocin for 7 or 10 days, with their alpha-synuclein fluorescence and puncta analyzed using confocal microscopy. *SHSY5Y* cells with the A53T SNCA mutation (SNCA-A53T) were treated with myriocin or underwent SPTLC1 knockdown, with aggresome levels assessed using an aggresome detection kit. A53T transgenic mice (M83) received myriocin injections three times weekly for 5 or 7 months from five months old. Motor function and spatial memory were evaluated using Open Field and Y-maze tests, while dopaminergic neurons in their substantia nigra and striatum were quantified by tyrosine hydroxylase staining. Alpha-synuclein forms were measured with DAB staining or immunofluorescence.*NL5901* exposed to myriocin for 7 days showed a decrease in the number of puncta and fluorescence intensity of a-syn in both the head and tail. The group exposed to myriocin for 10 days showed a decrease in the number of a-syn puncta in the head. Reduced aggresome was identified in SNCA-A53T cells following myriocin treatment or SPTLC1 knockdown. M83 mice showed reduced motor function compared to WT ($P<0.01$ by two-sample t-test). Even though the group treated with myriocin for 5 months showed non-altered motor activity
and spatial memory function, the groups treated with myriocin for 7 months showed enhanced motor function ($P<0.05$ by two-sample t-test). Increased TH-positive cells and fibers, decreased monomer/phosphorylated a-syn, and decreased Thioflavin-S-positive fibers were identified in the group treated with myriocin for 5 months.These data suggest that inhibition of ceramide synthesis may induce therapeutic effects regarding a-syn pathology and motor function in several PD models. Further investigation in this novel approach as a therapeutic and/or preventive intervention for PD is warranted.

Disclosures: E. Lee: None. **J. Lee:** None. **C. Oh:** None.

Poster

PSTR390

Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.10/C19

Topic: C.03. Parkinson’s Disease

Support: MJFF Grant MJFF-023284
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MJFF Grant MJFF-022928
MJFF Grant MJFF-024055
MJFF Grant MJFF-023878

Title: The Michael J. Fox Foundation’s Efforts to Develop Novel Antibodies for Understanding and Measuring Alpha-Synuclein Modifications in Parkinson’s Disease.

Authors: *N. POLINSKI[1], M.-B. FARES[2], L. PETRICCA[2], S. AGÜERA DE HARO[3], M. AEBI[2], H. A. LASHUEL[3], A. ARAGON-GONZALEZ[4], G. K. TOFARIS[4], S. STRADER[5], A. B. WEST[5], N. PENA[6], L. A. VOLPICELLI-DALEY[6], H. CHENG[7], T. KELK[8], R. KUMARAN[8], E. CLARK[1], G. THAKURIA[9], J. L. EBERLING[9];
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Abstract: Alpha-synuclein misfolding and aggregation play a central role in the pathogenesis of Parkinson’s disease (PD). Increasing evidence points to post-translational modifications of alpha-synuclein (aSyn) as important regulators of its aggregation, pathology formation, and pathogenicity. Several types of post-translational modifications have been identified and associated with physiological and aggregated forms of aSyn. However, very little is known about how, where, and when aSyn is modified due to a lack of high-quality and accessible reagents. To address this gap, The Michael J. Fox Foundation (MJFF) has taken an active role in designing, validating, and distributing various tools and models that can be used to investigate PD-related biology, including antibodies to modified forms of aSyn. Here we summarize MJFF-led efforts in partnership with several research teams from academia, industry, and tool manufacturers to develop and characterize antibodies to aSyn truncated at 1-119, aSyn truncated at 1-122, aSyn ubiquitylated at K45/K38/K60, and N-terminal aSyn. We shall provide data on how these antibodies were designed, their sensitivity and selectivity, and their performance in different applications and model systems. In addition, we will include information on how to access these antibodies, an overview of other PD-related tools and models currently in development at MJFF, and a snapshot of other resources MJFF makes available to the scientific community. Ultimately, MJFF’s investment in providing the research community with robust, well-characterized tools and models will speed research towards a cure for PD by enabling research, de-risking investment in PD research, and increasing reproducibility by providing the tools to researchers across labs.

Disclosures: N. Polinski: None. **M. Fares:** None. **L. Petricca:** None. **S. Agüera de Haro:** None. **M. Aebi:** None. **H.A. Lashuel:** None. **A. Aragon-Gonzalez:** None. **G.K. Tofaris:** None. **S. Strader:** None. **A.B. West:** None. **N. Pena:** None. **L.A. Volpicelli-Daley:** None. **H. Cheng:** None. **T. Kelk:** None. **R. Kumaran:** None. **E. Clark:** None. **G. Thakuria:** None. **J.L. Eberling:** None.

Poster

PSTR390

Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.11/C20

Topic: C.03. Parkinson’s Disease

Support: MJFF ASAP

Title: Nanoplastic accumulation as a Promoting Factor for α-Synuclein Aggregation in the Gut and Brain

Authors: *E. G. VIVERETTE[1], A. BANDI[2], L. SHIELL[2], A. B. WEST[3];
[1]Pharmacol. and Cancer Biol., Duke Univ., Durham, NC; [2]Duke Univ., Durham, NC;
[3]Pharmacol., Duke Univ., Durham, NC

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative movement disorder affecting millions worldwide that is pathologically characterized by α-synuclein deposition throughout the brain. In addition, α-synuclein aggregation has been observed outside of the brain, notably within the gut. Nanoplastics (NP) are a growing environmental concern defined as plastic particles with a diameter of less than 100 nm and varying size, shape, polymer type, and concentration in the environment. Secondary NPs are formed when larger microplastics break down in the environment through exposure to UV radiation, oxidation, and mechanical degradation. These secondary NPs are a concern to human health due to their newly acquired bioactive properties, many of which have not been studied in detail. Previous studies have suggested a connection between anionic NPs and α-synuclein aggregation.To determine the relationship between α-synuclein aggregation and environmentally realistic concentrations of secondary NPs, we preformed multiple molecular dynamics studies, including Surface Plasmon Resonance (SPR), Zeta potential analysis, and seeding assays on multiple common secondary NP pollutant types, including polyethylene, polypropylene, and polyacrylates. We will use these preliminary *in vitro* studies to inform long-duration *in vivo* studies to help understand NP exposures on PD-relevant endpoints and pathologies both in the gastrointestinal system and brain in mouse models. Relevant tissues are analyzed to determine the effect of lifetime NP accumulation on PD development and progression, as well as to establish a connection between NP accumulation and migration from the gastrointestinal system to the brain as a possible new environmental toxin that may lead to PD susceptibility or worsened progression.

Disclosures: E.G. Viverette: None. **A. Bandi:** None. **L. Shiell:** None. **A.B. West:** None.

Poster

PSTR390

Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.12/C21

Topic: C.03. Parkinson's Disease

Title: Assessment of a simple cleaning procedure to remove alpha-synuclein assemblies from laboratory materials and surfaces

Authors: *A. FRANCOIS[1], L. ROBINET DE PLAS[1], M. PICCA[1], R. BILLIRAS[1], A. CHOMEL[1], C. GAMBLIN[2], E. NONY[1];
[1]Servier Res. Inst., Gif-Sur-Yvette, France; [2]Theraxel, Beaune, France

Abstract: Misfolded and aggregated alpha-synuclein (aSyn) is a characteristic hallmark of Parkinson's disease as well as other synucleinopathies. The propagation of aSyn assemblies was demonstrated in multiple studies where the use of *in vitro* aSyn fibrils was able to seed endogenous aSyn when added to neuronal cultures or injected into rodent brain. These observations highlight that aSyn assemblies possess prion-like properties, meaning that aSyn should not be considered as innocuous. To reduce potential health hazards related to aSyn handling, it is important to use an efficient and easily applicable cleaning procedure. Previous published studies on the decontamination and inactivation of aSyn fibrils showed the effectiveness to immerse glass, stainless steel, plastic and aluminum into a washing solution containing either sodium dodecyl sulfate or Hellmanex™. Based on these published results, we modified our biosafety level 2 laboratory cleaning procedure with the addition of an aSyn decontamination step, prior to routine sanitization cleaning process. Briefly, our new cleaning procedure consists of: a) wiping potentially contaminated surfaces or non-disposable materials with poly-cellulose wipes impregnated with a solution of Hellmanex™, b) drying the surfaces with dry poly-cellulose wipes, c) performing routine sanitization cleaning process. The efficacy of this new cleaning procedure was evaluated on human aSyn pre-formed-fibril (PFF), human aSyn filament and human monomeric pS129-aSyn spotted at 100, 1000 and 5000 pg onto stainless steel plates, to mimic a contamination. We developed a collection method to recover the spotted aSyn onto stainless steel plates, as well as a quantification method relying on both U-PLEX Human aSyn and LEGEND MAX™ Human αSyn Aggregate ELISA kits. Between 61% to 85% of the spotted aSyn proteins tested were recovered with the use of our collection method. Following measurements of total aSyn, decontamination rates of 96.9% ±1.14, 97.9% ±0.62 and 98.8% ±0.57 were achieved with the use of a cleaning solution containing 0.5%, 1% or 2% Hellmanex™ respectively, for the different aSyn forms tested and the 3 quantities spotted. Concerning the quantification of the aggregated aSyn, the use of 2% Hellmanex™ allowed to remove 98.4% ±0.71 of aSyn filament and no remaining aSyn PFF was detectable. Based on these results, we recommend using a decontamination solution including 2% Hellmanex™ to clean non-disposable materials and surfaces from potential aSyn contamination. The collection and analytical methods described herein enable rapid routine surface checks to mitigate potential health hazards related to the aSyn handling.

Disclosures: A. Francois: A. Employment/Salary (full or part-time):; Servier Research Institute. **L. Robinet de Plas:** A. Employment/Salary (full or part-time):; Servier Research Institute. **M. Picca:** A. Employment/Salary (full or part-time):; Servier Research Institute. **R. Billiras:** A. Employment/Salary (full or part-time):; Servier Research Institute. **A. Chomel:** A. Employment/Salary (full or part-time):; Servier Research Institute. **C. Gamblin:** A. Employment/Salary (full or part-time):; Theraxel. **E. Nony:** A. Employment/Salary (full or part-time):; Servier Research Institute.

Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.01/C22

Topic: C.03. Parkinson's Disease

Support: MOH-OFLCG000207

Title: Glycerol 3-phosphate acyltransferase modifies α-Synuclein-induced neurotoxicity in Drosophila and mouse primary neuronal models of Parkinson's Disease

Authors: *M. REN, G. LIM, W. TANG, K.-L. LIM;
Lee Kong Chian Sch. of Med. - Nanyang Technological Univ., Singapore, Singapore

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder marked by the loss of dopamine-producing cells in the substantia nigra region and the pathological accumulation of Lewy bodies that are enriched with α-Synuclein (α-Syn) aggregates and lipid debris. Mutations in the α-Syn-encoding gene *SNCA* are causative of familial PD and *SNCA* genetic variations increase risk for sporadic PD. Although multiple cellular pathways have been implicated in α-Syn toxicity, the role of lipid metabolism in its pathology remains elusive. Our study aims to explore the potential role of lipid synthesis enzyme glycerol 3-phosphate acyltransferase (GPAT) in α-Syn aggregation and neurotoxicity, utilizing both *Drosophila melanogaster* and primary mouse neurons as models of PD. From a *Drosophila* genetic screen that involves the *Drosophila* synucleinopathy model and a collection of 92 genes related to human PD risk loci, we discovered that mino, encoding the mitochondrial GPAT, modifies α-Syn neurotoxicity. Silencing *mino* significantly suppresses α-Syn-induced PD phenotypes in *Drosophila*, including dopaminergic neuronal loss in the protocerebral anterior medial cluster (*eGFP* RNAi control mean 102 ± SD 9; *mino* RNAi 112±13, 3 weeks old males), associated locomotion defects (mean increase 1.4±0.1 cm by *mino* RNAi in climbing for 5 s) as well as circadian rhythm-related abnormalities, whereas *mino* overexpression yields the opposite effects. Mechanistically, we found that *mino* modulates the levels of mitochondrial reactive oxygen index (control 1.11 ± 0.13*; mino* RNAi 0.98 ± 0.07; *mino* overexpression 1.19 ± 0.10) and lipid peroxidation index (control 0.39 ± 0.07*; mino* RNAi 0.28 ± 0.06; *mino* overexpression 0.60 ± 0.10). Additionally, we found that apart from mitochondrial mino, knocking down the predicted endoplasmic reticulum-localized GPAT (*GPAT4* and *CG15450*) also suppressed α-Syn-induced decline of locomotor activities, rescued the dopaminergic neurons and reduced α-Syn higher-order oligomers (tetramers and above), suggesting a common role of GPAT in modulating α-Syn neurotoxicity. However, knocking down *Gnpat*, encoding peroxisome-localized glyceronephosphate O-acyltransferase, shows minimum effects only. Importantly, feeding α-Syn-expressing flies with FSG67, a GPAT inhibitor, reproduces the benefits of *mino* knockdown in locomotor activities. FSG67 also inhibited α-syn phospho-Ser129 aggregation and lipid peroxidation in mouse primary neurons transfected with α-Syn preformed fibrils. In summary, our study not only elucidates a novel and important lipid-related factor contributing to α-syn toxicity, but also offers a novel therapeutic direction for PD.

Disclosures: M. Ren: None. **G. Lim:** None. **W. Tang:** None. **K. Lim:** None.

Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

Location: MCP Hall A

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Program #/Poster #: PSTR391.02/C23

Topic: C.03. Parkinson's Disease

Support: European Research Council Grant agreement No. #951294
University of Bordeaux's IdEx "Investments for the Future" program/GPR BRAIN_2030

Title: Understanding diffusion in extracellular space in synucleinopathy

Authors: J. ESTAÚN-PANZANO[1], Q. GRESIL[2], E. DOUDNIKOFF[1], C. MAZZOCCO[3], M.-L. AROTCARENA[4], ***B. BIOULAC**[5], B. DEHAY[4], L. COGNET[6], E. BEZARD[7];
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Abstract: The pathogenic mechanisms underlying synucleinopathies involve the spread and propagation of α-synuclein aggregates throughout the central nervous system and at least in part, through the ECS. We have used freely diffusing single-wall carbon Nanotubes (SWCNTs) to try to understand the effect of synucleinopathy on extracellular diffusion, as it is emerging as a critical component for propagation, communication and regulation in health and disease.Using SWCNTs single-particle tracking, we unravelled a significant increase in Substantia Nigra pars compacta extracellular diffusion values in the context of a-synuclein pathology accompanied by nigral neuron degeneration. Nonetheless, this model made it hard to disentangle the direct effect of neurodegeneration and synucleinopathy. We compared our previous model, which features neurodegeneration in the SN (our classic PD patient-derived Lewy bodies extract), versus those exhibiting abundant synuclein aggregopathy (pre-formed fibrils (PFF)). Single particle tracking has become a powerful tool for characterising some of the fundamental ECS properties at the nanoscale. The recording of long trajectories in the so-called "transparency window" of tissue permits the reconstruction of ECS maps with nanometric resolution, including instantaneous diffusion coefficients and estimation of channel width.Our results show that (i) SN and striatum present different diffusional regimes; (ii) the SN neurodegeneration model presents, as expected, increased diffusion regimes in the SN but none in the striatum, (iii) the presence of secondary aggregates in the striatum in the PFF model also causes an increased diffusion regime in the striatum. The sole spread of the synucleinopathy can, therefore, affect ECS diffusional parameters, thereby likely further contributing to its propagation. We hypothesise that these aggregates can affect the surrounding microenvironment, triggering inflammatory responses,

which would widen the ECS.Understanding the distinct effects of these synucleinopathy models on the ECS is crucial for unravelling the mechanisms underlying synuclein propagation, neurodegeneration, and associated clinical manifestations. Elucidating the interplay between aggregated α-synuclein and the ECS may unveil potential therapeutic targets to modulate synucleinopathy progression.

Disclosures: J. Estaún-Panzano: None. **Q. Gresil:** None. **E. Doudnikoff:** None. **C. Mazzocco:** None. **M. Arotcarena:** None. **B. Bioulac:** None. **B. Dehay:** None. **L. Cognet:** None. **E. Bezard:** A. Employment/Salary (full or part-time):; Motac. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac, TREEFROG Therapeutics, SE Therapeutics.

Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.03/C25

Topic: C.03. Parkinson's Disease

Support: JSPS KAKENHI 21K07282

Title: The effect of subthalamic or pallidal neuromodulation for α synuclein propagation

Authors: *Y. SHIMO[1], A. OKUZUMI[2], A. NAKAJIMA[1], H. IWAMURO[3], N. HATTORI[1]; [1]Juntendo Univ., Tokyo, Japan; [2]Neurol., Juntendo Univ., Tokyo, Japan; [3]Dept. of Neurosurg., Juntendo Univ., Tokyo, Japan

Abstract: Misfolding and aggregation of alpha-synuclein (αsyn) in neurons is a key pathological feature of synucleinopathies such as Parkinson's disease (PD), dementia with Lewy bodies, and multiple system atrophy. Previously, it was discovered that αsyn transmits from cell to cell through synaptic transmission and depends on the activity of aggregated αsyn neurons (Okuzumi et al. 2021). Neuromodulation therapy, such as making lesions in the basal ganglia or deep brain stimulation, is a widely accepted therapy for advanced patients with PD. However, it remains unclear whether neuromodulation therapy affects the propagation of αsyn in the brain. To clarify this point, we injected αsyn preformed fibrils (αsyn PFF), seeds for αsyn deposit propagation, into the striatum of wild-type mice before making lesions of entopeduncular nucleus (EP which is homologous to the internal segment of the Globus Pallidus (GPi) in human) or subthalamic nucleus (STN), which are the major target structures of neuromodulation therapy for PD. We used C57BL/6J male mice. We unilaterally injected αsyn PFF into the mice striatum a day before lesioning the ipsilateral STN (n=3) or EP (n=3) with ibotenic acid (0.01 mg/ml, 2 micro L) stereotaxically. For the control group, saline was injected into the EP or STN in each mouse (n=3). After preparing paraffin sections of the brain for counting phosphorylated a-syn (p-syn) inclusions, whole-brain sections were imaged with a Keyence microscope (BZ-X810) using

bright field capture. For STN lesioned group, the total area of αsyn inclusions/unit area in the substantia nigra (SN: 443.63997±81.7231) and cerebral cortex (Cx:1582.962±489.7998) was significantly lower compared to the control group (SN: 5447.55 ± 3438.55, Cx: 10733.358 ± 3166.882, mean ± SD, $p<0.0001$ Mann- Whitney U test). However, there was no statistical difference in αsyn inclusion area of SN and Cx between the EP lesioned group and the control group ($p=0.3349$ for SN, $p=0.0858$ for Cx, Mann- Whitney U test). These results indicate that modulation of STN activity may play a substantial role in the propagation of αsyn compared to GPi and shed light on the mechanisms of αsyn propagation.

Disclosures: Y. Shimo: None. **A. Okuzumi:** None. **A. Nakajima:** None. **H. Iwamuro:** None. **N. Hattori:** None.

Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.04/C26

Topic: C.03. Parkinson's Disease

Title: Exploring the differential vulnerability of different dopaminergic neurons within the mouse midbrain to alpha-synuclein oligomers

Authors: *I. DEL POPOLO, M. J. WALL;
Univ. of Warwick, Coventry, United Kingdom

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterised by the loss of dopaminergic neurons (DNs) in substantia nigra pars compacta (SNpc). The pathogenesis of PD includes multifactorial dysfunctions, with mitochondrial dysregulation and alpha-synuclein aggregation being the major drivers of the pathology. PD research has predominantly focused on SNpc DNs degeneration, although there are evidence suggesting that DNs in the ventral tegmental area (VTA) are also affected in later stages of disease. Thus, we aimed to investigate the differential vulnerability of DNs midbrain to alpha-synuclein oligomers (o-Syn). Firstly, we performed immunohistochemistry to identify DNs in acute mouse brain slices using tyrosine hydroxylase as a marker for dopamine. We then used whole-cell patch clamp recordings to investigate the sensitivity of SNpc and VTA DNs to the effects of o-Syn. It was found that o-Syn preferentially affected SNpc DNs (n= 12) in contrast to VTA DNs (n=12) by increasing their conductance and altering their firing pattern. These effects in SNpc were partially reversed by glibenclamide (n= 9) (K_{ATP} channel blocker), suggesting that K_{ATP} channels are activated by o-Syn and therefore could be considered as a potential therapeutic target. To further investigate the effects of o-Syn on SNpc DNs, we used fast scan voltammetry to investigate dopamine release in dorsal striatum (containing dopamine terminals from the nigrostriatal pathway). Paired stimuli with single pulse stimulation were generated with four different inter-pulse intervals (5, 10, 20

and 60 seconds). It was found that o-Syn significantly increased the degree of paired pulse depression of dopamine release for all the intervals (n= 10). Thus, o-Syn not only affect the electrophysiology of SNpc neurons, but also modulates the release of dopamine from their terminals. Altogether, it appears that SNpc DNs are more vulnerable to the effects of o-Syn than VTA DNs.

Disclosures: I. Del Popolo: None. **M.J. Wall:** None.

Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

Location: MCP Hall A

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Program #/Poster #: PSTR391.05/C27

Topic: C.03. Parkinson's Disease

Support: NS108686
NS112540
NS092093
NS086074
ASAP-000592

Title: Exogenous alpha-synuclein aggregates cause tau-dependent synaptic deficits: Mechanistic insights for Lewy body dementia

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Abstract: Alpha-synuclein (αS) is currently the primary pathological protein implicated in Parkinson's disease dementia (PDD) and dementia with Lewy bodies (DLB), which are classified as Lewy Body Dementia (LBD). Previously we have shown that mutant A53T αS transgenic mice (TgA53T) have tau-dependent age-related cognitive deficits associated with GSK3β-dependent tau mislocalization to dendritic spines leading to synaptic dysfunction. However, in LBD, most cases are not linked to a known genetic mutation nor associated significant degeneration of hippocampus as seen in Alzheimer's disease. Because LBD is associated with high neuritic αS pathology in CA2, likely from afferents from the entorhinal cortex (EC), we hypothesize that in sporadic LBD, the neurites from EC neurons are releasing toxic αS that causes tau mislocalization and synaptic deficits in hippocampal neurons, leading to cognitive deficits. To test our hypothesis, we treated primary cultures of hippocampal neurons to αS preformed fibrils (PFF). The neurons were transfected to express DsRed and Tau-eGFP to

monitor the neurites/spines and tau, respectively. Live cell imaging at 24 hours post-PFF treatment shows that αS PFF, even at very low doses (0.05 μg/mL; ~3.5 nM), causes tau mislocalization. Treatment of αS monomer (4.0 μg/mL) had no effect. We also show that αS PFF-dependent mislocalization of tau occurs independently of endogenous αS but requires GSK3β activity. Longer-term analysis shows that αS PFF does not impact the integrity of the spines, even at 7 days. However, αS PFF causes significant loss of spines, as well as synaptic markers (PSD95, Synapsin), by 14 days post-PFF treatment. We tested our hypothesis in vivo by stereotactically injecting αS PFF to the CA1 region of the hippocampus of WT mice. Infusion of αS PFF caused deficits in mEPSC frequency, amplitude, and LTP at 48 hours post-infusion. More importantly, the synaptic and LTP deficits caused by αS PFF were abolished in mice lacking tau expression. Finally, analysis of hippocampal sections from sporadic PD and LBD cases confirms neuritic αS pathology within the CA regions of the hippocampus. Significantly, α-synucleinopathy cases present with tau mislocalization to the somatodendritic compartment in CA2 hippocampal neurons compared to controls. We conclude that in LBD, neuritic αS pathology may be releasing toxic αS species leading to tau mislocalization and synaptic deficits that lead to circuit dysfunction and cognitive deficits.

Disclosures: S.C. Vermilyea: None. **C. Perez de Nanclares:** None. **B. Singh:** None. **R. Schlichte:** None. **J. Meints:** None. **H. Clark:** None. **J.C. Troncoso:** None. **D. Liao:** None. **A. Araque:** None. **M.K. Lee:** None.

Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.06/C28

Topic: C.03. Parkinson’s Disease

Support: R15NS130532-01
1R03NS088395-01A1
1R15NS093539-01
1R21NS107960-01

Title: Impact of the Demyelinating Agent, Cuprizone, in the Preformed Fibril Model of Limbic Lewy Body Disease

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Abstract: Lewy body disorders are characterized by fibrillar α-synuclein and other cellular material deposited in somal inclusions known as Lewy bodies and synaptic, axonal, or dendritic inclusions known as Lewy neurites. Neurons with long, thin, and unmyelinated axons are more

likely to harbor α-synuclein+ inclusions compared to those with short, myelinated axons, but it is not known whether myelination *per se* confers neuronal resilience against α-synucleinopathy. We have observed that preformed α-synuclein fibrils do not appear to be taken up by or seed α-synucleinopathy in white matter tracts (*e.g.*, intrabulbar anterior commissure or internal capsule) in experimental models of Lewy body disease. These observations led us to test if forced demyelination exacerbates α-synucleinopathy after infusions of preformed fibrils in the bulbar anterior olfactory nucleus. To answer this question, we capitalized on a pharmacological demyelinating agent, cuprizone, commonly used to model multiple sclerosis. Dietary cuprizone is known to induce CNS-specific, partial demyelination. Genetically outbred, nontransgenic mice of both biological sexes were administered cuprizone (0.3%) or control diet for two weeks prior to infusing preformed fibrils or vehicle in the bulbar division of the anterior olfactory nucleus. Although there was no change in food consumption, mice on the cuprizone diet showed the expected reduction in bodyweight in vehicle and fibril-injected mice of both sexes. Cuprizone also reduced the levels of the two major protein constituents of the myelin sheath, myelin basic protein (MBP) and proteolipid protein (PLP), but only in male mice. There was no additive or synergistic effect of cuprizone and fibril exposure on these myelin markers in either sex. In fibril-treated mice, cuprizone only modestly increased the fraction of nonionic detergent-insoluble α-synuclein that was hyperphosphorylated at serine residue 129. Similarly, there was no robust exacerbation of neuron loss with cuprizone. These findings suggest that that cuprizone and fibril exposure do not act additively or synergistically at the doses used here, perhaps because they impact the same, saturated physiological processes in male mice subjected to experimental Lewy body disease and forced demyelination. Future work is warranted to test if neurons that are forcibly demyelinated are not as vulnerable to Lewy pathology as expected because they continue to retain other important protective features of myelinated neurons.

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Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

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Topic: C.03. Parkinson’s Disease

Support: NIH K08NS101118
R21NS127211
R01NS118146

Title: Crispr library identification of microglial genes that regulate uptake and endolysosomal trafficking of aggregated alpha-synuclein

Authors: *Y. SHIN[1], Y. CHEN[3], J. BALS[4], K. D. COTTER[2], J. HAINES[5], Y. LI[6], B. A. BENITEZ[7], A. A. DAVIS[8];
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Abstract: The defining feature of Parkinson Disease (PD) and related illnesses termed "synucleinopathies" is the accumulation of misfolded alpha-synuclein (α-syn) protein. Misfolded α-syn not only aggregates intracellularly but also can be released into the extracellular space and internalized by neighboring neurons, where they serve as seeds for further aggregation. This spread of aggregation ultimately leads to neuronal dysfunction and death. Microglia, the resident immune cells of the brain, are important modulators of this neuron-to-neuron transmission as they can clear protein aggregates in the brain, including α-syn fibrils. However, the precise molecular mechanisms behind how microglia clear α-syn aggregates are not fully understood. In this study, we performed an unbiased CRISPR screen using a murine microglia-derived cell line (BV2) to identify genes crucial for microglial uptake and endolysosomal trafficking of α-syn fibrils. BV2 cells were transduced with Brie CRISPR library to delete 19,674 genes across the mouse genome individually in single cells. The CRISPR-edited microglia were then treated with α-syn fibrils labeled with the pH-sensitive fluorophore pHrodo. Because deletion of genes critical to α-syn fibril uptake should lead to reduced cellular fluorescence, we used fluorescence-activated cell sorting followed by RNA sequencing (FACS-seq) to sort the cells exhibiting the lowest 3% fluorescence intensity and identify the guide RNAs present in those cells. Gene ontology analysis confirmed that this population was enriched for genes involved in the endolysosomal pathway, as expected. Notably, the screen identified several microglial proteins whose involvement in α-syn uptake has not been previously reported. Integrating these findings with human genetic data linked to PD risk can be an effective strategy for identifying and validating potential therapeutic targets for synucleinopathies.

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Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.08/C30

Topic: C.03. Parkinson's Disease

Title: The influence of GBA1 and CTSB deficiency on lysosomal compensation in the context of Parkinson's disease

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Abstract: Parkinson's disease (PD) is a neurodegenerative movement disorder characterized by the intracellular accumulation of alpha-synuclein (αSyn) into Lewy bodies and the progressive loss of dopamine neurons in the substantia nigra. It is widely thought that lysosomal dysfunction is an important facet of proteinopathy and cell loss in PD as mutations in several genes involved with autophagy and lysosome biology are causal for the disease. However, the specific mechanisms culpable are unknown. Activity of the lysosomal cysteine protease cathepsin B (CTSB) is decreased by *GBA1* and *LRRK2* mutations. Additionally, several genetic studies have identified *CTSB* as a locus of interest both in and out of the context of *GBA1*. This protease has been shown to cleave αSyn at several sites, breaking down the monomeric form of the protein but failing to do the same with αSyn fibrils. However, it has also been observed that decreased CTSB activity can suppress the ability of pre-formed αSyn fibrils to induce aggregation of endogenous αSyn, thus illustrating complex ways CTSB activity could influence the pathogenesis of PD and other synucleinopathies. This study aims to further elucidate how CTSB is involved in αSyn metabolism using CRISPR/Cas9 to knockout *CTSB* expression from wild-type and *GBA1* heterozygous-null cells. Results showed significant and mechanistically distinct alterations in the protein levels of several other lysosomal cathepsins in response to CTSB loss with and without *GBA1* deficiency. When assessed by immunoblotting, protein levels of proCTSD are elevated with CTSB loss, but those of CTSV are only increased with *CTSB* knockout in *GBA1* heterozygous null cells. Alternatively, CTSH appears suppressed in *GBA1* wild-type cells with CTSB absent, and CTSL levels do not vary in response to *CTSB* knockout. Furthermore, these changes may be additionally influenced by αSyn-related stress. These observations suggest a complex cellular response by the cell to compensate for the loss of CTSB activity, and that αSyn may exert additional pressure on an already dysfunctional system and provoke further adaptive responses. Further research is necessary to determine how this may relate to αSyn metabolism and how changes in CTSB function may contribute to PD development.

Disclosures: E. Chapman: None. **N. Subrahmanian:** None. **L. Laboissonniere:** None. **M.J. LaVoie:** None.

Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.09/C31

Topic: C.03. Parkinson's Disease

Title: Alpha-synuclein aggregation mechanisms in the gut of aged alpha-synuclein mice

Authors: *A. BANDI;
Duke Univ., Durham, NC

Abstract: α;;-Synuclein Aggregation Mechanisms in the Gut of Aged α;;-Synuclein Mice
Anjali Bandi, Elizabeth G. Viverette, Andrew B. West
Duke Center for Neurodegeneration and Neurotherapeutics, Department of Pharmacology and Cancer Biology, Duke University
Parkinson's Disease (PD) is a neurodegenerative disorder, affecting millions worldwide, pathologically characterized by the presence of α;;-synuclein inclusions throughout much of the brain in the late stages of the disease that impacts the survival of dopamine-producing neurons in the substantia nigra. Previous studies have shown that constipation, and mild indicators of irritable bowel syndrome, is one of the most frequent and earliest symptoms in many PD patients. About 50%~80% of PD patients suffer from constipation, making it critical to study the pathology of α;;-synuclein and other pathological markers of disease mechanisms in the gut alongside its impact on neurodevelopmental disease progression in the brain.
In this study, we are focusing on gut and brain α;;-synuclein phenotypes in transgenic mice that express physiological levels of human wild-type α;;-synuclein from a PAC transgenic in mice with *Snca* expression deleted. Pathological characteristics of α;;-synuclein in the colon will be correlated to α;;-synuclein changes in the brain in young animals to those aged with severe constipation phenotypes caused by the human α;;-synuclein expression. Gnotobiotic mice have been generated in this mouse strain to understand the relative contribution of PD-associated microbes as well as gut microbiomes from PD patients. We will be using machine-learning assisted pathological analysis together with antibodies specific for different types of α;;-synuclein pathology. The overall goal is to resolve how changes in the gut may influence α;;-synuclein phenotypes in the brain.

Disclosures: A. Bandi: None.

Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.10/C32

Topic: C.03. Parkinson’s Disease

Support: Parkinson's Foundation Launch Award

Title: Mild complex I deficiency promotes αlpha-synuclein phosphorylation and aggregation

Authors: *N. SUBRAHMANIAN[1], A. SHUTE[1], D. HALL[1], M. S. MOEHLE[2], B. I. GIASSON[3], M. J. LAVOIE[4];
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Abstract: Mitochondrial complex I (CI) is a proton-pumping NADH: ubiquinone oxidoreductase crucial for energy production and has been implicated in several neurodegenerative disorders. Parkinson's disease (PD) is characterized by the near selective loss of dopaminergic neurons in the substantia nigra (SN) and the abnormal pathological accumulation of hyperphosphorylated α-synuclein (αSyn) in affected neurons throughout the neuraxis. Mild CI deficits have been observed in the SN of idiopathic PD patients. Therefore, we explored whether CI deficiency could be a contributing factor in altered αSyn proteostasis. We modeled CI deficits by utilizing HEK293 lines exhibiting different levels of CI deficits: I) lines with partial CI deficit due to loss of the CI assembly factor NDUFAF2 or CI subunit NDUFV3 and II) lines with total loss of CI due to lack of CI subunits such as NDUFS4 and NDUFB10. To model αSyn pathology, cells overexpressing αSyn in the absence or presence of synthetic pre-formed fibrils (PFFs) were used to induce abnormal αSyn accumulation. αSyn phosphorylation (pS129) and aggregation were examined by three complementary methods: i) immunocytochemistry (ICC), ii) immunoblotting of TritonX-100 soluble and insoluble fractions, and iii) self-association of αSyn through bimolecular fluorescent complementation. CI deficiency resulted in a) an increased number of cells with detectable pS129 and an elevated pS129 intensity per cell was detected by ICC with both mild αSyn stress (- PFFs) and severe αSyn stress (+ PFFs); b) a higher number of cells exhibiting self-associated α-synuclein; and c) an increased proportion of total and pS129 αSyn in the Triton-X100 soluble fractions (-PFFs) and insoluble fractions (+PFFs), together implying increased propensity for αSyn aggregation due to CI deficits with not only severe, but also mild αSyn insult. The comparative analysis across multiple CI mutants with different levels of CI deficiency revealed a unique finding that the level of αSyn aggregation did not correlate with the severity of CI deficiency. Instead, effects on αSyn proteostasis due to mild CI deficits were comparable to those observed following a complete loss of CI activity. These results demonstrate that even mild CI deficits, as observed in idiopathic PD patient brains, have detrimental consequences on cellular αSyn proteostasis. Ongoing studies are being conducted to understand how CI deficiency alters αSyn homeostasis and neuronal loss in animal models and stem cell-derived neuronal systems. Our results provide crucial new insights into the influence of mitochondrial dysfunction on protein deposition and disease in PD and other synucleinopathies.

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Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.11/C33

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01NS132293-01

Title: Pff-induced lewy-like pathology yields deficits in cognitive flexibility and reductions in both cortical excitability and cortico-striatal transmission in mice

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Abstract: Lewy body dementia (LBD) is a complex group of neurodegenerative disorders characterized by the accumulation of α-synuclein aggregates in the brain, leading to various cognitive, motor, and psychiatric symptoms. Among these manifestations, executive dysfunction stands out as a prominent and debilitating feature, significantly impacting the daily functioning and quality of life of affected individuals. Despite the recognition of executive dysfunction as a hallmark of LBD, the role of α-synuclein aggregation in specific brain areas in executive dysfunction remains poorly understood. One brain area which likely contributes to executive function impairments is the prefrontal cortex, which is widely acknowledged as a nexus for executive function and an area that exhibits robust lewy pathology. To investigate the involvement of α-synuclein aggregation in the prefrontal cortex in LBD symptoms, we utilized a combination of pharmacological, electrophysiological, and behavioral approaches in the pre-formed fibril (PFF) mouse model of LBD at 6-weeks and three-months post-surgery. Increasing time from surgery in the current investigation corresponds with increased α-synuclein aggregation. The current investigation focused on the role that pathological α-synuclein has on layer V pyramidal neurons within the pre-limbic medial prefrontal cortex (PL-mPFC), an area shown to be functionally homologous in both humans and mice. Our findings reveal significant alterations in the intrinsic properties of PL-mPFC neurons, including increases in rheobase and decreases in intrinsic excitability that became more pronounced at later time-points. Moreover, we observed substantial reductions in prefrontal cortico-striatal transmission that became more pronounced at later time-points. Overall, our findings suggest that pathological α-synuclein induced neuronal hypoexcitability in the PL-mPFC. Furthermore, we used a visual discrimination task to assess cognitive flexibility in mice previously injected with PFFs. Our results reveal that both monomer and PFF animals are able to learn this task initially at 6-weeks, but that PFF mice show deficits in reversal. Furthermore, we found that at 3-months post-PFF injection, mice show deficits in acquisition of this visual discrimination task, further suggesting that increased burden of α-synuclein aggregates decreases executive function. Altogether, these results provide key insights into the intricate interplay between neuropathology and executive dysfunction in LBD, providing a possible avenue for targeted interventions and improved management strategies for affected individuals.

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Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.12/C34

Topic: C.03. Parkinson's Disease

Support: HMRF 15163051

Title: Investigating the pathological mechanisms for the spread of α-synuclein in Parkinson's Disease

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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide. Loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and Lewy bodies in neurons are two major pathological hallmarks of the Parkinson's disease (PD). The spread of α-synuclein to other brain regions may explain deficits of brain functions other than motor problems. However, it is still a major question in PD research field. While free radicals play an important role in the pathogenesis of PD, it is unclear whether this factor also makes impact on the spreading of α-synuclein to other brain regions. This study aims to investigate whether free radicals affect the spread of α-synuclein in experimental models of PD. In 6-hydroxydopamine (6-OHDA) mice model, our results show that at 3 weeks after the injection, there was significant motor function impairment and TH^+ cell loss (80.34 ± 2.48%) in the ipsilateral side of the SNpc, compared with control group. At 5 weeks after the injection, we observed significant increase of oxidative stress level in the CA1, CA2 and CA3 regions of the hippocampus in 6-OHDA group, compared with control group (all $P < 0.05$, n = 5). However, there was no significant change in cognitive impairment, neuronal loss, and phosphorylation of α-synuclein/tau proteins in the ipsilateral hippocampus. In another experimental PD model by intracerebral injection of adeno-associated virus expressing human α-synuclein (AAV-α-synuclein), although the spread of α-synuclein in the hippocampus was observed at 5 weeks after the injection of AAV-α-synuclein, no significant change of oxidative stress level and phosphorylation of tau proteins was found in the hippocampus (all $P > 0.05$, n = 4). Taken together, these results suggest that free radicals is not sufficient to induce neurodegeneration in the hippocampus and cognitive impairment. On the other hand, rapid increase of α-synuclein may not induce neuronal loss in the hippocampus. Therefore, we will further investigate if free radicals can deteriorate the pathogenesis of α-synuclein in PD.

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Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

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Program #/Poster #: PSTR391.13/C35

Topic: C.03. Parkinson's Disease

Support: NIH-NINDS award 1R01NS128467

Title: Identifying regional differences in the Lewy pathology interactome with in situ proximity labeling

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Abstract: Parkinson's disease (PD) is a complex neurodegenerative disorder in which dopaminergic neurons of the nigrostriatal pathway are destroyed. The main pathological feature of PD is the appearance of intracellular inclusions broadly defined as Lewy pathology (LP) which primarily contain alpha-synuclein (asyn) phosphorylated at the serine 129 residue (PSER129). Understanding the interactions of LP within the cellular milieu is critical to understanding mechanisms of PD initiation and progression. Here we determined the LP-interactome in the caudate, putamen, and substantia nigra (SN) of the PD brain using the in-situ proximity labeling method called biotinylation by antibody recognition (BAR). To do this, brain regions including the SN, caudate and putamen were manually dissected from formalin-fixed 40µm thick brain sections. Specimens were proximity labeled using BAR with anti-PSER129 antibody EP1536Y (BAR-PSER129). BAR-labeled proteins were purified and characterized by dot blot. BAR-PSER129 capture proteins were identified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Dot blots of BAR-labeled fractions confirmed enrichment of asyn and biotin (>1.5-fold increase). LC-MS/MS revealed 597 BAR-PSER129 enriched proteins. Pathway analysis of BAR-PSER129 proteins across the nigrostriatal pathway revealed PD KEGG pathway enrichment as well as Gene Ontology (GO) cellular components for extracellular vesicle (padj 6.449e-51) and cell junction (padj 5.774e-28). Top GO cellular components for SN, caudate, and putamen were extracellular exosome (padj 8.478e-23), secretory vesicle (padj 3.357e-7), and extracellular vesicle (padj 3.834e-27), respectively. BAR-PSER129 successfully identified LP-interactions in distinct brain regions and many of the BAR-PSER129 identified proteins were within known PD pathways. Vesicle pathways were a prominent feature of all nigrostriatal regions tested. Overall, BAR is a viable approach for identifying the pathological asyn interactome including identification of regional and subcellular pathological interactions in the human PD brain.

Disclosures: T.R. Tittle: None. **S. Choi:** None. **Y. Chu:** None. **J. Kordower:** None. **B.A. Killinger:** None.

Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.14/C36

Topic: C.03. Parkinson's Disease

Support: NIH Grant AG061251

Title: Deciphering The Role Of Heparan Sulfate In The Progression Of α-synuclein Pathology

Authors: *S. DIGRASKAR[1], J. HUH[2], P. DAS[2], L. A. VOLPICELLI-DALEY[3], R. M. COWELL[2], J. ESKO[4], P. AGUILAR CALVO[5];
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Abstract: The aggregation and deposition of misfolded α-synuclein (α-syn) are key characteristics of synucleinopathies such as Parkinson's Disease (PD) and Dementia with Lewy bodies (DLB). It has been proposed that α-syn aggregates are transmitted between cells trans-synaptically. However, pathological α-synuclein (α-syn) has been found in extracellular fluids (EC) of PD and DLB patients, and mounting evidence suggests that EC α-syn plays a pivotal role in the progression of α-syn pathology. Importantly, the receptors responsible for α-syn uptake are not yet fully understood. Heparan sulfate proteoglycans (HSPGs) are glycoproteins that colocalize with α-syn deposits in PD brains and act as receptors for the internalization of α-syn fibrils in immortalized cells. We hypothesize that HSPGs modulate α-syn pathology by mediating the *neuronal* internalization and aggregation of α-syn. Given that HSPGs interact with ligands through the negatively charged sulfate groups in their HS chains, we investigated how decreasing neuronal HS sulfation impacts the *in vivo* propagation of α-syn fibrils. First, we cultured neurons from mice conditional knockout for the N-Deacetylase/N-Sulfotransferase-1 (*Ndst1* $^{f/f}$), the enzyme responsible for the N-sulfation of HS chains, and infected them with lentiviral vectors expressing Cre recombinase to decrease HS sulfation. Cells were incubated with 488-tagged syn preformed fibrils (PFFs) and, fibril incorporation was measured using a confocal microscope. We found that depleting *Ndst1* expression profoundly reduced the neuronal incorporation of PFFs, which indicates that HSPGs are major receptors for the neuronal internalization of α-syn fibrils. To explore the contribution of HS in α-syn pathology *in vivo*, we injected PFFs into the striatum of mice that express lower *neuronal* HS sulfation (*NdstSynCre*$^{+}$) and physiological levels of sulfation (*NdstSynCre*$^{-}$). At 7 weeks post-injection, we observed significantly fewer α-syn lewy bodies, as well as fewer lewy neurites in the brains of *NdstSynCre*$^{+}$ mice. Moreover, our behavioral tests show that *NdstSynCre*$^{+}$ mice had less anxiogenic behavior than *NdstSynCre*$^{-}$ mice, suggesting that reducing neuronal HS sulfation might have delayed synuclein pathology. Our studies provide the first *in vivo* evidence of HSPGs as endogenous cofactors for the cellular incorporation and aggregation of α-syn, contributing to the clinical and pathological presentation of synucleinopathy. Based on these findings, our

research highlights the impairment of the HSPG: α-syn interactions as a potential therapeutic strategy.

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Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

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PICT-FONCyT-MINCyT (PICT 2020-00937)
CONICET
PICT-FONCyT-MINCyT (PICT 2018-01825)

Title: Protein trafficking defects as a possible contributing mechanism for alpha-synuclein-induced neurodegeneration

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Abstract: Intracellular trafficking is a critical process that requires a complex set of key proteins to perform efficiently. In neurons, the precise functioning of the intracellular trafficking machinery is essential due to the unique cytoarchitecture characterized by axons and dendrites extending a considerable distance from the soma. Defects in intracellular trafficking have been described as a common feature of several neurodegenerative diseases. In Parkinson's disease and synucleinopathies, a major pathogenic factor is the dysregulation of alpha synuclein (AS). Although the mechanisms by which this protein causes neurodegeneration are not clearly understood, there are several hypotheses, including the possibility that AS may cause defects in intracellular trafficking. In this study, we used a mammalian neuronal model to investigate whether AS affects protein trafficking. Using a novel tool with specific domains for synchronizing intracellular trafficking, we investigated changes in the biosynthetic pathway dynamics. Our results show that AS induces trafficking deficits selectively on different proteins. We used p75 neurotrophin receptor (p75NTR) and transferrin receptor (TfR) as model proteins. Our results provide evidence that AS disrupts p75NTR trafficking by reducing its fluorescence intensity in neuronal processes (reduction of 25,3% ± 5,6; three replicates with 5-6 neurons per condition, $p<0,0005$), while TfR fluorescence levels remained unchanged. This selective effect

led us to further investigate the influence of AS on the biosynthetic pathway, revealing differential effects on the Golgi vesicle fission machinery of these two proteins. According to our studies, AS causes defects in the p75NTR fission machinery of p75NTR, reflected by a decrease in the size of vesicles trafficking this receptor (reduction of 22,1% ± 3,1; three replicates with 5-6 neurons per condition, $p<0,05$). Importantly, these experiments were performed using both confocal and STED super-resolution microscopy. Finally, our evidence suggests that AS stabilizes the actin cytoskeleton, which affects the Golgi vesicle fission machinery for p75NTR cleavage. Surprisingly, expression of a constitutively active cofilin completely rescued p75NTR trafficking as reflected by fluorescence levels and vesicle size. This work provides new evidence about the mechanisms by which AS induces neurodegeneration, enhancing our understanding of Parkinson's disease and synucleinopathies.

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Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.16/C38

Topic: C.03. Parkinson’s Disease

Support: NINDS Grant 1R01NS128467
Michael J. Fox foundation Grant MJFF-022480
NINDS Grant R21NS109871
NINDS Grant ASAP-024442

Title: Phosphatase pretreatment differentiates aggregated from non-aggregated phosphorylated alpha-synuclein

Authors: *S. CHOI[1], T. TITTLE[1], D. GARCIA PRADA[1], J. KORDOWER[2], R. MELKI[3], B. A. KILLINGER[1];
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Abstract: Synucleinopathies, including Parkinson’s disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA) are neurodegenerative diseases characterized by the accumulation of alpha-synuclein (αsyn) aggregates in the nervous system. αSyn phosphorylated at serine 129 (PSER129) is a standard marker of αsyn aggregates and is considered rare in the healthy human brain. However, recent studies have shown that non-aggregated, non-pathological PSER129 occurs in the healthy mammalian brain and may result from neuronal activity. As a result, differentiating between aggregated and non-aggregated PSER129 is a critical point to be addressed in the synucleinopathy field. We hypothesize that treatment with the non-specific calf-

intestine alkaline phosphatase (CIAP) could be used to differentiate aggregated and non-aggregated PSER129. We developed and applied the CIAP treatment protocol using tissues from transgenic mice (e.g., M83) injected unilaterally with αsyn preformed fibrils (PFFs) or PBS into the olfactory bulb (OB) and human synucleinopathy tissues. Immunohistochemistry revealed that PSER129 was abundant across the neuroaxis of M83 mice injected with either PFFs or PBS. Following CIAP pretreatment, PSER129 was undetectable in PBS-treated mice. In contrast, PFF-treated mice showed strong PSER129 immunoreactivity in multiple brain regions, including the OB, piriform areas, entorhinal cortex, and amygdala (e.g., regions of the olfactory system), as well as in the hypothalamus, white matter, midbrain, and brainstem. The apparent CIAP-resistant PSER129 (CIAP-PSER129) in PFF-treated mice consisted of dense neurites and neuronal cell bodies and was also proteinase K resistant. In human synucleinopathy (PD and MSA) midbrain tissues, CIAP pretreatment did not significantly affect PSER129 immunoreactivity. Denaturation of aggregate-containing tissue samples (mouse and human) prior to CIAP pretreatment abolished PSER129 CIAP resistance. αSyn aggregates contain CIAP-resistant PSER129. Pretreatment of tissues with CIAP increases the sensitivity and specificity for detecting aggregated PSER129 and allows for differentiation of the two distinct PSER129 pools. CIAP-PSER129 revealed progressive prion-like αsyn spread across the entire neuroaxis of M83 mice injected with PFFs into the OB. CIAP pretreatment is non-destructive, making it particularly useful for downstream applications such as mass spectrometry-based protein identification. Future studies should focus on utilizing the CIAP method to explore the transition from non-aggregated to aggregated PSER129 in disease models and human synucleinopathy brains.

Disclosures: S. Choi: None. **T. Tittle:** None. **D. Garcia Prada:** None. **J. Kordower:** None. **R. Melki:** None. **B.A. Killinger:** None.

Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.17/C39

Topic: C.03. Parkinson’s Disease

Support: NIH/NINDS NS124226
NIH/NINDS R33 NS112441
NIH/NIA U01 AG074960

Title: The effects of norepinephrine on phagocytosis of alpha-synuclein in microglial cells

Authors: *A. A. OTTO[1], A. EALY[2], P. PADHI[2], G. ZENITSKY[2], H. JIN[2], V. ANANTHARAM[2], A. KANTHASAMY[2], A. G. KANTHASAMY[2];
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Abstract: Norepinephrine (NE) deficiency due to selective degeneration of locus coeruleus (LC) neurons during the prodromal stages of Parkinson's Disease contributes to numerous non-motor symptoms, including depression and anxiety. While LC is one of the primary regions that is affected by alpha-synuclein (αSyn) pathology, recent studies have elucidated that conformational-specific oligomeric forms of αSyn alter neuronal homeostasis and dysregulate LC-derived NE neurotransmission potentiating early PD pathophysiology. Microglia is quintessential for brain development, immune response, and disease recovery, yet studies elucidating whether NE deficiency worsens αSyn pathology due to functional alterations in microglial phagocytosis is unclear. As the brain-resident immune cell, microglia recognize misfolded proteins via pattern recognition receptor binding and subsequently activate inflammatory pathways in vitro and in vivo evidence, thereby implicating microglial-mediated clearance of αSyn may be a potential neuromodulatory target. To test this hypothesis, we first assessed whether NE improves the phagocytotic function of aggregated αSyn using an immortalized human microglia cell line (C20) by FITC-phagocytosis assay, western blotting, and immunocytochemistry. We observed a time and dose-dependent increase in aggregated pre-formed fibril forms of αSyn following 0.1, 1, and 10μM of NE in C20 microglia in ICC. We further observed that NE priming likely fragmented the aggregated pre-formed fibril forms of αSyn confirmed by colocalization experiments between LAMP-2, LC3, and Cell Light-Early Endosomal Marker, for lysosomal tracking and autophagy markers. Preliminary findings suggest that this phasic phagocytotic response occurs in a short timescale (15 min- 1H), following rapid degradation. Overall, C20 human microglia is suitable in vitro to study phagocytosis and our future study will explore αSyn PFF in primary mouse cells and mice models of PD. Future investigation will shed light on novel biotherapeutic strategies and understanding their disease mechanisms. Supported by NIH/NIA U01 AG074960, NIH/NINDS R33 NS112441, and NIH/NINDS NS124226

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Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.18/C40

Topic: C.03. Parkinson's Disease

Support: Sastry Foundation Endowed Research Fund

Title: Upregulation of the polyamine catabolic enzymes SAT1 and SMOX in Neurons Mitigates alpha-synucleinopathy in a Drosophila Model of Parkinson's Disease

Authors: *B. RANXHI[1], Z. BANGASH[1], Z. CHBIHI[1], S. V. TODI[1], P. A. LEWITT[2], W.-L. TSOU[1];
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Abstract: Spermine oxidase (SMOX) and spermidine/spermine N1-acetyltransferase (SAT1) are key enzymes involved in the metabolism of L-ornithine-derived polyamines, a group of highly conserved molecules that play critical roles in cellular processes such as DNA stabilization, gene expression, and ion channel regulation. These enzymes are integral to the polyamine catabolic pathway, maintaining cellular homeostasis and response to stress. Both SMOX and SAT1 are regulated by cellular polyamine levels and participate in feedback mechanisms that ensure polyamine homeostasis. Dysregulation of SMOX and SAT1 has been implicated in the pathogenesis of neurodegenerative diseases. Hence, targeting SMOX and SAT1 pathways may be a potential therapeutic strategy for mitigating polyamine dysregulation and ameliorating neurodegenerative disease progression. In this study, we introduced two novel Drosophila lines overexpressing SAT1 and SMOX. Neuronal overexpression of either SAT1 or SMOX in a Drosophila model of Parkinson's disease characterized by hSNCA overexpression results in extended lifespan and improved motility. Importantly, we observed a significant reduction in α-synuclein protein levels upon overexpression of SAT1 and SMOX. Furthermore, we examined the levels of LC3, a protein involved in autophagy, and found that overexpression of α-synuclein leads to a decrease in LC3 levels. However, overexpression of SAT1 enhances LC3 levels, suggesting enhanced autophagic activity (which may facilitate the clearance of α-synuclein aggregates and protect against neuronal cell death). Our findings suggest that targeting the polyamine pathway could offer a promising therapeutic approach. While further investigation is needed to elucidate the relationship between SAT1, SMOX, and LC3, our results underscore the importance for understanding L-ornithine-derived polyamine metabolism in the pathogenesis of Parkinson's disease.

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Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.19/C41

Topic: C.03. Parkinson’s Disease

Support: Sastry Foundation Parkinson’s Disease Research

Title: Polyamine Metabolism Impacts α-Synuclein-Dependent Neurodegeneration: A Drosophila Perspective on pathogenesis of Parkinson's Disease

Authors: Z. BANGASH, B. RANXHI, Z. CHBIHI, S. V. TODI, P. A. LEWITT, *W.-L. TSOU; Wayne State Univ., Detroit, MI

Abstract: L-ornithine-derived polyamines (PAs) are essential and highly conserved molecules mediating multiple cellular processes, including cell growth, nucleic acid synthesis, and apoptosis regulation. These PAs have been widely studied in oncology, gastrointestinal pathologies, and some neurodegenerative diseases, but their detailed contribution mechanisms to these conditions are not fully understood. Recent studies have shown significant increases in the serum concentrations of the L-ornithine-derived PA (putrescine, spermine, and spermidine), each correlated to Parkinson's disease (PD) progression and its clinical subtypes. Given the pivotal roles of these PAs and the critical need for their homeostatic regulation, our research aims to determine whether these biomarkers afford mechanistic insights into the neurodegenerative processes of PD. In this study, we investigated the role of PA metabolism in PD progression using *Drosophila melanogaster* models that overexpress human α-synuclein (α-syn), PD's pathological trigger protein, to systematically modulate PA metabolic pathways. We genetically knocked down key enzymes involved in PA interconversion pathways, including ornithine decarboxylase (ODC1), spermidine/spermine N1-acetyltransferase 1 (SAT1), spermidine synthase (SRM), and spermine oxidase (SMOX). Specifically, we observed a significant increase in fly longevity and fly motility when SMOX is suppressed. Furthermore, we assessed the integrity of the fly eyes when RNAi targeting PA metabolism enzymes are co-expressed with α-syn. Our results demonstrated that knockdown of SMOX and SRM enhanced eye integrity, while suppression of other enzymes such as PAOX, SMS, SAT1, and ATP13A3 resulted in attenuated eye integrity. This study provides novel insights into the neuronal and systemic changes associated with PA metabolism that may be relevant for PD pathogenesis. The *Drosophila* model of manipulating PA interconversion enzymes and their metabolites offers a valuable experimental platform for investigating the neurodegenerative process, particularly regarding α-syn aggregation. Our findings hold promise for the development of therapeutic interventions targeting L-ornithine-derived PA pathways in neurodegenerative diseases.

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Poster

PSTR391

Alpha-Synuclein: Mechanisms and Transmission

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.20/C42

Topic: C.03. Parkinson's Disease

Title: The formation, expansion and dissolution of alpha-synuclein inclusions is modulated by a genetic network acting through phase separation

Authors: E. EUBANKS[1], K. VANDERSLEEN[1], R. KIMELMAN[1], N. PATEL[1], B. SHARMA[1], J. LIU[2], S. ARCHAKAM[1], P. CHINCHILLA[1], J. ELLIOTT[3], M. DARESTANI FARAHANI[1], P. SOCHA[1], J. BAUM[1], M. MOURADIAN[4], Z. SHI[1], W. DAI[1], J. HARDY[5], ***E. KARA**[1]; [1]Rutgers Univ., Piscataway, NJ; [2]Neurol., Rutgers - Robert Wood Johnson Med. Sch., Piscataway, NJ; [3]Chem. & Chem. Biol., Rutgers Univ., Piscataway, NJ; [4]Neurol., Rutgers-Robert Wood Johnson Med. Sch., Piscataway, NJ; [5]Univ. Col. London (UCL), London, United Kingdom

Abstract: Introduction: α-synuclein (αSyn) accumulates in the brains of patients with Parkinson's disease (PD) and forms inclusions in neurons called Lewy Bodies (LBs). LBs initially form in the brainstem and, as the disease progresses, are also observed in rostral regions. It has been hypothesized that this apparent spread of pathology is caused by the prion-like cell-to-cell transfer (propagation) of αSyn.Results: To understand the mechanism underlying the propagation of αSyn, we did a high throughput screen. We cloned a construct encoding GFP-T2A-αSyn-RFP, with which we transiently transfected a HEK QBI cell line overexpressing wild type αSyn. The translated protein is cleaved at the 2A position, thus producing two independent proteins: GFP and αSyn-RFP. The latter then transfers to neighboring cells and the populations can be identified based on their colors: donor cells are RFP+GFP+ and recipient cells are RFP+GFP-. We used this model system to complete a genome wide, imaging based, arrayed siRNA high throughput screen that identified 38 genes whose knock down modifies the propagation of αSyn. Several of those genes have been implicated in the pathogenesis of neurodegeneration. Weighted gene coexpression network analysis (WGCNA) using gene expression data from multiple regions from healthy human brains showed that the 38 genes co-cluster with known PD genes in the same gene expression modules more frequently than expected by random chance. Follow up experiments in a novel tissue culture model system showed that αSyn molecules are mobile within inclusions as tested by FRAP experiments. This is consistent with the inclusions being liquid condensates formed via phase separation. Knock-down of two of the 38 genes increases the number and decreases the size of αSyn inclusions by modulating phase separation. One of those genes is also involved in pathways regulating the biogenesis of lipid droplets, and its knock-down affects the co-condensation between αSyn and lipid droplets. Analyses are in progress to determine whether the underlying protein networks are enriched in intrinsically disordered proteins, and whether dysregulation of phase separation on a larger scale is the common mechanism underlying αSyn dysregulation in PD.Conclusion: There is a link between αSyn aggregation, propagation and phase separation, and underlying pathways are regulated by genetic networks that dysfunction in PD.

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Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.01/C43

Topic: I.08. Methods to Modulate Neural Activity

Support: National Key R&D Program of China (2021YFF0702200)
STI 2030-Major Projects (2021ZD0200401)
Key R&D Program of Zhejiang Province (2021C03001)

Title: Development of a Long, Flexible, MRI-Compatible Stimulation Probe for Enhanced Deep Brain Stimulation and Functional MRI Integration in Nonhuman Primates

Authors: *H. WANG[1,2,3], Z. TANG[2,3], M. YE[1,2,3], L. LAN[2,3,4], B. QU[1,2,3], Z. LYU[2,3], Y.-Y. CHEN[5], H.-Y. LAI[1,2,3,6];
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Abstract: The combined application of local deep brain stimulation (DBS) and whole-brain functional magnetic resonance imaging (fMRI) is crucial for advancing neuroscience research and treating neurological and psychiatric disorders. However, conventional DBS probes often generate MRI artifacts due to magnetic susceptibility differences, and their large electrodes can inadvertently stimulate adjacent areas, potentially distorting the neural analysis or causing adverse effects. To address these issues, we developed a long, flexible, and MRI-compatible stimulation probe (LFMSP) that minimizes artifacts and unintended stimulation. We implanted LFMSP into the brain of a nonhuman primate (NHP) and used ultra-high-resolution 7T MRI to acquire T1-weighted imaging, T2-weighted imaging, diffusion-weighted imaging and fMRI. Targeting the nucleus accumbens/anterior limb of the internal capsule (NAc/vALIC) for stimulation, a region implicated in treating treatment-resistant depression. This 68-mm long probe with 16 stimulation electrodes (each 30-μm diameter) utilizes polyimide and ultra-thin layers of gold and copper to finely tune Young's modulus and magnetic susceptibility. Using this probe, we achieved precise local stimulation of the NAc/vALIC with biphasic currents below 100 μA, eliciting spatially localized fMRI responses (~ 13 mm^3) in the anterior cingulate cortex (ACC). The 200 μm inter-electrode spacing and various electrode combinations enabled selective stimulation across different areas. At stimulation current was below 100 μA, adjacent electrodes achieved spatial selectivity in various ACC response regions. Post-implantation MRI data showed no significant changes, indicating stable long-term interaction at the tissue-LFMSP interface. The LFMSPs consistently elicited stable BOLD responses in the ACC over three months. These results affirm that the LFMSP is MRI-compatible and effective for long-term, selective local stimulation in NHP, representing a significant advancement in brain research tools.

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Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.02/C44

Topic: I.08. Methods to Modulate Neural Activity

Support: NINDS R01NS125143

Title: Automated deep brain stimulation parameter selection via meta-active learning of evoked potentials

Authors: *E. R. COLE[1], M. SCHRUM[2], E. OPRI[3], L. CHEN[4], A. NASCIMENTO[5], F. ISBAINE[6], S. BORGHEAI[7], R. E. GROSS[8], M. GOMBOLAY[9], S. MIOCINOVIC[10]; [1]Biomed. Engin., Georgia Technol. and Emory Univ., ATLANTA, GA; [2]Univ. of California, Berkeley, Berkeley, CA; [3]BME, Univ. of Michigan, Ann Arbor, MI; [4]Col. of Computing, Georgia Inst. of Technol., Atlanta, GA; [5]Georgia Inst. of Technol., Atlanta, GA; [6]Neurosurg., Emory Univ., Atlanta, GA; [7]Emory Univ., Decatur, GA; [8]Neurosurg., Rutgers Univ., New Brunswick, NJ; [9]Sch. of Interactive Computing, Georgia Inst. of Technol., Atlanta, GA; [10]Neurol., Emory Univ., Atlanta, GA

Abstract: Introduction: Deep brain stimulation (DBS) parameters must be selected manually for each patient in a months-long trial-and-error process, delaying optimal clinical outcomes. Objective: We are developing a fully automated approach to optimize STN-DBS parameters for Parkinson's disease intraoperatively. Methods: Our approach uses meta-active learning (meta-AL), an AI method that learns from a prior patient database to interactively apply stimulation parameters, measure the effect on neural biomarkers in real-time, and iterate to optimize parameters most quickly in new patients. Its optimization goal is to maximize DBS local evoked potentials (DLEP) while minimizing EMG-measured motor evoked potentials (mEP)-electrophysiological biomarkers of pathway activation for symptom relief and side-effects, respectively. Results: Using annotated EMG recordings from 9 patients, we first develop and validate an automated mEP detection algorithm with 92% accuracy in identifying which DBS settings induced mEP. We then apply meta-learning to predict DLEP and mEP values as a function of stimulation parameters in 52 intraoperative patients (mean 28 settings/patient), showing that a meta-trained neural network can predict biomarker values for unseen parameter combinations with high cross-validation accuracy (DLEP: 0.879 correlation, mEP: 0.767). We then use this modeled data to provide the basis for a simulation environment where meta-AL can practice DBS programming, showing that meta-AL can identify optimal parameters of simulated patients *in silico* within 50 tested stimulation settings and more efficiently than various control

methods. Last, we demonstrate an open-source system for real-time biomarker recording and parameter selection in Python, with our next step being to deploy this method in patients. Conclusion: Long-term, our approach could reduce DBS programming time by providing expected best parameters before clinical testing and could enable effective programming for other DBS applications (epilepsy, neuropsychiatric diseases, etc.) where behavioral feedback is limited.

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Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.03/C45

Topic: I.08. Methods to Modulate Neural Activity

Support: DARPA: N65236-19-C-8017

Title: Floates: a passive implant for minimally invasive deep brain stimulation (dbs)

Authors: *V. JAIN[1], M. FORSSELL[1], P. GROVER[2], M. CHAMANZAR[3];
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Abstract: The use of deep brain stimulation (DBS) has greatly improved treatments for neurological and psychiatric disorders. However, the invasive nature of DBS procedures brings forth numerous challenges, including risks associated with implanted hardware. Traditional DBS systems, which involve electrodes and pulse generators, may lead to complications such as infections, bleeding, and tissue damage. There are also concerns about long-term issues like electrode malfunction. This necessitates regular monitoring and maintenance. Recent data highlights the urgent need for innovative solutions to tackle hardware-related problems. This work introduces FLOATES (Floating Line Operating Afar Transcranial Electrical Stimulation), a new hybrid DBS model that combines a non-invasive high-density surface electrode array with an invasive untethered insulated wire. This work builds upon our prior demonstration of high resolution steerable transcranial electrical stimulation (TES) in mice model.
To demonstrate proof-of-concept for FLOATES, deep brain regions i.e., subthalamic nuclei (STN) in C57Bl6 mice were targeted to induce motor evoked potentials (MEP) in forelimbs. Animals were divided in 3 different groups targeting STN using 1) Intracortical microstimulation (ICMS), 2) Transcranial stimulation using surface electrode arrays and 3) FLOATES, combination of surface electrode and untethered wire. C-fos staining was also performed to demonstrate targeted stimulation at the cellular level.

Results showed that motor thresholds resulting from stimulation through FLOATES are up to 3 times smaller than TES stimulation at the same location on the skull in the absence of the implanted wire. This demonstrates that transcranial current is coupled to the wire and reaches the distal end located in STN. This demonstrates the effectiveness of FLOATES in facilitating charge/current coupling through the wire to reach deep brain regions like the STN, eliciting behavioral responses without stimulating brain regions along the floating wire's path. C-fos staining also revealed that FLOATES resulted in targeted stimulation of STN and other connected regions controlling motor response.
FLOATES may effectively address hardware-related challenges associated with traditional DBS methods and offers potential therapeutic benefits. This innovative approach enables deep brain stimulation, while reducing the risks of tethered DBS systems, thus making it a promising option for treating movement disorders such as Parkinson's disease and essential tremor, as well as psychiatric disorders like depression, obsessive-compulsive disorder, and addiction.

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Poster

PSTR392

Deep Brain Stimulation: Parkinson’s Disease

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.04/C46

Topic: C.03. Parkinson’s Disease

Support: NIH/NINDS Grant (K23NS097576; P50 NS123103-01)

Title: The effect of deep brain stimulation and activation of the prefrontal cortico-STN hyperdirect pathway on response inhibition in patients with Parkinson’s disease

Authors: *R. JAFARI DELIGANI[1], B. HOWELL[2], C. MCINTYRE[2], S. MIOCINOVIC[1]; [1]Neurol., Emory Univ., Atlanta, GA; [2]Duke Univ., Durham, NC

Abstract: Deep brain stimulation (DBS) has variable effects on the ability to inhibit unwanted actions (response inhibition) in people with Parkinson’s disease (PD). This has been attributed to the location of stimulation within the subthalamic nucleus (STN), but much is still unknown. We hypothesized that: 1) maximizing the activation of prefrontal cortico-STN hyperdirect projections (prefrontal hyperdirect pathway) with DBS impairs proactive response inhibition compared to minimized activation of these projections; and 2) DBS -on (optimized by a clinician) improves motor response reaction time compared to DBS-off in people with PD. We used a cued Go/NoGo task where a left/right cue is presented on screen followed by a Go signal requiring a button press. On 25% of trials, a NoGo signal is given instead which mandates withholding the planned response. We measured median response times and percent NoGo response errors. Patients participated in two sessions (both off medication) within a two week period. Each session included two DBS settings (random order) with 30 minutes wash in period

as follows: session 1) clinical and sham (0.1 mA/2 Hz) and session 2) maximized (max) and minimized (min) activation of prefrontal hyperdirect pathway. To determine the optimal stimulating contact for max and min settings, we used predictions from patient-specific biophysical DBS models. The models used driving force predictor functions to estimate percent activation of prefrontal hyperdirect pathway in a connectomic atlas in patient (preoperative T1) space. Additional setting parameters were 2.5mA/60μs/130Hz (with amplitude reduction in 0.1 mA steps if setting was intolerable). We report results for 15 subjects (1 woman, age 60.6±10 years, disease duration 10.9±3.1 years). The response time (sham 453±113ms; clinical 417±110ms; max 428±127ms; min 432±115ms) had a trend toward shorter in the clinical setting compared to sham setting (p=0.09; paired Wilcoxon signed rank test). NoGo response errors (sham 7±6%; clinical 6±9%; max 11±9%; min 8±13%) did not differ between max and min settings. There was a trend toward higher errors in max compared to sham setting (p=0.09; paired Wilcoxon signed rank test). In nine patients, the absolute error rate increased more than 3% on max compared to sham. We conclude that STN DBS does not significantly improve response time in patients with PD. The ability to inhibit planned movements is also not affected by STN DBS overall. However, maximizing the activation of prefrontal hyperdirect pathway may lead to inhibition impairment in some patients.

Disclosures: R. Jafari Deligani: None. **B. Howell:** None. **C. McIntyre:** None. **S. Miocinovic:** None.

Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

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Topic: I.08. Methods to Modulate Neural Activity

Support: DARPA N65236-19-C-8017
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Chuck Noll Foundation for Brain Injury Research

Title: Transnasal electric stimulation of deep brain targets in human cadavers

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Abstract: Non-invasive brain stimulation techniques such as transcranial electric or magnetic stimulation (TES/TMS) are attractive due to their ease of implementation in subjects. However, because they rely on injecting energy through the scalp, and the energy is attenuated at larger distances, these methods have difficulty achieving meaningful effects outside of superficial brain

regions. Moreover, many deep brain regions are useful targets for neural stimulation, e.g. controlling reward networks (with applications to addiction or depression), or hormonal release. We propose using minimally invasive electrodes inserted in nasal cavities, enabling electrical stimulation through the skull base to reach targets in the deep brain. Two locations are considered for the placement of the electrodes: the olfactory cleft, where currents can achieve stimulation in the orbitofrontal cortex (OFC), and the sphenoid sinus, from which stimulation of the hypothalamus is possible.

To demonstrate the feasibility of such stimulation, we have performed experiments in human cadavers, measuring electric fields generated in the deep brain by electrodes inserted endoscopically through the nose. Commercial stereotaxic EEG (sEEG) electrodes were for stimulation in conjunction with gold cup electrodes placed on the scalp. For recording, additional sEEG probes were inserted in the brain to target the OFC or diencephalon. The sEEG probes consist of 10 electrodes with 5 mm separation. Electrode positioning was confirmed by post-experiment computer tomography. Stimulation consisted of current pulses of 10 mA amplitude injected between pairs of electrodes, with anode and cathode in one of the nasal locations or on the scalp.

With the anode in the olfactory cleft and cathode on the scalp, the electric field in the OFC, measured 8 mm away from the anode, reaches 150 V/m. With both electrodes in the olfactory cleft, separated by 15 mm, the field reaches 20 V/m at the same location, while placing both electrodes on the scalp results in 7 V/m. With the anode in the sphenoid sinus, electric fields in the diencephalon, 20 mm from the anode, reach 15 V/m with the cathode on the scalp, and 10 V/m with the cathode also in the sphenoid (at a 15 mm distance).

This work demonstrates that placement of stimulation electrodes in the nasal cavities is possible and that transnasal electrical stimulation can elicit electric fields with physiologically useful amplitudes in the deep brain.

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Poster

PSTR392

Deep Brain Stimulation: Parkinson’s Disease

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.06/C48

Topic: I.08. Methods to Modulate Neural Activity

Support: N65236-19-C-8017

Title: Reducing scalp pain during transcranial electrical stimulation of motor cortex in awake subjects

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Abstract: Transcranial electrical stimulation (TES) is a powerful non-invasive brain interfacing modality where current pulses are injected into superficial brain areas from electrodes placed on the scalp. While it has long been known that pulsed TES can directly activate neurons in motor cortex and elicit motor responses, it is not commonly used in awake subjects because unwanted activation of scalp nociceptors causes substantial pain during the injection of current. Transcranial magnetic stimulation (TMS), which can achieve similar outcomes without any painful sensation, is therefore the tool of choice for non-invasive activation of motor cortex in awake subjects. However, TES presents several advantages over TMS, as it allows better control of the injected field, requires simpler instrumentation that can more easily be miniaturized, and has a more direct effect on cortical neurons.
We investigated techniques to reduce the scalp pain during TES of motor cortex in awake volunteers. Electrodes were placed on the scalp above the motor area representing upper limbs (2 cm medial to C3), while electromyography signals were recorded in wrist and intrinsic hand muscles using high-density surface patches and bipolar electrodes. Stimulation consists of repeated injection of single monophasic current pulses. Subjects self-reported pain during stimulation via numeric 1-10 scale.
Different modifications to the stimulation conditions were used and their effect on motor responses and pain determined. The distance between the anode and cathode was reduced from 40% to 20% of the interaural distance, resulting in a larger motor threshold (40%±20% increase) without a quantitative change in the reported pain (difference: 0.01±1.1). However, the qualitative assessment of the scalp sensation indicates that the smaller distance elicits more local sensation, with less activation of temporalis or masseter.
Pulse widths were varied between 50 μs and 1000 μs. The motor threshold fits the equation $I=4655/w+20.2$ (where w is the pulse width in μs; $r2=0.96$). The reported pain at threshold exhibits considerable variability across pulse widths between participants, with the minimum pain at threshold achieved across the entire range of widths.
Finally, the addition of a “background hum”, a low-amplitude (1-5 mA), high-frequency pulse train (100-300 Hz) applied through electrodes placed near the motor electrodes reduced reported pain by 1.65±0.49 compared to the same condition without the hum.
This work demonstrates the possibility of TES of the motor cortex that is not prohibitively painful, enabling wider use in neuroscience research and clinical applications.

Disclosures: R. Tusi: None. **M. Forssell:** None. **J. Kim:** None. **V. Jain:** None. **P. Yadav:** None. **D.J. Weber:** None. **P. Grover:** None.

Poster

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Deep Brain Stimulation: Parkinson’s Disease

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Topic: I.08. Methods to Modulate Neural Activity

Title: Predicting neural responses to DeepFocus, a new technique for transnasal, deep-brain electrical stimulation

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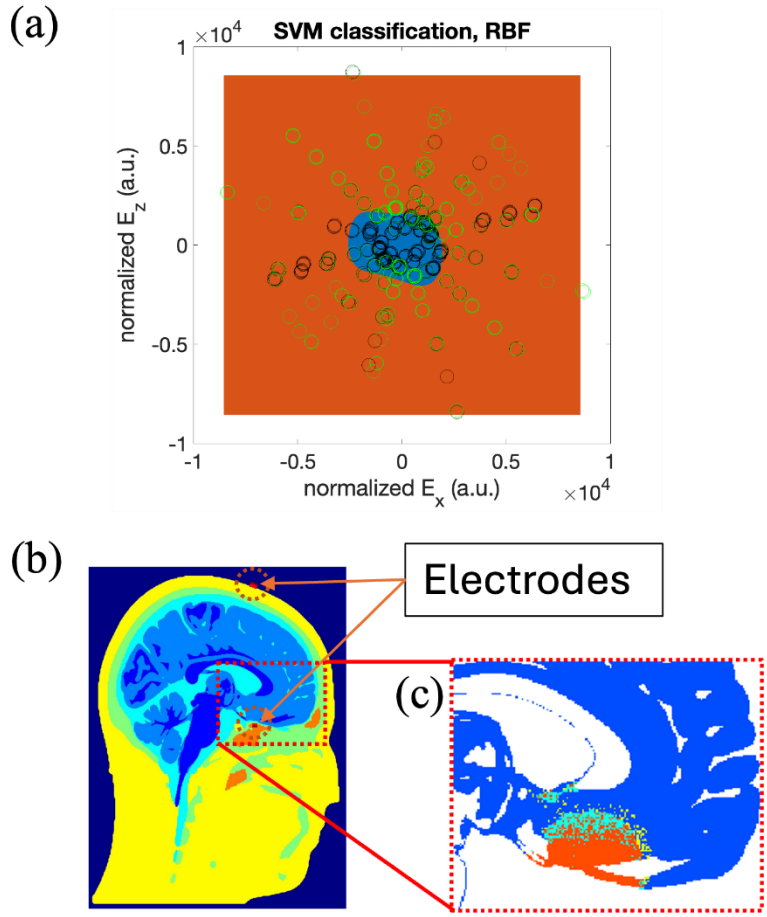
Abstract: A non-invasive alternative to deep brain stimulation could be an accessible way of treating neural disorders, but to be safe, it needs to avoid using prohibitively large currents. We propose DeepFocus, a new minimally invasive deep brain stimulation technique, utilizing electrodes placed in nasal cavities in conjunction with conventionally placed scalp electrodes. To demonstrate its feasibility, we have previously reported ex vivo experiments quantifying the current efficiency benefits of DeepFocus. Here, we utilize the ex vivo data in conjunction with finite element simulations to predict neural responses in a human head model. The simulation predicts responses considering the direction and magnitude of electric fields applied to the brain by DeepFocus.The ex vivo experimental dataset consists of local field potentials (LFPs) recorded in mouse brain slices following stimulation through electrodes placed at various locations relative to the slice, simplifying the geometry of DeepFocus. The simulated electric field components (E_x, E_z) at the target location on the slice, and the LFPs labeled as either “responses” or “non-responses”, were used to train a classifier using a support vector machine with a radial basis function kernel. We applied the classifier to the electric fields simulated using an MRI-derived human head model to predict the regions in which neural responses occur. In a 10 mm-thick sagittal section around the midline, we assumed that axons were oriented perpendicularly to the brain surface.Fig. 1 shows the simulated responses of a DeepFocus configuration with the anode in the sphenoid sinus and cathode on the scalp, including activation of Brodmann 25. Compared to a classic amplitude threshold, the classifier predicts an increase in response volume by 23% in the section considered. This implies that an optimized electric field can reduce the amount of current injected to reach the target area. While simple amplitude thresholds based on electric field simulations provide first-order approximations of neural response, more accurate modeling is obtained here.



(a) Predicted threshold boundary of neural responses. The responses were recorded at cortical layer 5 of the mouse brain. The circles represent the experimental data (black: non-response, green: response). SVM classification with an RBF kernel was used to classify the data. Responses and non-response areas learned by the classifier are represented with blue and orange, respectively. (b) Segmentation of the MRI-derived head model(Dark blue: white matter; blue: gray matter; cyan: cerebrospinal fluid; green: bone; yellow: skin; and orange: air). The two electrodes (red dots) are aligned vertically. The anode is placed on the sphenoid sinus and the cathode is placed on the scalp. (c) Responses predicted by amplitude thresholding of the electric field and by the SVM classifier. The orange area indicates the responses predicted by both methods,, the cyan area shows responses predicted by the SVM classifier only, and the yellow area by the amplitude threshold only.

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Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

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Program #/Poster #: PSTR392.08/C50

Topic: I.08. Methods to Modulate Neural Activity

Support: DARPA N65236-19-C-8017

Title: Tolerability of transcranial electrical stimulation for evoking motor responses in people with fibromyalgia: a pilot study

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Abstract: Fibromyalgia (FM) is a common, serious chronic pain condition of the central nervous system. While pharmaceutical options can be beneficial, alternative treatment is often sought due to the refractory nature of FM. Primary motor cortex (M1) stimulation, such as transcranial magnetic stimulation (TMS), has been linked to neuronal pain circuits and is known to affect pain reduction. Another option is pulsed transcranial electrical stimulation (TES), which may allow greater control than TMS due to its more direct effect on the brain. However, pulsed TES at current levels resulting in cortical responses may cause substantial scalp pain. Further, patients with FM have been reported to have lower electrical pain thresholds [Nørregaard et al. 1997], precluding them from prior applications of pulsed TES. In this study, we optimized TES parameters to reduce scalp pain while obtaining upper and lower limb motor responses on a participant with FM. We recruited one female (F1) who was diagnosed with FM. We placed 10 mm-diameter gold cup electrodes at scalp locations corresponding to M1 regions known to evoke motor responses in the upper and lower limbs when stimulated. To monitor motor responses, we placed high-density electromyography patches on the dominant (right) wrist flexor and extensor muscle groups or bilaterally on the tibialis anterior (TA). We also placed several bipolar electrodes on the right upper arm and hand or on both lower legs and feet. We repeatedly injected monophasic current pulses (width: 200-500 µs, amplitude: 3-85 mA, frequency: 0.33-3 Hz) while F1 reported her perceived pain on a 0-10 numerical rating scale. We adjusted the pulse parameters to increase the injected current while maintaining a self-reported pain level below 6. In addition to the pulse parameters, we included a background “hum” consisting of high-frequency, low-amplitude pulses near the active and return of the gold cup electrodes. We were able to elicit motor responses in the arm and legs at 1 Hz on F1. Motor responses in the arm were visible approximately 25 ms following stimulation at 45 mA with a 200 µs pulse width and were most prominent in the wrist flexors and the hand muscles. Motor responses in the legs were visible approximately 40 ms following stimulation at 80 mA with a 200 µs pulse width and were most prominent in the TA. Further, we were able to stimulate M1 at 3 Hz and evoke motor responses in the wrist flexors. Throughout the sessions, F1 did not report a pain level greater than

6. The ability to target and stimulate the M1 regions responsible for upper and lower limb control on a patient with FM brings us one step closer to developing a novel, non-invasive method of managing pain associated with FM.

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Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.09/C51

Topic: I.08. Methods to Modulate Neural Activity

Support: R01 MH123687

Title: Anterior striatum microstimulation enhances flexible learning in non-human primates

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Abstract: Neurons in the anterior striatum (aSTR, caudate head) encode prediction errors that are tuned to specific visual features of objects that caused the prediction error (Oemisch et al., 2019). These feature-specific prediction error signals may causally support learning which features are behaviorally relevant. We tested this by electrically microstimulating the aSTR of two rhesus macaques performing a feature-reward learning task. The experimental design varied the difficulty of learning to address a previous study that found no effect of cue-triggered stimulation on associating shapes with response locations (Williams & Eskandar, 2006). The experiment also varied the motivational incentives of choices to relate to studies suggesting that aSTR stimulation may increase the incentive of non-risky options irrespective of learning (Amemori et al., 2018; Santacruz et al., 2017). Our task required monkeys to learn in blocks of 30-50 trials the rewarded feature shown on one of three objects. In each trial monkeys selected an object by maintaining fixation on it for 0.7 s. Difficulty varied between blocks by varying features from 1-3 feature dimensions. Motivational incentives varied by offering either low/high gains for correct choices and imposing low/high losses for incorrect choices. High-frequency microstimulation was delivered 0.3-0.6 s during choice fixations prior to reaching learning criterion either restricted to correct choices (Stim.-Correct) or incorrect choices (Stim.-Error). Stim.-Correct or Stim.-Error blocks alternated with sham stimulation blocks.,We found that aSTR-stimulation overall improved learning in the most difficult conditions. Learning improvement was evident in 15 of 48 individual experimental sessions (Fisher's Exact test). In these sessions, learning improved with both Stim.-Correct and Stim.-Error stimulation conditions. The learning improvement was pronounced in difficult learning conditions with low

incentives on correct trials (Gain 2 compared to Gain 3) and low punishment after errors (Loss 1 compared to Loss 3). Stimulation improved learning particularly after error trials. Stimulation also significantly reduced choice reaction times and increased plateau performance for the most difficult condition. These results illustrate that aSTR causally supports flexible learning when learning requires credit assignment among multiple feature dimensions (difficult conditions), but not with simpler object shapes. The results also suggest that aSTR stimulation affects motivation which becomes evident by improving learning particularly when incentives were low.

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Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

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Topic: I.08. Methods to Modulate Neural Activity

Support: 5R01NS112176-04
1R42NS125895-01A1
1R01NS129549-01

Title: Elucidating DBS mechanisms of action for addiction using the MAVEN in a large animal model

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Abstract: Introduction: Over 40 million in the U.S. suffer from substance use disorder (SUD). Opioids are the leading cause of overdose, contributing over 100,000 overdose deaths and 800,000 nonfatal overdoses in 2022 alone. Dopamine (DA) extracellular levels in limbic and cortical structures (e.g., nucleus accumbens, NAc; prefrontal cortex, PFC) have been shown to mediate automated habitual and drug-seeking behaviors with diminished reliance on reinforcement. The Mayo Clinic Neural Engineering and Precision Laboratory (NEPS) previously developed a voltammetric technique, multiple cyclic square wave voltammetry (MCSWV), for quantifying tonic extracellular DA levels in the brain in near real-time. Herein, using the Multifunctional Apparatus for Voltammetry, Electrophysiology, and Neuromodulation (MAVEN) research platform, we investigate the mechanism of deep brain stimulation (DBS) in addiction.**Methods**: An anesthetized swine model was used for CT- and MRI-guided stereotactic

implantation of the quadripolar human electrode in the VTA followed by the sensing carbon fiber microelectrode (CFM) in the NAc. The depth of the CFM in the NAc was adjusted to obtain a robust stimulation-evoked DA signal as measured by fast-scan cyclic voltammetry (FSCV). Following this, MCSWV was initiated and tonic DA concentrations were recorded at baseline, during high-frequency VTA stimulation, and in the presence of addictive substances at increasing doses. Post-mortem analysis was performed to confirm lead placement. **Results**: MAVEN was successful in recording tonic and phasic DA recordings in the pig. Administration of pentobarbital resulted in robust increases in the tonic DA concentration as measured by MCSWV. Post-mortem histological assessment confirmed accuracy of lead placement in the NAc (sensing CFM) and VTA (depth electrode). **Conclusions**: In a swine model, MAVEN was used to record tonic DA concentrations in the NAc at baseline, following administration of addictive substances, and following high-frequency DBS of the VTA, in near real-time. This represents the pre-clinical validation of MAVEN prior to translation to human clinical studies.

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Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

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Topic: I.08. Methods to Modulate Neural Activity

Support: 5R01NS112176-04
1R42NS125895-01A1
1R01NS129549-01

Title: Development of carbon-fiber microelectrode for dopamine concentration measurements in a large animal (Pig) model during deep brain stimulation

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Abstract: Background: Voltammetric recordings of neurotransmitters is increasing in popularity due to its high spatiotemporal resolution compared with traditional modalities such as microdialysis. Voltammetric sensing electrodes commonly utilize carbon-fiber; however, due to the highly technical nature of carbon-fiber microelectrode (CFM) fabrication, propensity for biofouling, and dimension requirements that spare surrounding normal brain tissue, its application has been limited in human investigations.The Mayo Clinic Neural Engineering and

Precision Laboratories (NEPS) recently developed a waveform, multiple cyclic square wave voltammetry (MCSWV), capable of tonic dopamine measurements *in vivo* in near real-time. Herein, we present the validation of the CFM in a large animal model in anticipation of its translation to human clinical studies.
Methods: The CFM's were fabricated by attaching a short portion of carbon fiber onto the same type of micro-electrode as those use for human micro-electrode recording for electrophysiology. The elongated part of the carbon fiber was coated with pedot nafion and cut to approximately 40 micron in length. The electrodes were implanted stereotactcally in a large animal model (pig) of DBS neurosurgery, using MRI and CT guidance. The Mayo developed Muntifunctional Apparatus for Votametry Electrophysiology and Neuromodulation (MAVEN) device was used for the electrochemistry.
Results: M-CSWV recordings demonstrated successful recording of tonic dopamine in the nucleus accumbens (NAc) of the pig at baseline, in response to high frequency stimulation of the ventral tegmental area (VTA) and with pentobarbital. Post calibration *in vitro* beaker tests were performed post mortem to convert units of charge to concentration (nanomoles) during data analysis, which showed previously demonstrated dopamine signal, as evaluated by MAVEN software.
Conclusions: We were successful in the fabrication and testing of the carbon fiber microelectrode for continuous measurements of tonic dopamine concentrations using M-CSWV, while maintaining dimensions same as used for human microelectrode recording. These results in a large animal model of DBS support the continued efforts of translating this technology into humans.

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Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

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Topic: I.08. Methods to Modulate Neural Activity

Support: 2022ZD0210300
2018SHZDZX05
2021SHZDZX
23PJ1414400

Title: Spatially precise and chronically stable deep brain stimulation via a novel ultra-flexible microelectrode in a mouse model of parkinson's disease

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Abstract: Deep brain stimulation (DBS) is one of the most important clinical advances in treating neurological and psychiatric disorders. However, the poor biocompatibility of rigid clinical DBS electrodes often leads to glial scar accumulation and electrode drifting over long-term implantation, resulting in compromised spatial resolution and chronic stability of neuromodulation. To tackle these challenges, we developed a spatially refined and long-term stable deep brain stimulation strategy using ultra-flexible microelectrodes to achieve precise neuromodulation in a mouse model of Parkinson's disease (PD). We hypothesize that the seamless tissue-electrode integration should create a glial scar-free interface for neuromodulation at low currents, thereby eliminate off-target effects caused by current spread and frequent parament adjustment during chronic implantation. The ultra-flexible microelectrodes engineered for neuromodulation exhibited excellent electrochemical properties and electrical stability for long-term electrical stimulation in vivo, with a maximum charge injection capacity (~1.2 mC/cm^2) comparable to that of rigid electrodes and enduring over 100 million current pulses. To measure the spatial resolution of neuromodulation, we implanted ultra-flexible microelectrodes and traditional rigid electrodes in the visual cortex of GCaMP6s-transgenic mice and monitored neural responses using two-photon calcium imaging during intracortical microstimulation. Compared to rigid electrodes, The current thresholds for neural activation were significantly lower (1 μA v.s. 10 μA) for ultra-flexible microelectrodes, and the activation range could be precisely tuned at sub-100-μm resolution by adjusting current levels. Furthermore, the neuronal response patterns were largely non-overlapping between two adjacent sites, validating the high spatial resolution of ultra-flexible microelectrode stimulation. Based on these findings, we performed unilateral DBS of the subthalamic nucleus in PD mice. The behavioral outcomes demonstrated that the ultra-flexible microelectrodes outperformed rigid electrodes from several aspects: 1) achieving therapeutic effects with smaller currents simultaneously at multiple electrode sites, 2) inducing more natural locomotion without obvious side effects, and 3) stable stimulation parameters over long-term implantation. Taken together, our results demonstrate the capability of ultra-flexible microelectrodes for precise and long-term stable neuromodulation for neurological disorders, providing a new path to more effective clinical therapies with minimized side effects.

Disclosures: Z. Wang: None. **C. Liu:** None. **C. gan:** None. **F. zan:** None. **C. Ren:** None. **Z. Zhao:** None.

Poster

PSTR392

Deep Brain Stimulation: Parkinson’s Disease

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Program #/Poster #: PSTR392.13/C55

Topic: C.03. Parkinson's Disease

Support: R01-NS094206
P50-NS123109

Title: Differential effects on the pedunculopontine nucleus during targeted deep brain stimulation of the subthalamic nucleus

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Abstract: The subthalamic nucleus (STN) is interwoven with and surrounded by axons of passage from and to various brainstem nuclei, including the pedunculopontine nucleus (PPN). We hypothesized that these axons are activated during deep brain stimulation therapy targeting the STN for treating parkinsonian motor signs. In two parkinsonian non-human primates, high frequency stimulation was delivered through a directional lead of macroelectrodes implanted within the STN while recording unit-spike activity with microelectrodes in the PPN. Recorded cells exhibited heterogeneous responses to stimulation including modulation of spike rates and spike patterns. Spike rate changes first occurred with relatively low stimulation amplitudes and at higher stimulation amplitudes, antidromic and orthodromic spike activity became apparent. For electrodes positioned near the lenticular fasciculus, orthodromic short-latency inhibition occurred in the spike patterns of cells in PPN, whereas antidromic responses were observed when stimulating across multiple locations within and adjacent to the STN. These results suggest that activity patterns in the PPN depend on the recruitment of axonal pathways in, and around, the STN during deep brain stimulation therapy. These findings may provide context for interpreting behavioral outcomes of DBS therapy for treating symptoms of Parkinson's disease.

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Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

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Topic: I.08. Methods to Modulate Neural Activity

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2022R1A2C2005062
NRF-2022R1I1A4063209

Title: Micro Temporal Interference Stimulation on Cultured Neuronal Cells

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Abstract: In recent years, there has been increasing interest in temporal interference stimulation (TIS) as a potential therapeutic approach for deep brain stimulation. Unlike conventional methods requiring invasive electrode placement within the brain, TIS operates by externally overlapping two high-frequency currents to generate a targeted low-frequency electric field within specific brain regions. Consequently, this low-frequency electric field offers a non-invasive alternative to traditional methods for stimulating deep brain regions. However, there is currently insufficient research on how TIS parameters impact individual neurons. This study explored the impact of TIS on hippocampal neurons cultured on a microelectrode array (MEA). Two high-frequency sinusoidal waves, differing by 20 Hz in frequency, were applied to the MEA to assess the microscale effectiveness of TIS. Results showed that TIS significantly increased neuronal activity compared to single high-frequency stimulation. Simulation studies were also conducted to assess the feasibility of targeting specific regions on the MEA for stimulation. Ultimately, the study aimed to determine whether TIS could be extended to invasive multichannel electrodes.

Disclosures: J. Sohn: None. **S. Hwang:** None. **S. Jun:** None.

Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

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Brain Research Foundation with a Fay/Frank Seed Grant (KJM)
NIH U01-NS128612 (KJM)

Title: Parameterization of intraoperative microelectrode recordings for spike isolation and analysis

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Abstract: Deep brain stimulation (DBS) is a direct therapeutic approach to brain circuit disorders, providing sophisticated treatment options for a wide range of neurological and neuropsychiatric conditions. The accurate placement of permanent leads to the planned structure or substructure is critical to the efficacy of DBS treatment. Current practice for DBS targeting is based on a combination of anatomical borders visible on pre-operative magnetic resonance imaging (MRI) and functional targeting based on intraoperative stimulation testing and electrophysiological mapping. However, brain shift, imaging limitations, and individual anatomical variations limit the effectiveness of using imaging alone. Functional electrophysiology mapping is accomplished through extracellular intraoperative microelectrode recordings (MER). MER involves inserting a single-channel microelectrode into the computed brain target to identify neuronal structure boundaries. The microelectrode is advanced manually into the deep brain in small, typically sub-millimeter steps, stopping at each step to record neural activity in the region of the electrode tip. However, MER can often be subjective, time-consuming, and require significant expertise to interpret neural activity. While different cell-types or firing patterns have been identified for different anatomical targets (i.e. internal globus pallidus; GPi), mathematical description of different action potential shapes could greatly aid in creating objective principles for DBS functional targeting using MER. Here we parameterize single-unit action potentials using a canonical response parameterization machine learning technique. We calculate a spike-similarity metric for all sorted units across serial measurements for GPi DBS procedures and perform hierarchical clustering to identify unique spike shapes. We found anatomically significant clusters of spike shapes which could form the basis of an intraoperative functional targeting system.

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Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.16/C58

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH RO1 EY35533
NIH R01 MH122258

Title: Single pulse electrical stimulation reveals strong interactions between visual pathways

Authors: *M. G. YÁÑEZ-RAMOS[1], G. OJEDA VALENCIA[1], H. HUANG[1], N. GREGG[2], Z. QADIR[1], M. MONTOYA[1], G. A. WORRELL[2], K. J. MILLER[3], K. N. KAY[4], D. HERMES[1]; [1]Physiol. and Biomed. Engin., [2]Neurol., [3]Neurosurg., Mayo Clin., Rochester, MN; [4]Radiology, Univ. of Minnesota, Twin Cities, St Paul, MN

Abstract: Visual streams have classically been characterized by ventral and dorsal pathways for perception and action. Recent studies have indicated an additional lateral visual pathway comprising areas LO1, LO2, TO1, and TO2. These pathways are sometimes interpreted as independent feedforward information streams. However, studies using post-mortem dissection and diffusion MRI based connectograms indicate that the lateral LO-TO regions are anatomically connected to dorsal and ventral areas. To better understand these anatomical connections, we applied single pulse electrical stimulation in human patients with stereo-electroencephalography (sEEG) electrodes implanted for clinical purposes. This yielded cortico-cortical evoked potential (CCEP) measurements that allow us to quantify the directionality, strength, and temporal dynamics of the connections between the lateral, ventral, and dorsal visual pathways. CCEP data were collected in 9 patients with electrodes covering visual areas. We characterized the stimulation evoked potentials using the Canonical Response Parameterization (CRP) method and calculated the responses' reliability, signal-to-noise ratio, and duration. We found that the lateral visual pathway receives strong inputs when stimulating either the dorsal or the ventral pathway. Interestingly, stimulation of the dorsal pathway (including intraparietal sulcus) resulted in responses of shorter duration in the lateral pathways (average 155 ms, +/- 36 ms SE) compared to stimulation of the ventral pathway (average 265 ms, +/- 31 ms SE). We also found that the lateral pathway has strong outputs to the dorsal as well as the ventral pathway. The duration of the evoked responses after lateral pathway stimulation were comparable across dorsal (161 ms, +/- 30 ms SE) and ventral (181 ms, +/- 90 ms SE) pathways.

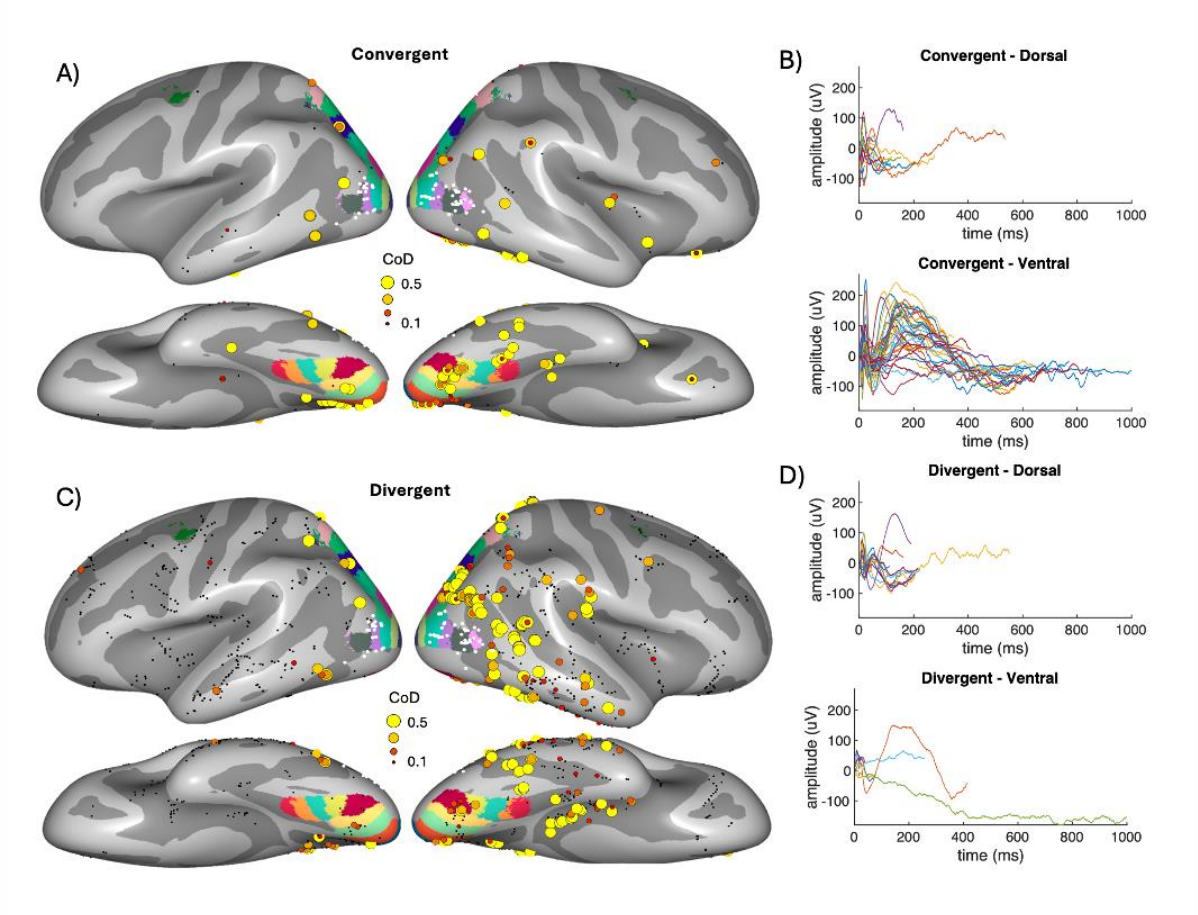

Figure 1. Lateral pathway inputs and outputs. A) MNI brain inflated with visual areas from the Wang and Kastner atlas. In this convergent framework, we stimulated electrode pairs and measured inputs to electrodes in the lateral visual pathway (LO-TO, white dots). Circles in hot colormap represent the reliability of the evoked responses (Coefficient of Determination, CoD) elicited in LO-TO by a stimulated electrode pair. Black dots indicate stimulated electrode pairs that did not elicit a significant response in LO-TO. B) Evoked response waveforms measured in LO-TO after stimulating electrodes in the dorsal (top) and ventral (bottom) pathways localized by the Wang and Kaster atlas. B) In the divergent framework, electrode pairs in LO-TO (white dots) were stimulated and colored electrodes indicate significant outgoing evoked responses. D) Evoked response waveforms measured in the dorsal (top) and ventral (bottom) pathways localized by the Wang and Kaster atlas after LO-TO stimulation.

Disclosures: M.G. Yáñez-Ramos: None. **G. Ojeda Valencia:** None. **H. Huang:** None. **N. gregg:** None. **Z. Qadir:** None. **M. Montoya:** None. **G.A. Worrell:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NeuroOne Inc., Cadence, UNEEG Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cadence Neuroscience Inc., and NeuroOne Inc.. F. Consulting Fees (e.g., advisory boards); Liva Nova, Neuropace, NeuroOne, Cadence. **K.J. Miller:** None. **K.N. Kay:** None. **D. Hermes:** 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.; Neuralink.

Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

Location: MCP Hall A

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Program #/Poster #: PSTR392.17/C59

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH U01-NS128612

Title: Capturing and decoding canine cortical activity with implantable device

Authors: *F. LAMPERT[1], F. MIVALT[2], I. KIM[3], N. F. INCE[4], J. KIM[2], M. A. VAN DEN BOOM[5], V. KREMEN[2], V. A. COENEN[6], G. SCHALK[7], P. BRUNNER[8], G. A. WORRELL[3], K. J. MILLER[4];
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Abstract: Rationale: Currently available brain-computer interface (BCI) ecosystems often require a multi-disciplinary teamwith extensive knowledge in neurophysiology and hardware and software processing, that limits clinicians and re-searchers in utilization of BCI applications. Some of key requirements for any BCI system are to have a sufficientnumber of channels and to reliably track local neural activity over extended periods of time. We are developinga system consisting of the CorTec BrainInterchange (BIC) and the opensource BCI2000 software environmentthat provides a seamless integration of the BIC with real-time signal visualization and analytical pipeline. Herewe evaluate the long-term efficacy and performance of the BIC-BCI200 system almost one year after the implan-tation by performing a series of simple somatosensory, visual and social reinforcement tasks on a beagle canineand measuring the response on the brain surface with electrocorticography (ECoG) construct comprising of 32electrodes. Methods: Local responses were decoded by detecting broadband spectral changes, previously shown to be associ-ated with capturing local neural activity. Precisely, power spectral shift at frequencies above ∼60Hz was captured,as broadband spectral changes are obscured at low frequencies by coincident oscillations. Results: The outcomes of the basic sensory tasks reveal local neuronal activity, indicated by changes in broadbandspectral patterns, in brain regions documented as in the literature. During the visual task, there was notable acti-vation in the marginal gyrus. The somatosensory task elicited significant activation in the *pre-* and *post-cruciategyri*, as well as the *ectosylvian/rostral composite gyri*. A plain verbal and combined tactile & verbal reinforce-ment tasks, induced activation in the anterior *ectosylvian gyrus*. Alongside broadband changes in spectral power,we identified task-specific low-frequency oscillations. Particularly, a ∼15Hz rhythm, similar to human occipitalalpha waves, emerged over the *marginal gyrus* exclusively during the "lights-off" phase, and a ∼24Hz rhythm,reminiscent of

human beta waves, was selectively suppressed during somatosensory stimulus and amplified duringreinforcement tasks. Conclusion: These results demonstrate the feasibility of the device even after almost a year in situ and agree withour previous findings from analogous tasks 2 months post-implant. This underscores the potential of our open-source BCI ecosystem, capable of the closed-loop stimulation, and holds promise for personalized therapies forpatients with neurological disorders.

Disclosures: F. Lampert: None. **F. Mivalt:** A. Employment/Salary (full or part-time):; Cadence Neuroscience Inc. **I. Kim:** None. **N.F. Ince:** None. **J. Kim:** None. **M.A. van den Boom:** None. **V. Kremen:** None. **V.A. Coenen:** None. **G. Schalk:** None. **P. Brunner:** None. **G.A. Worrell:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cadence Neuroscience Inc. **K.J. Miller:** None.

Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.18/C60

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH U01-NS128612

Title: The Insula Encodes Somatotopic Representation of Motor Execution with a Robust Connectomic Map to Primary Motor Cortex

Authors: *P. KEREZOUDIS[1], M. JENSEN[2], B. KLASSEN[3], G. A. WORRELL[1], N. F. INCE[4], D. HERMES[5], K. J. MILLER[4];
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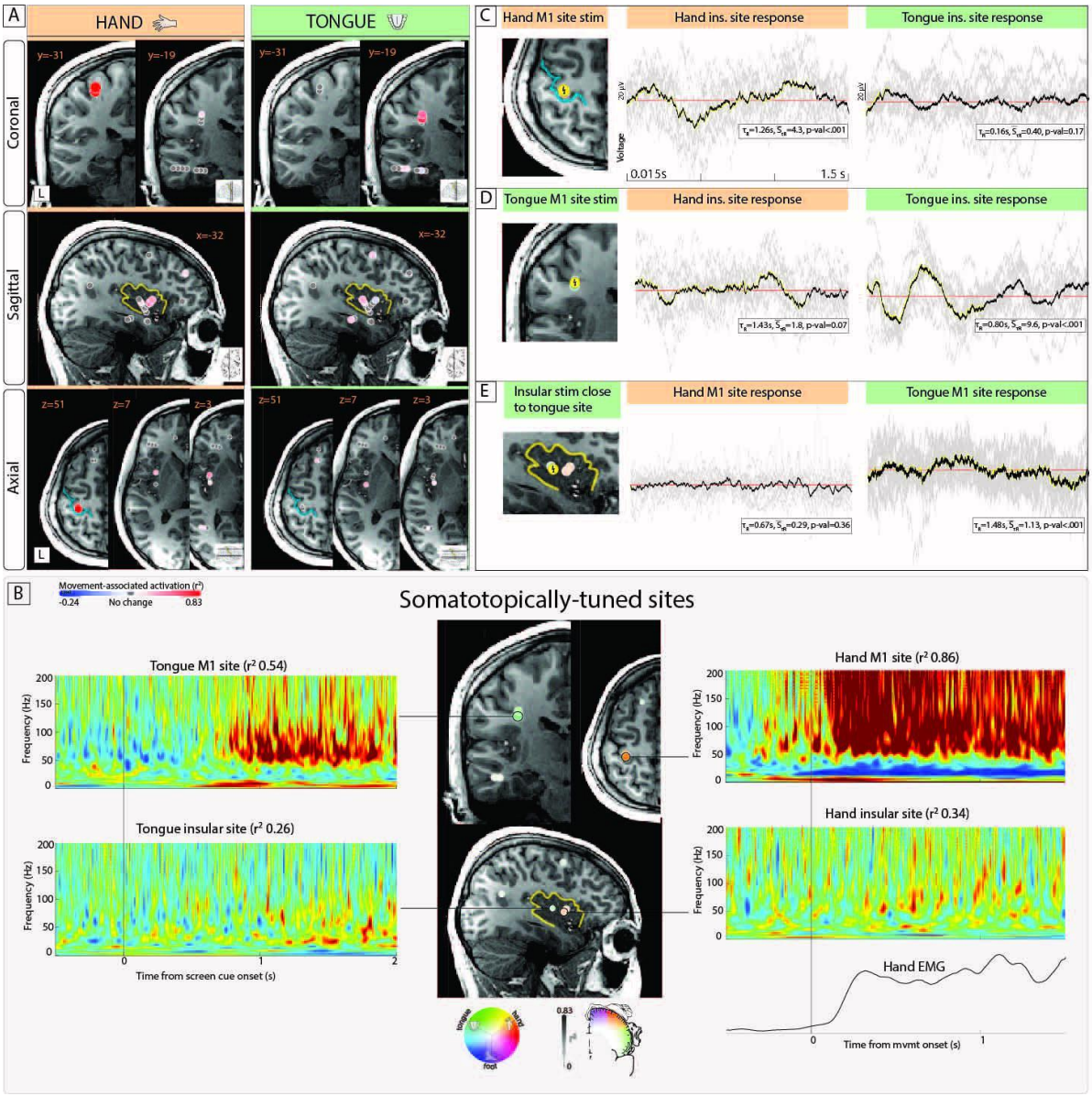
Abstract: Background: Research to date has revealed the critical role of the insular cortex in integrating incoming sensory stimuli with the emotional state of the individual. However, its functional representation of the movement of different body parts has not yet been comprehensively explored. Objective: To analyze the temporal, spectral and spatial dynamics of insula's involvement in motor execution. Methods: Stereoelectroencephalography was recorded in 18 patients with epilepsy, aged 12-37 years. Each performed visually-cued simple repetitive movements of the hand, tongue, or foot while electromyography (EMG) was captured. Changes in the high-frequency broadband power spectrum (65-115 Hz) were compared between behavior and rest to quantify degree of activation during motor execution. When available, connections between somatotopically-tuned sites in the insula and M1 cortex were studied using single pulse electrical stimulation (SPES). Results: Insular broadband activation during tongue movement

was observed most commonly (100% of subjects), followed by hand (83%) and foot (50%), with a similar distribution in each hemisphere. Across subjects, a clear somatotopic pattern emerged in the insula; tongue-tuned sites tended to cluster in the superior part of anterior and posterior long gyri, while hand-tuned sites were located anterior to them. Insular activity lagged behind primary motor cortex but preceded EMG onset in most cases. SPES-evoked potentials showed robust connectomic relationships between modality-specific somatotopic regions in the insula and primary motor cortex.Conclusion: There is a robust connectomic map between somatotopic regions in the insula and their counterparts in primary motor cortex. This relationship must be considered for resective, ablative and neuromodulation strategies involving this region.



Disclosures: P. Kerezoudis: None. **M. Jensen:** None. **B. Klassen:** None. **G.A. Worrell:** None. **N.F. Ince:** None. **D. Hermes:** None. **K.J. Miller:** None.

Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.19/C61

Topic: I.08. Methods to Modulate Neural Activity

Support: R01-MH-122258

Title: Single pulse electrical stimulation reveals varying connectivity between anterior and posterior hippocampus to medial prefrontal cortex

Authors: *G. OJEDA VALENCIA[1], N. GREGG[2], H. HUANG[3], B. N. LUNDSTROM[1], M. MONTOYA[3], B. H. BRINKMANN[1], J. VAN GOMPEL[4], P. BRUNNER[5], G. A. WORRELL[1], K. J. MILLER[4], D. HERMES[3];
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Abstract: Electrical stimulation of the hippocampus is used to modulate brain networks involved in neuropathologies. These stimulation therapies primarily target the hippocampus and its interconnected networks. However, given the complex nature of the hippocampus, identifying unique features of the anterior and posterior regions is important to help understand the impact of neuromodulation on these structures. Non-human studies have shown that the rodent homolog of the anterior hippocampus (aHC) is more strongly connected to the medial prefrontal cortex (mPFC) compared to the posterior hippocampus (pHC). To investigate this in humans, we studied five patients with refractory epilepsy who had stereo-EEG electrodes implanted in several brain regions, including the hippocampus and mPFC, but without seizure onset zones in the later areas.We delivered single-pulse stimulation to the aHC and pHC and measured cortico-cortical evoked potentials (CCEPs) from the mPFC. Using the waveform agnostic Canonical Response Parameterization (CRP) method we characterized CCEP features and reliability. This method allows for the investigation of long (~500 ms) and short latency CCEPs (<50 ms). We tested whether stimulation in the aHC elicited more reliable responses compared to the pHC in the mPFC. The results showed that stimulation of the aHC elicited significantly more reliable responses in the mPFC compared to stimulation of the pHC, in each of the five subjects. These findings match the connectivity patterns observed in rodents and indicate that the aHC-to-mPFC connection is consistently more reliable compared to the pHC-to-mPFC in humans. Given these findings, neurostimulation therapies targeting the anterior hippocampus might induce stronger network modulation on the medial prefrontal cortex.

Disclosures: G. Ojeda Valencia: None. **N. gregg:** None. **H. Huang:** None. **B.N. Lundstrom:** 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.; Neuroelectrics, Rapport, Medtronic. D.

Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Neuropace. F. Consulting Fees (e.g., advisory boards); Medtronic. **M. Montoya:** None. **B.H. Brinkmann:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Medtronic. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cadence. F. Consulting Fees (e.g., advisory boards); Eisai. **J. Van Gompel:** A. Employment/Salary (full or part-time):; Mayo Clinic. 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. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Cadence, UNEEG, NeuroOne, Medtronic. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroOne, Cadence. Other; Scientific advisory boards for : Neuropace, LivaNova, Cadence, and NeuroOne. **P. Brunner:** None. **G.A. Worrell:** A. Employment/Salary (full or part-time):; Mayo Clinic. 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. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); UNEEG, NeuroOne, Medtronic, Cadence. Other; Scientific advisory boards for : Neuropace, LivaNova, Cadence, and NeuroOne. **K.J. Miller:** None. **D. Hermes:** 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.; Neuralynk.

Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.20/C62

Topic: I.08. Methods to Modulate Neural Activity

Title: Cortical suppression through synchronisation during brain computer interfacing

Authors: *F. E. PERMEZEL[1], M. JENSEN[2], D. HERMES[3], K. J. MILLER[4];
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Abstract: This research examines the neural behavior associated with motor imagery and brain-computer interface (BCI) feedback, utilizing electrocorticography (ECoG). The study primarily explores how 12-20Hz rhythm entrainment with broadband amplitude reflects cortical synchronization and suppression. It investigates these phenomena by analyzing 12-20Hz rhythm entrainment during both resting and active phases of a motor task and during a BCI imagery

feedback task. In the experiment, a patient utilized speech-related broadband power increases in a speech motor area to control a BCI system through imagery of repeating words. The study demonstrated more extensive broadband power fluctuations in the speech motor area during the BCI imagery task. A significant discovery of the study is the finding of increased coupling of 12-20Hz rhythm phase with broadband power during the rest phase in the BCI imagery feedback task, compared to the rest phase of the motor task, suggesting potential "cognitive control" over cortical suppression. These results suggest a promising direction for future research in the BCI field, particularly in the use of phase-amplitude coupling (PAC) to assess deliberate cortical suppression during 'active rest' states.

Disclosures: F.E. Permezel: A. Employment/Salary (full or part-time):; Mayo Clinic DBS Fellow. **M. Jensen:** None. **D. Hermes:** None. **K.J. Miller:** None.

Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.21/C63

Topic: I.08. Methods to Modulate Neural Activity

Title: The importance of selecting a proper reference and/or re-reference scheme for single-pulse electrical stimulation experiments

Authors: J. ADKINSON[1], H. HUANG[4], M. HASEN[2], I. A. DANSTROM[2], K. BIJANKI[3], N. GREGG[5], K. J. MILLER[5], S. A. SHETH[1], D. HERMES[4], ***E. BARTOLI**[2];
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Abstract: Single-pulse electrical stimulation (SPES) experiments assess functional effective connectivity in human intracranial recordings by testing for causal influence between brain regions. The pulse-evoked potentials (PEPs) measured at distant electrode locations from the stimulation site are quantified (typically by their amplitude at specific latencies) and interpreted as a proxy of connectivity for each pair of stimulated-recorded locations. This technique is a simple, robust and well-established tool to map brain networks. However, data processing steps for SPES experiments can be challenging (Levinson et al., 2024). Re-referencing is performed to remove the contribution of the recording reference, but it often alters the original signals and distorts the average PEP waveform. Re-referencing is an important step of data processing as it ensures comparability of findings across datasets and removes the presence of evoked potentials shared across all recordings, a quality that prevents false-positive connectivity results. Here, we focus on this latter scenario by showcasing the occurrence of biased connectivity results in 1 human participant (male, 25 years old, 182 sEEG electrodes) due to the presence of a common evoked potential signal across several electrodes. We test two alternative solutions to this

problem: 1) Neutral reference selection: repeating the SPES experiment after selecting a different reference location 2) Data-driven re-referencing: adjusted common average referencing for cortico-cortical evoked potential data, CARLA (Huang et al., 2024) The correlation between the pulse-evoked waveforms recorded from 182 sEEG electrodes was on average r = .38 in the original dataset. Both the neutral reference and the data-driven re-referencing were successful at reducing the overall correlation between the evoked responses from different electrodes (r=0.12 and r=0.02), and effectively reduced the number of electrode pairs showing a significant waveform correlation by 12.6% and 11.4%, respectively. Our results demonstrate the importance of selecting a neutral reference location, or, when not experimentally possible, employing careful data-driven re-referencing to obtain unbiased connectivity results from PEPs.

Disclosures: J. Adkinson: None. **H. Huang:** None. **M. Hasen:** None. **I.A. Danstrom:** None. **K. Bijanki:** None. **N. gregg:** None. **K.J. Miller:** None. **S.A. Sheth:** None. **D. Hermes:** None. **E. Bartoli:** None.

Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

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Topic: I.08. Methods to Modulate Neural Activity

Support: UH2/UH3 NS95495
R01-NS112144
R01-NS92882
U24NS113637

Title: Longitudinal Monitoring of Network Excitability in Mesial Temporal Lobe Epilepsy

Authors: *V. KREMEN[1], N. GREGG[1], V. SLADKY[2], F. MIVALT[1], I. BALZEKAS[2], B. N. LUNDSTROM[2], E. K. ST.LOUIS[2], D. HERMES[3], K. J. MILLER[4], J. VAN GOMPEL[4], B. H. BRINKMANN[2], G. A. WORRELL[2];
[1]Neurol., [3]Physiol. and Biomed. Engin., [4]Neurosurg., [2]Mayo Clin., Rochester, MN

Abstract: Rationale: Epilepsy is characterized by aberrant network excitability, manifesting as spontaneous recurrent seizures and interictal epileptiform spikes (IES). We examine the temporal dynamics of seizures and IES in mesial temporal lobe epilepsy (mTLE) in relation to biomarkers derived from electrical stimulation and its evoked potentials (EPs) and explore their utility in seizure forecasting, which represents a long-sought yet unrealized objective within epilepsy clinical care. Methods: We conducted an investigation using a chronically implanted device capable of continuous intracranial EEG (iEEG) monitoring, wireless data transmission, and electrical stimulation in five people with drug resistant mTLE. Each subject had electrodes implanted to target two pivotal nodes within the mTLE network: the hippocampus (Hc) and the

anterior nucleus of the thalamus (ANT). We examined the evoked responses in the Hc with ANT electrical stimulation, providing a novel method to assess ANT-Hc network excitability. These assessments were performed with subjects in their natural home environments over the course of several months, offering unique insights into the temporal dynamics of epileptic networks in mTLE. Results: Our data reveal a bimodal peak in seizure probability during daytime, specifically heightened during morning and evening hours, whereas IES rates are increased at night, particularly during non-REM slow-wave sleep. Our analyses delineate distinct neurophysiological profiles: seizures are more frequent during wakefulness, and IES rates increase during slow-wave sleep. Furthermore, our findings show that the ANT-Hc EPs demonstrate notably shorter latencies during wakefulness as opposed to slow-wave sleep. This observation suggests reduced limbic network excitability and connectivity during deep sleep phases. Interestingly, while maintaining similar latency, an increase in the amplitude of responses and the energy of the signal preceding seizures was observed, possibly indicating heightened excitability prior to seizures onset. Conclusion: The results highlight the complex interplay between circadian biology and epileptic activity and may help inform targeted therapeutic strategies that leverage temporal fluctuations for optimal epilepsy management. They also provide valuable implications for understanding seizure generation and propagation mechanisms in mTLE. The consistent measurement of EPs over extended periods holds promise as a reliable biomarker for assessing changes in network excitability and seizure risk, potentially transforming the predictive capabilities in epilepsy.

Disclosures: V. Kremen: None. **N. gregg:** None. **V. Sladky:** None. **F. Mivalt:** None. **I. Balzekas:** None. **B.N. Lundstrom:** 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 Deep Brain Stimulation Therapy for Epilepsy Post-Approval Study (EPAS), Neuropace RNS System Responsive Stimulation for Adolescents with Epilepsy (RESPONSE) Study, Neuroelectrics tDCS for Patients with Epilepsy Study. Other; UNEEG, NeuroOne, Epiminder, Medtronic, and Philips Neuro. **E.K. St.Louis:** None. **D. Hermes:** None. **K.J. Miller:** None. **J. Van Gompel:** None. **B.H. Brinkmann:** F. Consulting Fees (e.g., advisory boards); UNEEG, NeuroOne, Epiminder, Medtronic, and Philips Neuro. **G.A. Worrell:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; investigators for the UNEEG Inc. investigational device trial. F. Consulting Fees (e.g., advisory boards); UNEEG, NeuroOne, Epiminder, Medtronic, and Philips Neuro..

Poster

PSTR392

Deep Brain Stimulation: Parkinson’s Disease

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NIH/NINDS (R21-NS128307)
NIH/NIMH (R01-MH120194)
NIH/NIMH (R01-MH122258)
McDonnell Center for Systems Neuroscience
Fondazione Neurone

Title: Activation of Cingulate Cortex Subregions Modeled using Scalp EEG.

Authors: *P. DEMAREST[1,2], G. TAN[3,4,5], W. ENGELHARDT[6,2], K. PARK[7,2], X. LIU[6,2,5], N. BRYSON[6,2,5], H. CHO[6,2], H. PARK[6], J. R. SWIFT[6], M. ADAMEK[8], L. N. EISENMAN[9], A. S. GREENBLATT[9], P. BRUNNER[6,2], J. T. WILLIE[6], E. C. LEUTHARDT[6,2];
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Abstract: Brain-computer interfaces (BCIs) use neurofeedback to provide a non-pharmacological treatment option for chronic pain. For example, scalp electroencephalography (EEG) is commonly used to identify electrophysiological correlates of pain perception, and the biomarkers detected in such studies are leveraged in BCI-based neurofeedback therapies. Our previous work demonstrated that increased frontal EEG theta activity alleviates chronic pain symptoms. The source of this activity is hypothesized to originate in the anterior and middle cingulate cortex, a central modulator orchestrating multiple components of the experience of pain (including affect and unpleasantness). However, the principal limitation of these studies is the low interpretability of scalp EEG, as it cannot directly record from brain regions involved in pain processing and resolve the dynamics of these regions. Stereoelectroencephalography (SEEG) has become the standard for monitoring activity within deep-brain structures in humans. SEEG can be used to characterize cingulate cortex network activity recruitment through direct intracranial electrical stimulation. Typically, SEEG is combined with simultaneous scalp EEG recordings. This combination can model the activation of cingulate cortex networks from electrophysiology recorded on the scalp surface. In our study, ten patients with intractable epilepsy underwent passive bipolar single-pulse stimulation of either the anterior cingulate cortex (ACC), midcingulate cortex (MCC), or posterior cingulate cortex (PCC) at 3mA or 6mA during simultaneous scalp EEG recordings. Our findings show that stimulation of different cingulate cortex subregions produces distinguishable surface EEG patterns. Stimulation of the MCC evoked the highest magnitude response on surface EEG, with a bi-hemispheric frontal propagation starting from contralateral F3, while stimulation of the ACC revealed the lowest

magnitude evoked responses. The MCC shows highly integrated network recruitment (Global Field Power 1.5), where the evoked potential propagated across frontal, middle, and parietal locations. Stimulation of the ACC showed frontal-specific propagation, while stimulation of the PCC showed propagation across parietal locations. In summary, these results validate the connectivity of the ACC to limbic systems, the MCC to frontal-motor-parietal networks, and the PCC in default mode networks. These results further indicate that activation of the ACC and MCC likely contributes to detectable activity at frontal electrodes, further validating our hypothesis that these frontal patterns are associated with activity from these regions.

Disclosures: P. Demarest: None. **G. Tan:** None. **W. Engelhardt:** None. **K. Park:** None. **X. Liu:** None. **N. Bryson:** None. **H. Cho:** None. **H. Park:** None. **J.R. Swift:** None. **M. Adamek:** None. **L.N. Eisenman:** None. **A.S. Greenblatt:** None. **P. Brunner:** None. **J.T. Willie:** None. **E.C. Leuthardt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurolutions, Sora Neuroscience, Inner Cosmos.

Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.24/C66

Topic: I.08. Methods to Modulate Neural Activity

Support: RO1NS124650

Title: Evoked resonant neural activity for localizing Globus Pallidus interna of patients with Parkinson's Disease: preliminary results

Authors: *H. HEYDARI[1,2], L. BRANCO[3], C. SWAMY[1], B. T. KLASSEN[4], K. J. MILLER[1], A. VISWANATHAN[5], N. F. INCE[1];
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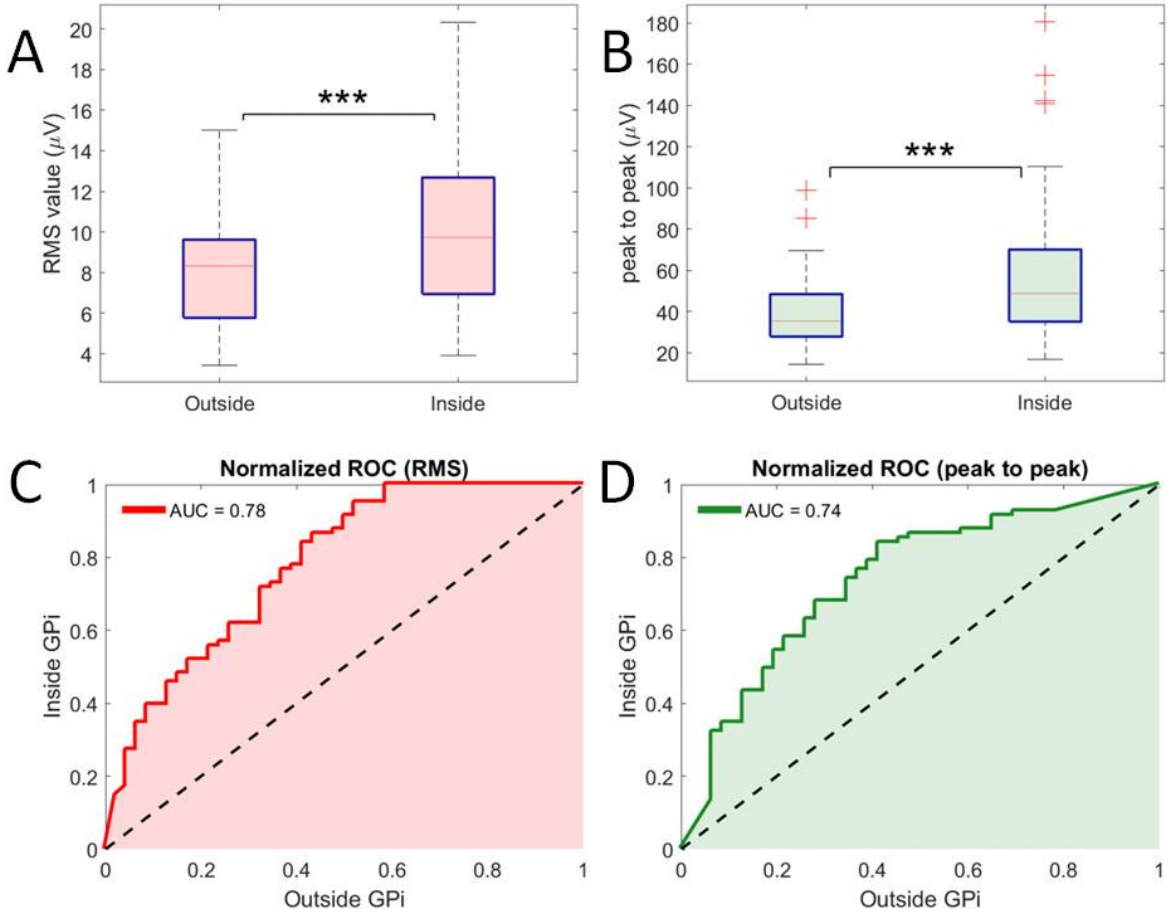
Abstract: Introduction Evoked resonant neural activity (ERNA) is a promising biomarker for localizing the Globus Pallidusinterna (GPi) during deep brain stimulation (DBS) surgery for Parkinson's disease (PD). Key advantages of ERNA include the ability to record ERNA in the anesthetized patient and the prominent signal amplitude compared to other typical biomarkers, such as beta band and high frequency oscillations. **Objectives** Evaluate the potential of employing ERNA as a quantitative biomarker to assess DBS contact location with respect to GPi. **Methods** Awake neurophysiological recordings were obtained from 16 GPi (8 patients) during DBS surgery after securing the directional leads. Monopolar stimulation at 130 Hz, 2mA, was delivered through the bottom and top contacts of the chronic electrodes while recording

LFPs at 15 kHz. Stimulation occurred9 times in bursts of 20 pulses, followed by a 200-millisecond gap, where ERNA may be present.Peak to peak amplitude and Root Mean Square (RMS) value were calculated for each trial, and the median over trials was taken. The metrics from top and bottom stimulations were averaged for final values. LeadDBS was used to quantify each DBS contact as located inside versus outside the GPi. **Results** Contacts located within the GPi (82 out of ***n***=128) had a significantly higher RMS value compared with those outside the GPi (10.09 ± 3.68 versus 8.13 ± 2.90 µV, p-value = 8.44e-7, two-sample t-test from normalized data). Additionally, the peak-to-peak amplitude of the ERNA was significantly higher in the contacts inside of GPi (57.63 ± 32.87 versus 39.60 ± 17.59 µV, p-value= 2.20e-5, two-sample t-test from normalized data). Receiver operating characteristic (ROC)curves demonstrate discriminative power comparing Inside versus outside of GPi from RMS value(AUC=0.78, Fig. 1C) and peak-to-peak amplitude (AUC=0.74, Fig. 1D). **Conclusions** ERNA recorded from contacts within the GPi may be an effective biomarker to confirm GPi DBSlead implantation intraoperatively.



Disclosures: H. Heydari: None. **L. Branco:** None. **C. Swamy:** None. **B.T. Klassen:** None. **K.J. Miller:** None. **A. Viswanathan:** None. **N.F. Ince:** None.

Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.25/C67

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH/NIBIB (P41-EB018783)
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NIH/NIMH (R01-MH122258)
McDonnell Center for Systems Neuroscience
Fondazione Neurone

Title: Real-time feedback of complex physiology and rapid experimental parameter optimization with BCI2000

Authors: *W. ENGELHARDT[1,2,3,4], J. MELLINGER[5], H. HUANG[1,2,3,4], P. DEMAREST[1,2,3,4], J. R. SWIFT[1,2,3,4], P. BRUNNER[1,2,3,4];
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Abstract: BCI2000 is a general-purpose software platform that enables data acquisition and real-time feedback. It provides a standardized environment to monitor experiments and generate scientific insight for the investigator. BCI2000 acquires, analyzes, and visualizes the participant's behavior and neurophysiological signals in real-time. This includes the participant's fixation on the screen, signal quality, and other behavioral data. This streamlined data collection allows for the efficient exploration of experimental parameter space and leads to the generation of robust scientific discoveries.
The field of neuromodulation has increasingly large parameter spaces, which require feedback to the investigator to properly explore. To best explore the parameter space, BCI2000 visualizes neurometric and psychometric functions. For example, real-time evoked potentials can be visualized in invasive neurostimulation experiments. This informs the experimenter about underlying effective connectivity and allows for the selection of subsequent stimulation locations dynamically throughout the experiment. This platform also enables real-time computation of biomarkers, which is often conducted during offline analysis. For example, phase-amplitude coupling analysis enables researchers to visualize functionally co-activated areas in real time, allowing for the optimal selection of locations for neuromodulation experiments.
Additionally, it is commonly desired to titrate optimal parameters for individual participants with

use of psychometric or neurometric curves. This titration is based on a model, such as perception threshold, task difficulty, or reaction time, which all vary across participants. To this end, accurate measures of the participant's behaviors are required to enable experimental consistency across participants (e.g., determining perception threshold). Instead of generating these curves offline outside of the experimental context, BCI2000 enables the generation of these curves in real-time.
In conclusion, BCI2000 is a unique platform that provides researchers with real-time tools to determine biomarkers and minimize the time required for parameter optimization. This, in turn, simplifies the experimental workflow and improves the potential for scientific discovery and insight.

Disclosures: W. Engelhardt: None. **J. Mellinger:** None. **H. Huang:** None. **P. Demarest:** None. **J.R. Swift:** None. **P. Brunner:** None.

Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.26/C68

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant R01MH122258

Title: Thalamic deep brain stimulation modulation of network effective connectivity and excitability

Authors: *N. GREGG[1], G. OJEDA VALENCIA[2], H. HUANG[2], V. KREMEN[3], B. N. LUNDSTROM[1], K. J. MILLER[4], G. A. WORRELL[1], D. HERMES[2];
[1]Mayo Clin., Rochester, MN; [2]Physiol. and Biomed. Engin., Mayo Clin., Rochester, MN; [3]Neurol., Mayo Clin., Rochester, MN; [4]Neurosurg., Mayo Clin., Rochester, MN

Abstract: Deep brain stimulation (DBS) is a viable treatment option for a range of disorders of network excitability, such as epilepsy, Parkinson's disease, and deafferentation pain. The effects of DBS are observed over multiple timescales, spanning seconds to weeks or months. The mechanisms through which DBS modulates large-scale networks are unresolved. Single pulse electrical stimulation and the resulting evoked responses provide a measure network effective connectivity and excitability. Ten patients with drug resistant epilepsy were enrolled in the study. During clinical stereotactic-EEG including a thalamus lead, single electrical pulses were delivered to the thalamus at baseline and following a trial of high-frequency (145 Hz) repetitive duty-cycle thalamic stimulation. Thalamocortical evoked potential root-mean-square power assessed effective connectivity from the stimulated thalamus subfield to each recording contact. Thalamic stimulation induced changes in thalamocortical effective connectivity (comparing baseline to post-high frequency stimulation) were quantified by Cohen's D effect size, and

statistical significance by paired-sample t-test, $p<0.05$ significance level. Interictal epileptiform discharges were quantified by an automated classifier. This study was approved by the Mayo Clinic IRB and all patients provided informed consent. Prolonged delivery of high-frequency stimulation (>1.5 hours of active stimulation (not including the off-phase of duty-cycle stimulation)) resulted in a significant decrease in amplitude of evoked responses recorded from remote network regions, and modulation intensity was correlated with baseline connectivity strength. When stimulation durations were shorter, outcomes were variable. In contrast, the impact of stimulation on interictal epileptiform discharges was immediate. These results indicate that the impact of DBS on effective connectivity within the targeted network accumulate gradually over the course of several hours, which may reflect neuronal plasticity mechanisms, while modulation of interictal epileptiform discharges is immediate and may reflect the direct electrical effects of high-frequency stimulation. Electrophysiological biomarkers of various neuromodulation effects may facilitate data-driven characterization and optimization of neuromodulation.

Disclosures: N. gregg: F. Consulting Fees (e.g., advisory boards); NeuroOne. **G. Ojeda Valencia:** None. **H. Huang:** None. **V. Kremen:** None. **B.N. Lundstrom:** None. **K.J. Miller:** None. **G.A. Worrell:** None. **D. Hermes:** None.

Poster

PSTR392

Deep Brain Stimulation: Parkinson's Disease

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.27/C69

Topic: I.08. Methods to Modulate Neural Activity

Support: Minnesota Partnership Grant for Biotechnology and Medical Genomics MNP#21.42
NIH Grant U01-NS128612

Title: Investigating spectral features of somatotopic representation in the human motor thalamus

Authors: *B. T. KLASSEN[1], M. BAKER[2], K. J. MILLER[3];
[1]Mayo Clin., Rochester, MN; [2]Mayo Clin., Jacksonville, FL; [3]Neurosurg., Mayo Clin., Rochester, MN

Abstract: In the human motor cortex, movement is associated with widespread power suppression of low frequency band (LFB) oscillations in the stereotypic beta range (12-30Hz) paired with somatotopy-specific increases in non-oscillatory broadband power captured in high frequency bands (HFB; 65-115Hz). Similarly, the motor thalamus has somatotopic representation, with lower limb representation lateral to upper limb, which is in turn lateral to axial representation. We have previously shown LFB suppression and HFB increases in the motor thalamus associated with simple hand movements from serial microelectrode recordings

during deep brain stimulation (DBS) surgery. However, It remains unclear whether HFB power increases are also organized by somatotopy in the motor thalamus, and whether it can be captured from intraoperative recordings. Therefore, we investigated the spectral features of somatotopic representation in patients undergoing implantation of DBS leads in the bilateral ventralis intermedius thalamic nuclei (VIM). Specifically, we recorded thalamic local field potentials from the bilateral directional DBS leads while subjects performed a simple motor task (hand, tongue, or foot movement) visually cued on a screen in front of them. Movement and rest epochs were segmented according to surface EMG recordings and signed r^2 cross-correlation values were calculated for each movement type at each recording site for the LFB and HFB. Overall, we found movement-specific organization to LFB suppression and HFB increases in power in the VIM. These findings suggest that movement-associated broadband power could be a useful tool for intraoperative functional-mapping of the human motor thalamus and targeting for therapeutic intervention.

Disclosures: B.T. Klassen: None. **M. baker:** None. **K.J. Miller:** None.

Poster

PSTR393

Parkinson's Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.01/C70

Topic: C.03. Parkinson's Disease

Support: NIH Intramural Research Program

Title: Intermittent fasting exacerbates systemic metabolic abnormalities in an alpha-synuclein-based Parkinson's disease animal model

Authors: *T.-S. FANG;
Beth Israel Deaconess Med. Ctr., Boston, MA

Abstract: Weight loss is common in Parkinson's Disease (PD). Accumulating evidence suggests that many individuals with PD exhibit abnormalities in systemic energy metabolism prior to motor symptom onset, but the relationships between such abnormalities and the characteristic α-synuclein pathology are unclear. We previously discovered autonomic nervous system dysfunction in presymptomatic mice overexpressing a familial PD α-synuclein mutation (A53T) in neurons and that this same PD mouse model exhibits a marked reduction in adipose tissue. In this study, we interrogated the metabolic status of cohorts of wild type (WT) and PD mice maintained on control or alternate day intermittent fasting (IF) diets for two months beginning at 10 weeks of age. MRI analysis data revealed that the PD mice have less adipose tissue, especially visceral fat, compared with WT mice when maintained on a standard ad libitum diet, and that IF accentuates fat loss. Measurements of metabolic parameters indicated reduced energy expenditure (EE) in PD mice compared to WT mice fed ad libitum, but not when maintained on

IF. WT and PD mice exhibited increased spontaneous activity when on the IF diet compared to the control diet. As expected, the respiratory exchange ratio was reduced on fasting days in both the WT and PD mice. PD mice consumed less oxygen than WT mice when on the ad libitum diet, but not when on IF. Motor performance was significantly impaired in PD mice on both diets compared to WT mice, and mortality was hastened in PD mice on IF compared to those fed ad libitum, suggesting the contribution of a presymptomatic "wasting" phenotype to disease progression and death in this PD mouse model. This research was supported by the Intramural Research Program of the National Institute on Aging.

Disclosures: T. Fang: None.

Poster

PSTR393

Parkinson's Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.02/C71

Topic: C.03. Parkinson's Disease

Support: Michael J Fox Foundation Target Validation Grant - MJFF 021205

Title: Functional changes in gut microbiota precede the onset of motor and non-motor pathology in an alpha synuclein model of Parkinson's disease.

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Abstract: Parkinson's Disease (PD) is the fastest growing neurodegenerative disease globally and manifests as a synucleinopathy with reduced motor output. Non-motor symptoms, including gastrointestinal dysfunction, precede or coincide with hallmark motor symptoms, suggesting a role of the gut-brain axis in the onset and progression of PD. Multiple international cohort studies have identified changes in the gut microbial composition in PD, but the underlying functional and pathological significance of these microbial changes remains poorly understood. In this exploratory study, we evaluated the early changes in the gut microbial composition associated with PD in an α-synuclein transgenic mouse model of PD synucleinopathy. M83 α-synuclein mice, express the full length A53T variant of human α-synuclein mice, making it an excellent model to study synuclein pathology in PD. We utilised high-resolution functional metagenomics with ultra deep sequencing to ensure identification of low-abundance and novel taxa. The microbial community and metabolic pathways in the sequenced samples were profiled using the Microba community profiler and Microba genes and pathway profiler respectively. A significant decrease in the microbial richness and Shannon diversity (p-value <0.05) was observed in PD mice (n = 7) when compared with WT mice (n = 6), which surprisingly, was

evident at early prodromal stages of disease, prior to the onset of motor and non-motor symptoms in this model. Gut dysbiosis in M83 synuclein transgenic mice was characterized by the decrease in microbes responsible for the maintenance of blood glucose levels, metabolic inflammation, cholesterol metabolism, and short-chain fatty acid (SCFA) production and an increase in butyrate-producing bacteria and specific opportunistic pathogens in our dataset. Metabolic pathways associated with degradation of protective metabolites such as SCFAs were upregulated in the gut microbiota of synuclein transgenic mice. Our results in a mouse model of PD synucleinopathy provide further evidence for the hypothesis that pathological gut microbial changes could contribute to the onset of motor and non-motor pathology and that gut microbial metabolic shifts could be an early driver of disease pathology and progression in PD.

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Poster

PSTR393

Parkinson's Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.03/C72

Topic: C.03. Parkinson's Disease

Support: PNRR-MAD-2022- 12375960
GR-2021-12372698
InflammaPark #1750818

Title: Astrocytes-driven α-synucleinopathy triggers neuroinflammation and peripheral immune cell infiltration in a parkinson's disease model

Authors: *M. NANNONI[1,2,3], S. BIDO[2], S. G. GIANNELLI[2], M. LUONI[2,3], G. RUFFINI[2], A. CALDERONI[2], S. MUGGEO[2], V. BROCCOLI[2,3];
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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the selective loss of dopaminergic (DA) neurons into the substantia nigra (SN) and the presence of intracytoplasmic inclusions (Lewy body). The role of neuroinflammation in PD development has attracted increasing interest in the last decade, as demonstrated by the evidence of gliosis in post-mortem brain tissue of PD patients. To date, the effect exerted by glial cells accumulating αSynuclein (αSyn) in the neurodegenerative process has not yet been clarified. While the role of αSyn aggregates in neurons has been extensively studied, their presence in astrocytes as confirmed by PD brain autopsies, remains largely unexplored. We exploited a cre/LoxP inducible system to selectively accumulate A53T-αSyn in astrocytes and we characterized our model through immunostaining analysis. To further assess how the accumulation of A53T-αSyn in astrocytes could affect their behavior, we studied the astrocyte morphology using a double

labeling system. We injected an AAV Myr-mGFP reporter into the SN for labelling DA terminals and a tail injected AAV Myr-mCherry flex reporter for the selective labelling of astrocytes. The selective overexpression of αSyn over-expression in striatal astrocytes resulted in a 31% loss of nigral DA neurons and neuroinflammation, accompanied by massive immune infiltration. We examined the molecular signature of astrocytes in our model and subsequently profiled the immune response. Furthermore, we investigated the role played by astrocytes in recruiting the peripheral lymphocytes in presence or absence of microglia which were depleted by chronic administration of PLX3397. The treatment allowed us to determine the direct contribution of astrocytes to the recruitment of peripheral immune cells and the progression of neurodegeneration. Both morphological changes and immune involvement were mirrored by changes in the gene expression profile. The study highlights critical involvement of the astrocytes in PD, acting as a trigger for driving the immune response. These results lay the groundwork for the development of new therapeutic strategies aimed at reducing astrocyte activation and the recruitment of lymphocyte infiltrate with the goal of slowing down the disease progression and protecting DA neurons.

Disclosures: M. Nannoni: None. **S. Bido:** None. **S.G. Giannelli:** None. **M. Luoni:** None. **G. Ruffini:** None. **A. Calderoni:** None. **S. Muggeo:** None. **V. Broccoli:** None.

Poster

PSTR393

Parkinson's Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.04/C73

Topic: C.03. Parkinson's Disease

Support: NIH/NIEHS, 5 R01 ES024745-07 (MCH)
NIH/NIEHS, 5 R01 ES033462-02 (MCH)
MJFF-020697 (MCH)

Title: Characterizing the impact of innate immune cell restricted Nlrp3 activity in a rotenone-based model of Parkinson's disease

Authors: *E. CARLONI[1], I. FOX[1], M. C. HAVRDA[2];
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Abstract: Parkinson's disease (PD) is a neurodegenerative motor disorder characterized by bradykinesia, rigidity, and tremors. It affects 1% of the world population over 60 years old. Environmental factors and normal aging increase the risk of PD. Immune cells of both the central and peripheral immune systems are posited to drive the well-documented neuroinflammatory pathology observed in PD. The NLRP3 inflammasome is a protein complex that is activated in response to cellular damage and stress such as that caused by many environmental toxicants. We

model PD-related exposure using the pesticide rotenone, a mitochondrial complex I inhibitor that activates the NLRP3 inflammasome and is associated with an increased risk of PD in agricultural workers. Mice systemically exposed to rotenone harbor detectable changes in peripheral immune cell phenotype and develop *Nlrp3*-dependent neuroinflammation and nigral cell loss. We do not yet know the specific contributions of peripheral and central nervous system immune cells in the development of PD symptoms in rotenone-treated mice. We developed cell type-specific *Nlrp3* gain-of-function models to determine whether peripheral *NLRP3* is required for the development of neuroinflammation and neurodegeneration in rotenone treated mice. We generated mice with *Nlrp3* restricted to microglia (*Tmem119/NLRP3*^{L351P}). *WT*, *Nlrp3*$^{-/-}$and *Tmem119/NLRP3*^{L351P} were systemically exposed to rotenone for 14 days and subsequently aged to 18 months. Behavioral tests and tissue collection occurred at baseline, 15 days post rotenone and 300 days post rotenone exposure. We identified behavioral and histological changes in *WT* and *Nlrp3*$^{-/-}$ mice in the open field paradigm differentially modified by microglia-restricted *Nlrp3*. In parallel studies, we characterized the impact of rotenone exposure on the peripheral myeloid compartment in WT and *Nlrp3*$^{-/-}$ mice. Initial studies indicate changes in monocyte neutrophil ratios and phenotypes resulting from *Nlrp3* loss and rotenone exposure. These collective studies provide a window into complex interactions that exist between the central and peripheral immune systems in the context of PD-related exposure.

Disclosures: E. Carloni: None. **I. Fox:** None. **M.C. Havrda:** None.

Poster

PSTR393

Parkinson's Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.05/C74

Topic: C.03. Parkinson's Disease

Support: European Union's Horizon 2020 research and innovation programme under grant agreement No 848002

Title: The assessment of anxiety and depression comorbidities and the DRN electrophysiology in a primate model of Parkinson's Disease

Authors: A. SADOUN[1], M. DEFFAINS[2], Q. LI[3], E. BEZARD[4], F. E. GEORGES[5], ***E. PIOLI**[6]; [1]MOTAC Neurosci., Floirac, France; [2]The Hebrew Univ., Jerusalem, Israel; [3]Motac Neurosci., Beijing, China; [4]Inst. of Neurodegenerative Dis., Bordeaux, France; [5]IMN-UMR-CNRS-5293, Bordeaux, France; [6]MOTAC, Bordeaux, France

Abstract: The study aimed to examine the anxiety/depression-like phenotype and the potential role of dorsal raphe nucleus (DRN) in the gold standard primate model of motor symptoms in PD, the so-called 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) macaque model. Parkinson's disease (PD), in which there is widespread degeneration of catecholamine and

serotonergic systems, is characterised by both motor and non-motor symptoms, such as impairment in cognitive performance, anxiety and depression. The anxiety / depression-like phenotype was evaluated in 11 MPTP and 6 normal macaques (*Macaca mulatta*) by ethological scan in both familiar and unfamiliar Human Intruder Test.The in vivo extracellular recordings of DRN neurones were also recorded in anesthetised normal (n=4) and MPTP (n=4) macaques. Single-unit activity was assessed by sorting spike trains from each 250-6000Hz band-pass filtered signal via offline spike detection and sorting method. Threshold-crossing method for spike detection principal component analysis for spike feature extraction and K-means clustering method were applied. The intruder test results showed that MPTP animals presented a significant decrease in attention to the environment and reaction to their social environment which likely may correspond to a depressive-like state. Besides, the results related to the stressful situation (intruder) compared to a less stressful situation (familiar intruder) suggested that the MPTP animals may show more anxiety signs compared to the control. DRN neurons of MPTP group showed an increase in firing frequency compared to the control group.The rich behavioural repertoire of non-human primates makes them ideal translational models in which neuropsychiatric conditions can be studied. The present results that the MPTP-treated macaque replicates some depressive-like behaviour associated with PD.

Disclosures: A. Sadoun: A. Employment/Salary (full or part-time):; Motac. **M. Deffains:** None. **Q. Li:** A. Employment/Salary (full or part-time):; Motac neuroscience. **E. Bezard:** A. Employment/Salary (full or part-time):; Motac neuroscience. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac, TREEFROG Therapeutics, SE Therapeutics. **F.E. Georges:** None. **E. Pioli:** A. Employment/Salary (full or part-time):; Motac neuroscience.

Poster

PSTR393

Parkinson’s Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.06/C75

Topic: C.03. Parkinson’s Disease

Support: Aligning Science Across Parkinson's (Grant No. ASAP-020505) through the Michael J. Fox Foundation for Parkinson's Research (MJFF)
MICIN / AIE / 10.13039/5011000011033 (Grant No. PID2020-120308RB-I00)
CiberNed Intramural Collaborative Projects (Grant No. PI2020/09)

Title: Development and characterization of a non-human primate model of disseminated synucleinopathy

Authors: *J. CHOCARRO[1,2,3], A. J. RICO[1,2,3], G. ARIZNABARRETA[1,2,3], A. HONRUBIA[1,2,3], J. L. LANCIEGO[1,2,3];
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Abstract: The presence of a widespread cortical synucleinopathy is the main neuropathological hallmark underlying clinical entities such as Parkinson's disease with dementia (PDD) and dementia with Lewy bodies (DLB). There currently is a pressing need for the development of non-human primate (NHPs) models of PDD and DLB to further overcome existing limitations in drug discovery. Here we took advantage of a retrogradely-spreading adeno-associated viral vector serotype 9 coding for the alpha-synuclein mutated gene (A53T) to induce a widespread synucleinopathy in cortical and subcortical territories innervating the putamen. Four weeks post-AAV deliveries animals were sacrificed and a comprehensive biodistribution study was conducted, comprising the quantification of neurons expressing alpha-synuclein, rostrocaudal distribution and their specific location. In brief, cortical afferent systems were found to be the main contributors to putaminal afferents (superior frontal and precentral gyrus in particular), together with neurons located in the caudal intralaminar nuclei and in the substantia nigra pars compacta (giving rise to thalamostriatal and nigrostriatal projections, respectively). Obtained data extends current models of synucleinopathies in NHPs, providing a reproducible platform enabling the adequate implementation of end-stage preclinical screening of new drugs targeting alpha-synuclein

Disclosures: J. Chocarro: None. **A.J. Rico:** None. **G. Ariznabarreta:** None. **A. Honrubia:** None. **J.L. Lanciego:** None.

Poster

PSTR393

Parkinson's Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.07/C76

Topic: C.03. Parkinson's Disease

Title: A53t alpha-synuclein overexpression rat model of parkinson's disease: behaviour and electrophysiology phenotyping

Authors: *L. KONDRATAVICIUTE[1], H. N. CHAU[3], I. SKELIN[4], M. KAPADIA[5], S. SUMARAC[2], L. FRANCO VERGARA[2], L. ZIVKOVIC[2], C. TAN[1], L. MILOSEVIC[2], T. A. VALIANTE[6], L. V. KALIA[1], S. K. KALIA[3];
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Abstract: Parkinson's disease (PD) is a neurodegenerative movement disorder characterized by the loss of midbrain dopaminergic neurons and the formation of alpha-synuclein (a-Syn) aggregates. Patients diagnosed with PD exhibit a wide array of pathologies, such as motor impairment, depression, anxiety, anosmia, and altered neuronal activity in basal ganglia structures. The accumulation of a-Syn is central to PD pathology, but its precise role in pathogenesis remains unclear. Here, we investigated the emergence of motor and non-motor behavioural deficits and electrophysiological signatures of circuit dysfunction in rats overexpressing a mutant form of a-Syn linked to early-onset PD. Adult female Sprague-Dawley rats were unilaterally or bilaterally injected into the substantia nigra (SN) with adeno-associated virus (AAV) expressing either human mutant A53T a-Syn or empty vector (EV). Electrophysiological recordings including single unit and local field potentials were performed under isoflurane anesthesia from the subthalamic nucleus (STN) at 3 and 6 weeks post-unilateral injection. Behavioral experiments (sucrose preference, novelty suppressed feeding, habituation/dishabituation, open field, and grid walking) were conducted on a separate cohort of animals at 3 and 6 weeks post bilateral AAV injection. Post-mortem immunofluorescence staining for tyrosine hydroxylase and a-Syn was performed to confirm neurodegeneration. The unilateral loss of dopaminergic neurons in the SN of A53T a-Syn expressing animals coincided with the gradual emergence of pathological oscillations in the STN. Concurrently, A53T a-Syn overexpressing animals exhibited trends in changing firing rates and bursting activity in the STN compared to healthy EV control animals. Rats with bilateral expression of A53T a-Syn exhibited progressive deterioration of motor function, accompanied by a diminished responsiveness to palatable stimulation observed 6 weeks post-injection. These findings suggest that mutant a-Syn overexpression in the SN is sufficient to cause STN dysfunction, aberrant circuit oscillations within the basal ganglia and motor impairments. The observed alterations highlight the suitability of the viral-mediated A53T a-Syn overexpression model for investigating not only dopaminergic neurodegeneration but also circuit dysfunction and non-motor behaviour which will have potential value in the development of disease modifying therapeutics.

Disclosures: L. Kondrataviciute: None. **H.N. Chau:** None. **I. Skelin:** None. **M. Kapadia:** None. **S. Sumarac:** None. **L. Franco Vergara:** None. **T.A. Valiante:** None. **L.V. Kalia:** None. **S.K. Kalia:** None.

Poster

PSTR393

Parkinson’s Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.08/C77

Topic: C.03. Parkinson’s Disease

Support: SIP20230740
SIP20241457

Title: Effects of globus pallidus lesion with low doses of 6-OHDA on motor activity and anxiety over time in rat.

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Abstract: The globus pallidus (GP) is a basal ganglia structure playing a key role in the control of movement and in limbic and cognitive processes. GP receives dopaminergic projections from the *pars compacta* of substantia nigra. In previous studies we have shown that using low doses of 6-Hydroxidopamine (6-OHDA) lesioned rats showed memory deficits and increased anxiety without altering spontaneous motor activity 30 days after surgery. However, there are studies that indicate that some behaviors produced by dopaminergic lesions can be compensated for over time. The present study aimed to investigate if the effects of intrapallidal injection of 6-OHDA keep up for a long time. One group of rats received 6-OHDA (7 ug in 0.5 uL) where as another group only received the vehicle (0.5 uL). 30, 60, 90, 120 and 150 days(d) post lesion, rats were tested in motor trials: rotation induced by apomorphine (0.5 mg/kg, sc), the beam and the rotarod tests. Anxiety tests: plus maze and defensive burying behavior, were only measured at 30 and 150 d post lesion. The rats were euthanized at 150 d after dopaminergic lesion to determine the number of TH(+) neurons using an immunohistochemical technique. The rats administered with vehicle in the GP did not show significant changes over time in any of the tests measured, while in the rats with 6-OHDA contralateral turns were produced at day 30, and increased significantly in 60 d remaining without change until 150 d. In the beam test, lesioned rats did not change their speed to cross it, however they stumbled more when crossing it. Rotarod performance decreased from 30 d, having the lowest performance in 60 d and remained without significant changes over time. In the plus maze rats decreased time in open arms and increases burying behavior at 30 d indicating an increase in anxiety compared to the control group and these results not changes in 150 d. The conclusion is that lesion with low doses of 6-OHDA in the GP anxiety appears before motor deficits and neither of them show any type of compensation. In the case of motor deficit there was a greater deterioration over time. This could be similar of what happens in Parkinson's disease, in which non-motor symptoms appear years before motor symptoms develops

Disclosures: M. Garcia-Ramirez: None. **H. Tellez Vazquez:** None. **D. Matadamas:** None. **E.C. Chuc-Meza:** None.

Poster

PSTR393

Parkinson’s Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.09/C78

Topic: C.03. Parkinson's Disease

Support: CNPq[200336/2023-1]
Capes [88887.694614/2022-00]

Title: Evaluation of the mechanisms involved in the induction of pain in Parkinson's disease, evaluation of animal model.

Authors: *L. G. PEREIRA[1], F. T. VIERO[2], G. TREVISAN[1];
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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder of the central nervous system, characterized by neurodegeneration of dopaminergic neurons. In addition to the motor symptoms of PD, patients also suffer from non-motor symptoms, such as pain. It is crucial to emphasize that the mice models conventionally used for PD research often present substantial motor deficits, which can compromise the accurate assessment of nociception. Thus, the main objective of this study was to standardize an experimental model of PD that enables a practical assessment of nociception. We used the bilateral injection model of 6-hydroxydopamine (6-OHDA) of 1 µl of 6-OHDA (4 µg/µl) into the striatum to induce a model of PD, aiming to investigate non-motor symptoms. Male and female mice of the C57BL/6 were divided into the 6-OHDA and Sham groups. Behavioural analysis was performed at baseline and on the 7^{th}, 14^{th}, 21^{st}, and 28^{th} days after induction. The rotating cylinder test was conducted to evaluate possible motor function and balance deficits. The mechanical allodynia was assessed using the Von Frey filament's up-and-down method. We used the acetone test on the plantar surface to measure cold allodynia. Heat allodynia was assessed using the heating plate test at a constant temperature of 38º degrees (CEUA Nº 3164260423). Our results demonstrated no significant differences in forced locomotion between the groups on the days evaluated after the induction of PD, indicating the absence of locomotor damage. In the mechanical allodynia, when analyzing PD male mice, a nociceptive peak was observed on the 21^{st} day after induction ($p<0.0001$), while PD female mice showed this peak on the 7th day after induction. Furthermore, PD female mice manifested greater cold allodynia than PD male mice on the 14^{th} day after induction ($p<0.0001$). In the heat allodynia, PD female mice exhibited differences on the 14^{th}, 21^{st}, and 28^{th}days with a peak of heat allodynia on the 14^{th} day after induction compared to Sham animals ($p<0.0001$). On the other hand, PD male mice showed differences on the 7^{th}, 14^{th}, 21^{st}, and 28^{th} days, with a peak of heat allodynia on the 14^{th} day after induction compared to Sham animals ($p<0.001$). In conclusion, the use of the bilateral 6-OHDA injection model has proven to be a significant step forward in inducing a PD model in C57BL/6 mice, allowing the investigation of nociception. Behavioural assessment at different time points post-induction provided a comprehensive understanding of the observed nociceptive changes. The present study provides results for future research and paves the way for more refined approaches to understanding and treating pain in PD.

Disclosures: L.G. Pereira: None. **F.T. Viero:** None. **G. Trevisan:** None.

Poster

PSTR393

Parkinson's Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.10/C79

Topic: C.03. Parkinson's Disease

Support: VLAIO Baekeland: HBC.2022.0631

Title: Prodromal Mouse Model of Parkinson's Disease recapitulating REM Sleep Behavior Disorder and Olfactory Dysfunction: A Replication Study

Authors: *A. ANTHONISSEN[1,2], H. MAURIN[1], I. LENAERTS[1], S. EMBRECHTS[1], D. CRAUWELS[1], W. H. DRINKENBURG[3], P. VERSTREKEN[2], J. D. PITA ALMENAR[1], A. AHNAOU[1];
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Abstract: Parkinson's Disease (PD) is a heterogeneous multisystem disorder characterized by a long prodromal phase with non-motor symptoms (e.g. sleep and smell abnormalities) allowing early diagnosis and precision medicine. Recent studies revealed that people suffering from REM sleep behavior disorder (RBD) and/or olfactory dysfunction (OD) are at higher risk of developing PD later in life implicating them as ideal candidates to test precision therapies. Establishing a translational mouse model recapitulating RBD and/or OD followed by the development of motor deficits may improve our understanding of the molecular and electrophysiological mechanisms underlying the phenoconversion of RBD to PD. Therefore, we conducted a longitudinal phenotyping study in BAC-SCNA mice expressing the human α-Synuclein gene (SCNA) in bacterial artificial chromosome (BAC) to reproduce early RBD-like behavior and olfactory dysfunction (Taguchi et al., 2020). Bi-monthly polysomnographic video recordings, electrophysiological recordings, olfactory habituation-dishabituation test, as well as gait and motor behavior using Catwalk and rotarod tests were performed. We confirmed RBD-like behavior at 8 months, whereas the olfactory deficit did not appear at the age of 11 months. Ongoing immunohistochemistry (IHC) of misfolded α-Synuclein (α-Syn) aggregation, dopaminergic neurodegeneration, and neuroinflammation will enable correlation between spatial pathology progression and functional impairments in BAC-SCNA mice.

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Poster

PSTR393

Parkinson's Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.11/C80

Topic: C.03. Parkinson's Disease

Support: WoodNext Foundation
Maurine Cox Endowed Chair

Title: Investigating the impact of prior-gut dysfunction on nigrostriatal neurodegeneration in a parkinsonian rat model of 6-hydroxydopamine

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Abstract: Parkinson's disease (PD) is the most common movement disorder, characterized by motor dysfunction due to a loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc). Emerging evidence suggests an active role for gut pathology in the progression of PD, however, little is known about gut pathology prior to PD onset. Epidemiological evidence points to an increased risk for PD among people with irritable bowel disease (IBD). To experimentally assess the impact of prior gut dysfunction on a parkinsonian model, animals were exposed to dextran sodium sulfate (DSS), a well-studied model of IBD, prior to the 6-OHDA treatment. Adult (5-7m), male Sprague Dawley rats were randomly assigned to water treatment or DSS treatment for 3 cycles via drinking bottles. Water-treated rats received only water for the duration of the experiment, while DSS-treated rats repeated 3 cycles of 6 days of 4% DSS followed by 6 days of water. As expected, DSS-treated rats lost weight during each 6-day cycle of DSS, but regained weight when returned to 6 days of water. Following either water or DSS treatment, rats were randomly assigned to receive a unilateral injection of 6-OHDA into the dorsolateral striatum (DLS) or a unilateral injection of 0.2% ascorbic acid (control). To assess progressive SNc DA loss in 6-OHDA injected rats, animals received an injection of the the dopamine receptor agonist, apomorphine, and apomorphine-induced contralateral rotations were assessed at 7 days pre-6-OHDA injection, and at 7, 14, 21, and 28 days post. At 29 days post, we performed cylinder test as an additional measure of motor deficits. Additionally, we collected serum from all rats at each of the timepoints to measure gut-related metabolites and cytokines. Finally, rats were terminated at 30 days post and brains collected. Our data shows that over the course of the 3 cycles, DSS-treated rats drank more compared to water-treated animals. Additionally, DSS-treated rats had elevated levels of the chemokine, IP-10, after treatment completion ($p=0.0174$). Preliminary results show that 6-OHDA treated rats display a progressive time-dependent increase in apomorphine-induced rotations, but Water and DSS groups did not differ from each other. A recent study shows that IP-10 is associated with worse performance on cognitive tests in PD patients. Outcomes of these studies will improve our understanding of the relationship of prior gut dysfunction and onset of PD.

Disclosures: C. Kaufhold: None. **R. Srinivasan:** None. **F. Sohrabji:** None.

Poster

PSTR393

Parkinson's Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.12/C81

Topic: C.03. Parkinson's Disease

Title: Pre-clinical use of animal models for non-motor aspects of Parkinson's disease

Authors: *T. H. JOHNSTON[1], M. P. HILL[1], P. A. HOWSON[1], J. S. SCHNEIDER[2]; [1]Atuka Inc., Toronto, ON, Canada; [2]Dept Pathol, Anat. & Cell Biol, Thomas Jefferson Univ., Philadelphia, PA

Abstract: There are a considerable number of non-motor (NM) symptoms of Parkinson's disease (PD) including highly debilitating co-morbidities such as cognitive dysfunction, anxiety/depression, constipation, loss of sense of smell, and sleep disorders. The MPTP-lesioned nonhuman primate (NHP) model of PD was originally developed to recapitulate the primary motor symptoms of PD. It is now appreciated that iterations of that model can also be used to study NM symptoms. Additionally, newer alpha-synuclein based models in rodents can be used to study olfactory dysfunction/hyposmia and constipation/GI motility dysfunction. In a chronic low-dose MPTP NHP model, deficits in attention, working memory and executive dysfunction can be detected prior to the onset of significant motor dysfunction. In a variation of that model, mild motor deficits can be superimposed on early-appearing cognitive deficits. The human intruder test (HIT) can be used with MPTP-lesioned NHPs to study emotional reactivity, anxiety and depression-related behaviors. Home cage continuous activity monitoring reveals sleep disorders in MPTP-treated NHPs. A recently developed model of vagal motoneuron synucleinopathy in the mouse results in significantly decreased gastric motility. Historically, non-motor symptoms of PD have been difficult to treat. The increasing ability to model these NM symptoms of PD in animals provides an excellent opportunity to understand underlying pathology and to aid the development and evaluation of new therapeutics.

Disclosures: T.H. Johnston: A. Employment/Salary (full or part-time):; Atuka Inc. **M.P. Hill:** A. Employment/Salary (full or part-time):; Atuka Inc. **P.A. Howson:** A. Employment/Salary (full or part-time):; Atuka Inc. **J.S. Schneider:** F. Consulting Fees (e.g., advisory boards); Atuka Inc..

Poster

PSTR393

Parkinson's Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.13/C82

Topic: C.03. Parkinson's Disease

Support: Kenneth Campbell Foundation
International Brain Research Organization (IRBO)

Title: Olfactory system alterations in a novel model of dopaminergic loss by 6-OHDA injections in adult zebrafish

Authors: *L. PUTT, N. SANCHEZ GAMA, E. CALVO-OCHOA;
Biol. and Neurosci., Hope Col., Holland, MI

Abstract: Parkinson's Disease (PD) is a neurodegenerative disorder characterized by dopaminergic neuronal loss and severe motor impairment. Olfactory loss is prevalent in over 95% of those with PD and is a symptom that precedes motor deficits in most individuals. However, the mechanisms linking PD and olfactory dysfunction are not well understood. It has been proposed that dopaminergic loss causing retrograde degeneration in the olfactory system might be underlying this phenomenon. Zebrafish provide an ideal model to study these processes, as their olfactory system is analogous to those of mammals and its olfactory bulb (OB) contains a large population of dopaminergic neurons that mediate olfactory function. Moreover, zebrafish exhibit lifelong neurogenic capability (i.e., generation of new neurons) and a high degree of neuroplasticity, which makes it an ideal model to study possible repair processes in neurodegenerative diseases, such as PD. In this work, we sought to generate a novel model of dopaminergic loss in the OB of zebrafish by injection of 6-hydroxydopamine (6-OHDA) into the cerebrospinal fluid at the ventricular zone at the interphase between the olfactory bulbs and the telencephalon. We aimed to target dopaminergic neurons in the OBs but not in posterior motor nuclei to not alter locomotion. We assessed dopaminergic neuronal loss, markers of inflammation, morphological changes of olfactory axons, and pre-synaptic markers following the injections using immunohistochemistry. We also evaluated olfactory function by means of behavioral assays. We observed a dramatic increase in the number of apoptotic cells in the OB 1- and 3-days post injection (dpi) as well as a significant loss of dopaminergic neurons, confirming a successful lesioning method. This was accompanied by morphological changes in olfactory glomeruli (i.e., sites of synaptic connections between olfactory axons and dopaminergic dendrites). Further, there was a stark increase in the neuroinflammatory response, by means of GFAP staining at both time points. We also found disturbances in olfactory-mediated behavior suggesting olfactory functional alterations. Our results show that we successfully optimized a novel method for injection of 6-OHDA to target dopaminergic neurons in the olfactory system. We can use this model to further study the relationship between dopaminergic loss and olfactory alterations.

Disclosures: L. Putt: None. **N. Sanchez Gama:** None. **E. Calvo-Ochoa:** None.

Poster

PSTR393

Parkinson's Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.14/C83

Topic: C.03. Parkinson's Disease

Title: Mechanical and thermal thresholds in the MPTP macaque model of Parkinson's disease

Authors: A. SADOUN[1], E. Y. PIOLI[2], ***M. LANDRY**[3], E. BEZARD[3], Q. LI[4];
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Abstract: Many PD patients suffer from some form of acute or chronic pain, including musculoskeletal, fluctuation-related, central, nocturnal, orofacial, and peripheral pain. There is no direct correlation between motor impairment and altered pain thresholds, indicating that motor dysfunction and pain may represent different pathophysiological processes in the progression of PD. Although the pathophysiology of pain in PD remains poorly understood, clinical examination of some PD patients demonstrates a significant decrease in tactile and thermal thresholds together with a reduction in mechanical pain perception and a substantial loss of epidermal nerve fibres and Meissner corpuscles. Some authors found a correlation between PD disease severity and the reduction of pain and cold perception, suggesting that sensitivity tests could be an additional indicator of PD evolution/severityMechanical (using automatic Electronic Von Frey apparatus) and thermal sensitivity (Heat and cold: contact feedback-controlled Peltier thermode (NTE-3 Thermal Sensitivity Tester) were investigated in a large cohort of MPTP-treated macaque monkeys in OFF and ON L-DOPA conditions.MPTP-treated macaques displayed alterations in their mechanical and thermal thresholds with minimal benefit from dopamine-replacement therapy.The use of the MPTP macaque model of PD, the gold standard for antiparkinsonian and antidyskinetic treatment validation, could be extended to test therapeutic strategies to combat PD pain

Disclosures: A. Sadoun: A. Employment/Salary (full or part-time):; Motac. **E.Y. Pioli:** A. Employment/Salary (full or part-time):; Motac neuroscience. **M. Landry:** None. **E. Bezard:** A. Employment/Salary (full or part-time):; Motac neuroscience. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac, TREEFROG Therapeutics, SE Therapeutics. **Q. Li:** A. Employment/Salary (full or part-time):; Motac neuroscience.

Poster

PSTR393

Parkinson's Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.15/C84

Topic: C.03. Parkinson's Disease

Support: NIH BRAIN Grant RF1NS128896
NIH RO124764
McCamish Blue Sky Grant 2023

Title: Sensory-behavioral deficits in Parkinson's disease: Insights from a 6-OHDA mouse model

Authors: S. R. LINEN[1], N. H. CHANG[1], E. J. HESS[2], G. B. STANLEY[1], ***C. WAIBLINGER**[1];
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Abstract: Parkinson's disease (PD) is characterized by the degeneration of dopaminergic neurons in the striatum, predominantly associated with motor symptoms. However, non-motor deficits, particularly sensory symptoms, often precede motor manifestations, offering a potential early diagnostic window. The impact of non-motor deficits on sensation behavior and the underlying mechanisms remains poorly understood. In this study, we examined changes in tactile sensation within a Parkinsonian state by employing a mouse model of PD induced by 6-hydroxydopamine (6-OHDA) to deplete striatal dopamine. Leveraging the conserved mouse whisker system as a model for tactile-sensory stimulation, we conducted psychophysical experiments to assess sensory-driven behavioral performance during a tactile detection task in both the healthy and Parkinson-like states. Our results unveil that, beyond anticipated motor impairments, dopamine depletion significantly impaired tactile sensation behavior. Varied behavioral deficits, encompassing detection performance, task engagement, and reward accumulation, were observed across lesioned individuals. These findings suggest that the clinical variability in PD symptoms may stem from a complex interplay of sensory and motor impairments, alongside motivational factors. The implementation of a sensory detection task is a promising approach to quantify the extent of impairments associated with dopamine depletion in the animal model. This facilitates the exploration of early non-motor deficits in PD, emphasizing the importance of incorporating sensory assessments in understanding the diverse spectrum of PD symptoms.

Disclosures: S.R. Linen: None. **N.H. Chang:** None. **E.J. Hess:** None. **G.B. Stanley:** None. **C. Waiblinger:** None.

Poster

PSTR393

Parkinson's Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.16/C85

Topic: C.03. Parkinson's Disease

Support: NIH NIEHS R01 ES0314

Title: Alterations in cognitive function and mitochondrial bioenergetics in aging mice lacking Atp13a2

Authors: *K. M. CROUCHER[1,2], J. K. LEPP[1], S. M. SCOTT[1], C. M. BITTNER[1], E. J. HAMAD[1], S. E. ROGERS[1], C. H. ONG[1], S. M. FLEMING[1];
[1]Pharmaceut. Sci., Northeast Ohio Med. Univ., Rootstown, OH; [2]Biomedical Sciences Graduate Program, Kent State University, Kent, OH

Abstract: ATP13A2 is a lysosomal polyamine transporter with loss of function mutations linked to multiple neurodegenerative disorders including Parkinson's disease, Kufor-Rakeb Syndrome, Neuronal Ceroid Lipofuscinosis, Hereditary Spastic Paraplegia, and Amyotrophic Lateral Sclerosis. *In vitro* studies suggest loss of *ATP13A2* can impair mitochondrial function and increase vulnerability to neurodegenerative conditions. Previous *in vivo* work shows deficits in mitochondrial function in the nigrostriatal pathway and sensorimotor dysfunction in older *Atp13a2* knockout mice. The present study sought to expand the behavioral and mitochondrial analyses in aging *Atp13a2* knockout mice to include assessments of cognitive and neuropsychiatric behaviors and measurement of mitochondrial function in extranigral brain regions including the prefrontal cortex (PFC) and cerebellum (CBL). Male and female *Atp13a2* wildtype (WT), heterozygous (Het), and knockout (KO) mice aged 3, 12, and 18 months were included in the study. Cognitive function was assessed using an object recognition test that measures both attention and memory and the elevated plus maze was used to measure emotional reactivity. Sensorimotor function was also assessed. In the brain, mitochondrial fractions from the PFC and CBL were isolated and the oxygen consumption rate (OCR) of mitochondria (5ug/well) was measured in triplicate using the Seahorse XFp Flux Analyzer in response to ADP, Oligomycin, FCCP, and Rotenone/Antimycin-A in the presence of the energetic substrates glutamate, succinate, and malate. Alpha-synuclein and mitochondrial proteins were also measured in the PFC and CBL. Behavior analysis showed a decrease in total investigation time in 18 month male KO compared to WT in the object recognition test suggesting a decrease in attention in these mice. In addition, sensorimotor function was impaired in 18 month KO mice compared to WT replicating previous findings. Mitochondrial bioenergetics analysis revealed a blunted OCR response in 3 month KO mice in both the PFC and CBL. At 12 months OCR was increased in Het mice in the PFC and at 18 months OCR was increased in the Het mice in both the PFC and CBL. Protein analysis showed an increase in alpha-synuclein in the PFC of 18 month KO and Het compared to WT and in the CBL of 18 month Het compared to WT. Overall, these data indicate impaired function of ATP13A2 *in vivo* alters cognition and disrupts mitochondrial function in the prefrontal cortex and cerebellum.

Disclosures: K.M. Croucher: None. **J.K. Lepp:** None. **S.M. Scott:** None. **C.M. Bittner:** None. **E.J. Hamad:** None. **S.E. Rogers:** None. **C.H. Ong:** None. **S.M. Fleming:** None.

Poster

PSTR393

Parkinson's Disease: Animal Models and Non-Motor Studies

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.17/C86

Topic: C.03. Parkinson's Disease

Support: CIHR Grant 507489
NSERC Discovery Grant 506730
CIHR Canada Graduate Scholarship-Master's

Title: Progression of olfactory function in Parkinson's Disease following alpha-synucleinopathy initiation in the anterior olfactory nucleus

Authors: *R. TRAN, J. ARSENAULT, Z. CHEN, J. KIM;
Univ. of Toronto, Toronto, ON, Canada

Abstract: Parkinson's Disease (PD) is typically diagnosed upon the emergence of severe motor symptoms in stage 3/4 of the disease. At the onset of motor deficits, approximately 50% of cells in the caudal substantia nigra are degenerated. Notably, 90% of PD patients exhibit olfactory deficits, preceding motor symptoms by at least four years in the earliest stage of disease progression. The aggregation of alpha-synuclein (a-syn), the physiological hallmark of PD, initially appears in the olfactory system, specifically within the anterior olfactory nucleus (AON). The AON, a hub receiving direct inputs from the olfactory bulb and hippocampus, integrates bottom-up olfactory sensory information and top-down contextual information from the respective regions to facilitate various olfactory functions such as detection, discrimination and memory. Despite the early accumulation of a-syn within the AON in PD, its precise role in pathology and symptom manifestations remains elusive. The current study aims to address this gap by examining the progressive spread of a-syn following bilateral intracerebral injections of a-syn preformed fibrils (PFFs) or phosphate buffer saline (PBS) in the AON of transgenic (Tg) A53T mice and its consequent effects on olfactory and motor function. PFFs trigger the misfolding of endogenous a-syn and transmit to anatomically connected regions, modelling a-syn pathology in PD. Olfactory function is assessed longitudinally using a robust go/no-go paradigm we developed. As anticipated, baseline olfactory detection, discrimination and contextual odour learning did not differ between Tg and wildtype mice without PFF injections. Interestingly, the mixed 2-way ANOVA revealed a potential increase in olfactory sensitivity in PFF-injected mice from 1 to 3 months post-injection at low odourant concentrations, aligning with our prior findings indicating enhanced olfactory detection upon AON inhibition. We postulate that olfactory memory and motor deficits will manifest in PFF-injected mice, accompanied by severe a-syn pathology and neuroinflammation in regions interconnected with the AON. The outcomes of the current study will contribute to the understanding of the mechanisms underlying olfactory deficits in PD and aid in the development of a reliable and cost-effective diagnostic tool for identifying PD patients at the earliest possible stage.

Disclosures: R. Tran: None. **J. Arsenault:** None. **Z. Chen:** None. **J. Kim:** None.

Poster

PSTR393

Parkinson's Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.18/C87

Topic: C.03. Parkinson's Disease

Support: DoD NETP 13204752 to Thyagarajan Subramanian
NIDDK R01 DK124098 to Thyagarajan Subramanian
NINDS R01 NS104565 to Thyagarajan Subramanian
Anne M. and Phillip H. Glatfelter III Family Foundation to Thyagarajan Subramanian and Kala Venkiteswaran

Title: Polysomnographic analysis of REM sleep without atonia in the paraquat and lectin rat model of Parkinson's disease

Authors: *C. SWAIN[1], K. LE[2], V. PESHATTIWAR[2], D. POKHAREL[1], T. SUBRAMANIAN[3], K. VENKITESWARAN[1];
[1]Neurosciences and Neurol., Univ. of Toledo Col. of Med. and Life Sci., Toledo, OH; [2]Neurol., Univ. of Toledo Col. of Med. and Life Sci., Toledo, OH; [3]Neurology, Neurosciences and Bioengineering, Univ. of Toledo Col. of Med. and Life Sci., Toledo, OH

Abstract: REM sleep is a phase of sleep in both humans and rodents characterized by rapid eye movement and atonia. REM sleep behavior disorder (RBD) is a clinical condition in humans where atonia is lost during REM sleep, and dream enactment often occurs. Studies show anywhere between 35 and 91% of idiopathic RBD cases may phenoconvert to a neurodegenerative disease, most commonly a Lewy body disease such as Parkinson's disease or Dementia with Lewy bodies. RBD therefore represents an important and specific risk factor for the development of Parkinson's disease, as it presents prior to the onset of motor symptoms. Our lab is investigating the presence of RBD-like sleep abnormalities using video polysomnography (vPSG) in the paraquat and lectin (P+L) rat model of Parkinson's disease. A preliminary study was done in which adult male Sprague-Dawley rats (n=3) were implanted with stainless steel screws above the supraorbital notch and parietal cortices for EOG and EEG, respectively, and a wire electrode in the neck muscle for EMG. 24 hour PSG recordings with concurrent video analysis was done to determine sleep architecture following 7 day oral administration of 1mg/kg paraquat and 0.05% lectin. vPSG recordings were taken at baseline, immediately following, two, and four weeks post-P+L treatment. EOG, EEG, and EMG waveforms were segmented into 10s epochs and features of EOG, EEG, and EMG power of specific frequencies were analyzed to determine when the rat was in REM sleep. EMG activity during REM sleep was analyzed, and the percentage of REM epochs with EMG activity was calculated as the percentage of REM epochs without atonia. A repeated measure ANOVA showed a significant effect of time on REM sleep without atonia. Experiments are ongoing to confirm our findings with a larger cohort of rats using methods we have previously described (Iyer, 2019) in a 90 day P+L rat model, in which we will investigate the progression of sleep abnormalities with respect to the development

of motor deficits. A discovery of RBD-like sleep abnormalities prior to the onset of motor deficits would indicate the P+L rat model recapitulates a progression of both nonmotor and motor symptomatology consistent with the clinical condition of Parkinson's disease in humans.

Disclosures: C. Swain: None. **K. Le:** None. **V. Peshattiwar:** None. **D. Pokharel:** None. **T. Subramanian:** Other; Speaker's bureau-Supernus, Neurocrine, Acadia; Royalty CRC Press; Founder of StereoRx. **K. Venkiteswaran:** None.

Poster

PSTR393

Parkinson's Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.19/C88

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01NS124777

Title: Simultaneous PET and phMRI studies of modulation between striatal dopamine D2 and metabotropic glutamate 5 (mGluR5) receptor function in rat brain

Authors: *I. CHEN[1], B. G. JENKINS[2], Z. I. SUAR[3], *I. I. CHEN[4], A.-L. I. BROWNELL[5]; [1]A.A.Martino's Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Charlestown, MA; [2]Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Boston, MA; [3]Massachusetts Gen. Hosp., Charlestown, MA; [4]Athinoula A. Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Charlestown, MA; [5]Radiology, Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Simultaneous PET and functional MRI (fMRI) studies can help disentangle modulation and interaction between dopamine (DA) and metabotropic glutamate (mGlu) receptors. The results of the receptor modulation can be readily utilized in the drug development for dopamine-glutamate regulation related disorders, including Parkinson's disease (PD. The simultaneous acquisition of PET and MRI using Bruker Biospec 4.7T system allows for the assessment of receptor binding potential (BP) alongside neural responses via fMRI. In a cohort of normal control rats (n=10, male/female=5/5) aged between 2 to 8 months, we investigated the impact of dopamine innervation changes on mGluR5 binding potential. Dynamic PET images were obtained using F18-FPEB (F18-3-fluoro-5-[(pyridin-3-yl)ethynyl]benzonitrile) over an 80-minute period, with d-amphetamine (AMPH) administered 20 minutes post F18-FPEB injection. We observed a basal binding potential of F18-FPEB in the striatum of 5.51±0.06. No statistically significant difference was detected between male (5.67± 0.78) and female (5.35 ±1.04) rats, and age did not influence F18-FPEB BP. Following AMPH administration, there was a displacement in F18-FPEB binding, with a delta BP of 1.37 ± 0.41. No gender disparity was noted (male 1.24±0.58, female 1.51 ± 0.66). fMRI revealed significant increases in regional cerebral blood volume (rCBV) following AMPH challenge, particularly in major dopaminergic areas, consistent with our previous findings. The significant rCBV increases are correlated with dopamine

increases leading to displacement in mGluR5 binding. Furthermore, we utilized F18-Fallypride to investigate potential alterations in dopamine D2 receptors during the pre-symptomatic stage of A53T transgenic PD rats (n=9, male/female=6/3) compared to normal control rats (n=3). The F18-Fallypride BP was reduced in the transgenic rats (control 3.32±0.39, PD 2.16±0.42, $p<0.09$), suggesting early changes in dopamine D2 receptor availability. Summary: These results suggest that interplay between mGluR receptors and dopamine can be imaged dynamically and longitudinally in a transgenic model of PD.

Disclosures: I. Chen: None. **B.G. Jenkins:** None. **Z.I. Suar:** None. **I.I. Chen:** None. **A.I. Brownell:** None.

Poster

PSTR393

Parkinson's Disease: Animal Models and Non-Motor Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.20/Web Only

Topic: C.03. Parkinson's Disease

Support: SKAN grant

Title: Establishing Parkinson's Disease mouse model to study the early molecular mechanism of nonmotor symptoms.

Authors: *L. DIWAKAR[1], M. CHOPRA[2], S. ANILKUMAR[2], A. STEZINSUNNY[2];
[1]Ctr. for Brain Res., Indian Inst. of Sci., Bengaluru, India; [2]Ctr. for Brain Res., Bangalore Urban, India

Abstract: Title: Establishing Parkinson's Disease mouse model to study the early molecular mechanism of nonmotor symptoms.Manasvi Chopra 1, Shobha Anilkumar 1, Albert Stezin Sunny 1, and Latha Diwakar 1.1Centre for Brain Research, Indian Institute of Science, Bangalore, India.
Abstract: Parkinson's Disease (PD) is the second most common disorder characterized by the loss of dopaminergic neurons in the nigrostriatal system. Although PD primarily causes motor symptoms it is now known that non-motor symptoms like anxiety, depression, anosmia, sleep disorders, and constipation occur much before a decade. Defining the timeline for the onset of motor and non-motor symptoms and associated mechanisms of disease progression is crucial for effective pharmacologic intervention in PD. We were interested in studying the molecular mechanisms of such nonmotor symptoms in animal models of PD. As a first step, we opted for a subchronic dose of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) for 60 days of treatment to achieve a progressive substantial loss of dopamine-producing neurons in substantia nigra pars compacta and dopaminergic projections in the striatum i.e. nigrostriatal pathway. We were able to show clear emergence of non-motor symptoms at 15 days followed by motor symptoms after 30 days of MPTP injection. Further, we saw an increase in α-synuclein and

phospho-synuclein as well as significant loss of Tyrosine hydroxylase (TH) neurons after 60 days of MPTP treatment. There was a loss of TH and Dopamine transporter fibers also indicating the pathogenesis of PD. We also observed a significant loss of dopamine in the striatum after 60 days of MPTP compared to saline-injected mice. We explored the possible involvement of neuroinflammation in the emergence of nonmotor symptoms. The present sub-chronic MPTP model of PD gives us scope to investigate in detail the role of the extra nigrostriatal pathway to better understand the emergence of nonmotor symptoms.

Disclosures: L. Diwakar: None. **M. Chopra:** None. **S. Anilkumar:** None. **A. StezinSunny:** None.

Poster

PSTR394

Huntington's Disease: Preclinical and Clinical Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.01/Web Only

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: University of Colorado Alzheimer's and Cognition Center (CUACC) plus philanthropy
Colorado Clinical and Translational Sciences Institute (CCTSI)
Huntington's Disease (HD) Pilot Grant

Title: Elevated levels of mosaic aneuploidy in the development and progression of Huntington's disease

Authors: M. ELOS[1,2], J. CANEUS[1,3,2], M. AHMED[1,2], P. GRISSOM[1,2], N. JOHNSON[1,2], H. POTTER[1,2], ***H. J. CHIAL**[4,5];
[1]Univ. of Colorado Alzheimer's and Cognition Ctr., Dept. Neurol., Linda Crnic Inst. for Down Syndrome, Aurora, CO; [2]University of Colorado Anschutz Medical Campus, Aurora, CO; [3]Sanofi, Orlando, FL; [4]Univ. of Colorado Anschutz Med. Campus, Aurora, CO; [5]University of Colorado Alzheimer's and Cognition Center, Dept. Neurology, Linda Crnic Institute for Down Syndrome, Aurora, CO

Abstract: Huntington disease (HD) is a neurodegenerative disorder of the central nervous system with an autosomal dominant inheritance pattern. HD arises due to neurodegeneration in the caudate nucleus, putamen, cortex, thalamus, and cerebellum. The neurodegeneration arises from the aggregation of mutant Huntingtin (HTT) protein caused by a CAG trinucleotide repeat expansion (>35 repeats) mutation in the *HTT* gene on the short arm of chromosome 4. HTT protein is required for proper mitotic spindle orientation, chromosome segregation, and cell cycle regulation. Molecular mechanisms underlying HD pathogenesis remain elusive. Here, we investigated whether chromosome segregation defects that lead to mosaic aneuploidy and consequent apoptosis contribute to neuronal loss in HD. Single cell suspensions of human brain

samples (cortex and cerebellum) and fibroblast cell lines from HD donors and age-matched control donors were processed for FISH analysis with a human chromosome 21 probe, NeuN staining, and TUNEL staining. We also carried out karyotype analyses of the HD fibroblast cell lines. Single cell suspensions of brain cells from mouse models of HD (cortex and cerebellum) were processed for FISH analysis with mouse chromosome 5 and 16 probes. We observed significantly higher levels of mosaic aneuploidy in brain cells, including neurons, and higher levels of apoptosis in aneuploid brain cells from the cortex and cerebellum of HD donors compared to control donors. We also discovered significantly higher levels of mosaic aneuploidy and a trend towards higher levels of apoptosis in the fibroblast cell lines from HD donors compared to age-matched control donors. Karyotype analyses showed that 50% of the HD fibroblast cell lines exhibited chromosomal abnormalities, whereas none of the control fibroblast cell lines exhibited these abnormalities. We also observed significantly higher levels of mosaic aneuploidy in brain cells from the cortex and cerebellum of HD mice compared to wild-type littermate controls. These data provide evidence that chromosome segregation defects that lead to genomic instability may cause HD neurons to undergo apoptosis. Studies from our lab and others suggest that aneuploidy may be a shared mechanism underlying many neurodegenerative disorders: Alzheimer's disease, Frontotemporal dementia, Niemann-Pick type C, and HD. Our findings highlight the need to further investigate the biological mechanism(s) underlying HD pathology and set the stage for novel therapeutic approaches to HD. Future studies will focus on developing cell lines with inducible expression of trinucleotide repeat expanded HTT for use in functional studies and drug screens.

Disclosures: M. Elos: None. **J. Caneus:** None. **M. Ahmed:** None. **P. Grissom:** None. **N. Johnson:** None. **H. Potter:** None. **H.J. Chial:** None.

Poster

PSTR394

Huntington's Disease: Preclinical and Clinical Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.02/C89

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant R01NS040068
NIH Grant U01NS105509
NIH Grant U01NS103475
CHDI Foundation Grant A3917
CHDI Foundation Grant A6266

Title: Cross-sectional analysis of cortical changes in Huntington's disease using 3T MRI

Authors: *H. BOCKHOLT[1], **A. M. KEY**[2], J. D. CLEMSEN[1], B. T. BAKER[1], M. S. RUDRUD[2], H. JOHNSON[3], J. S. PAULSEN[2];

[1]Georgia State Univ., Atlanta, GA; [2]Univ. of Wisconsin, Madison, Madison, WI; [3]Univ. of Iowa, Iowa City, IA

Abstract: Background: Huntington's Disease (HD) is linked to significant brain changes, especially in the basal ganglia, but also in cortical areas, which may serve as vital biomarkers[3,4]. This study[1] evaluates cortical depth alterations as potential surrogate markers in clinical trials.
Methods: Using 3T MRI, this cross-sectional study measured changes in the cortical areas of HD patients with advanced techniques provided by FreeSurfer[2], compared with clinical disease progression indices. Results: Participants were categorized into severity groups—control (251), low (212), medium (307), and high (339), predominantly aged 34 to 45 years, mostly female. Key findings included:•Low vs. Medium Severity: Decreases in the left entorhinal and right inferior parietal areas ($p=0.0281$ and $p=0.0090$ respectively) suggest these regions are particularly affected at mid-stages of HD.•Low vs. High Severity: Significant reductions in the left entorhinal and left medial orbitofrontal areas ($p=0.00016$ and $p=0.0142$ respectively) highlight these as critical regions impacted in more advanced stages.•Control vs. High Severity: The left entorhinal area showed notable deterioration compared to control groups ($p=0.00018$), underscoring its sensitivity to HD progression.These pairwise comparisons indicate statistically significant differences in cortical areas correlating with disease severity, demonstrating progressive cortical deterioration across HD's spectrum. Conclusion: This study highlights the potential of cortical measurements as reliable biomarkers of disease progression in HD clinical trials. High-resolution 3T MRI measurements of specific regions like the entorhinal and medial orbitofrontal areas provide significant insights into neurodegenerative changes, potentially enhancing therapeutic efficacy assessment. Future Work: Further studies will explore longitudinal analyses and a broader range of cortical metrics, using both 3T and 1.5T MRI scans for consistency across different MRI field strengths and sites. Imaging data harmonization using SynthSeg across multiple sites will be explored to validate these cortical measurements as robust surrogate biomarkers. References: 1. PREDICT-HD Consortium, 2. https://surfer.nmr.mgh.harvard.edu/fswiki/FreeSurferMethodsCitation, 3. https://doi.org/10.1016/j.nicl.2020.102211, 4. https://doi.org/10.1002/hbm.26125

Disclosures: H. Bockholt: None. **A.M. Key:** None. **J.D. Clemsen:** None. **B.T. Baker:** None. **M.S. Rudrud:** None. **H. Johnson:** None. **J.S. Paulsen:** None.

Poster

PSTR394

Huntington’s Disease: Preclinical and Clinical Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.03/C90

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Assessing pathological cortical laminar architecture in Huntington's Disease using ultra-high-resolution ex vivo MRI imaging.

Authors: Q. SMITH[1], P. C. NOPOULOS[2], ***T. KOSCIK**[3];
[1]Arkanasas Children's Res. Inst., Little Rock, AR; [2]Psychiatry, Univ. of Iowa Hlth. Care, Iowa City, IA; [3]Arkansas Children's Res. Institute/University of Arkansas for Med. Sci., Little Rock, AR

Abstract: Maladaptive cortical development has been hypothesized as a source of pathology in Huntington's Disease (HD). It is unclear whether observations in mouse models and fetal tissue that demonstrate abnormalities in neuronal migration result in a persistent phenotype beyond the developmental period. Specifically, disrupted neuronal migration has been observed where neurons that typically migrate to layers II/III remain in deeper cortical layers. As layers II/III are implicated in gain control in cortical circuits, their reduction is consistent with hyperexcitable properties of the cortex in HD. Our goal is to map cortical laminar architecture in end stage HD to map the presence and regional distribution of abnormalities in cortical laminar architecture. As laminar architecture is too small to be visualized conventional MRI resolution and the expanse of the cerebral cortex is not practically quantified using intensive histological methods. We have developed a set of high-throughput, ultra-high-resolution, ex vivo MRI techniques to image the entire brain at ~150 micron resolution (~2-300x standard MRI resolution). To date we have scanned 7 HD brain specimens and neurotypical, age- and sex-matched controls. Following image cleaning (denoising, intensity inhomogeneity correction, and averaging), we labeled cortical lamina using semi-automated techniques where intensity profiles across and perpendicular to the cerebral cortical surface indicate layer boundaries. These data suggest that superficial cortical laminae (layers II/III) are significantly thinner in HD than in neurotypical controls. This is consistent with disrupted neuronal migration to upper layers. In addition, deeper cortical layers are significantly reduced in cases of extreme CAG repeat expansion, juvenile onset forms of HD. Our results demonstrate that maladaptive cortical development is a persistent phenotype in HD and disease-course modifying CAG repeat length affects cortical laminar phenotypes.

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Poster

PSTR394

Huntington's Disease: Preclinical and Clinical Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.04/C91

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant R01NS040068
NIH Grant U01NS105509

NIH Grant U01NS103475
CHDI Foundation Grant A3917
CHDI Foundation Grant A6266

Title: Cross-sectional analysis of cerebral white matter parcellation in Huntington's disease using 3T MRI scans with FreeSurfer

Authors: *B. BAKER[1], J. D. CLEMSEN[2,1], H. JOHNSON[3], J. S. PAULSEN[4], H. J. BOCKHOLT[2,1];
[1]TReNDs, Atlanta, GA; [2]Georgia State Univ., Atlanta, GA; [3]The Univ. of Iowa, Iowa City, IA; [4]Univ. of Wisconsin, Madison, Madison, WI

Abstract: Background: In Huntington's Disease (HD), cerebral white matter exhibits significant degenerative changes that may aid in understanding disease progression. This cross-sectional study utilizes 3T MRI scans and FreeSurfer software to parcellate cerebral white matter and investigate its correlation with disease severity.
Methods: We employed high-resolution 3T MRI to achieve detailed parcellation of cerebral white matter regions in HD patients[2] and matched controls using FreeSurfer[1]. This approach allowed for precise quantification of volumetric changes and provided insights into the structural integrity of specific white matter regions. The study categorized participants into four severity groups—control (251), low (212), medium (307), and high (339)—with a mean age range from 34 to 45 years, predominantly female.
Results: Initial findings from this cross-sectional study reveal distinct volumetric reductions in the cerebral white matter of HD patients, which appear to correlate with clinical measures of disease severity. Total WMH volume was significantly different in individuals with high disease severity compared to medium severity (corrected p=0.04695), and low severity (corrected p=0.02193). Additionally, total white matter volume in the left entorhinal cortex showed significant differences between participants with high disease severity when compared with low severity (corrected p=0.02193).
Conclusion: The use of 3T MRI for white matter parcellation offers valuable insights into structural changes in HD. Our study highlights the potential of this approach to assess the extent of white matter deterioration and its implications for disease progression; however, the scope is limited to cross-sectional data from 3T scans, which may not capture the full variability of HD impacts across different field strengths.
Future Work: To enhance our understanding and validate our current findings, future research will include longitudinal studies and incorporate 1.5T scans. This will broaden the spectrum of detectable changes and improve the generalizability of our results. Additionally, we plan to utilize SynthSeg, an advanced neuroimaging tool, to ensure consistent and accurate parcellation across different scanner types and settings, enabling a more comprehensive analysis of HD's impact on cerebral white matter.
Keywords: Huntington's Disease, cerebral white matter, FreeSurfer, 3T MRI, cross-sectional study, neuroimaging, disease progression, brain structure, SynthSeg
References:1. Freesurfer, https://surfer.nmr.mgh.harvard.edu/fswiki/FreeSurferMethodsCitation2. PREDICT-HD Consortium

Disclosures: B. Baker: None. **J.D. Clemsen:** None. **H. Johnson:** None. **J.S. Paulsen:** None. **H.J. Bockholt:** None.

Poster

PSTR394

Huntington's Disease: Preclinical and Clinical Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant U01NS103475
NIH Grant U01NS105509
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CHDI Foundation Grant A3917
CHDI Foundation Grant A6266

Title: Neuroimaging associations of smell deficits in prodromal Huntington's disease

Authors: M. S. RUDRUD[1], W. H. ADAMS[2], B. T. BAKER[3], J. CLEMSEN[3], A. M. KEY[1], H. J. BOCKHOLT[3], H. JOHNSON[4], D. K. BURKS[1], *J. S. PAULSEN[1];
[1]Univ. of Wisconsin, Madison, Madison, WI; [2]Loyola Univ. Chicago, Chicago, IL; [3]TReNDS Ctr., Georgia State Univ., Atlanta, GA; [4]The Univ. of Iowa, Iowa City, IA

Abstract: Introduction: The symptomatic expression of Huntington's Disease (HD) includes the degradation of many motor and cognitive abilities. Although clinical diagnosis typically occurs around 30-50 years of age, many studies have shown olfactory impairments over a decade earlier. The current study investigates the proposed degradation of the olfactory system in the prodromal stage of the disease. HD pathology research reports that the olfactory bulb has anatomical changes with disease manifestation, and the huntingtin protein has been shown to aggregate in the olfactory bulb. This study aimed to assess the association of neuroimaging and olfactory performances to determine whether MRI can provide in vivo measures of the olfactory system's degradation in HD. Methods: Participants completed the University of Pennsylvania Smell Identification Test (UPSIT), and recommended cut-offs were used to determine impairment. Univariable and multivariable generalized linear mixed effects models were used to estimate the odds of the outcome occurring as a function of elapsed time as well as the following: participants' smell score; age by cytosine, adenine, and guanine product (CAP) score; sex; race; ethnicity; highest occupation; years of education; American National Adult Reading Test (ANART) score. For each multivariable model, the explanatory variable of interest was participants' smell score, and a receiver operating characteristic (ROC) curve was used to identify the optimal smell score needed to detect the outcome. Covariates included in this model were determined *a priori* and included elapsed time, CAP score, and years of education. Over 4400 visits were available from the PREDICT-HD study to calculate olfactory functioning at baseline and over time. Results: There were no differences in caudate and volumes at baseline between those with an impaired versus normal smell response. Using longitudinal data, even when stratified by risk status, there was generally no substantial difference between those with

an impaired versus normal smell response in the delta caudate and putamen response over time. Conclusion: Tracking neuroimaging deficits in the prodromal stages of HD is highly robust in the basal ganglia (i.e., caudate and putamen). Hardware variations, such as 7T MRI scanners, additional MRI outcomes, or alternative analysis methods, such as connectivity and/or diffusivity measures, may be required to assess the pathology-reported impairments associated with olfactory function and the olfactory circuit in prodromal HD.

Disclosures: M.S. Rudrud: None. **W.H. Adams:** None. **B.T. Baker:** None. **J. Clemsen:** None. **A.M. Key:** None. **H.J. Bockholt:** None. **H. Johnson:** None. **D.K. Burks:** None. **J.S. Paulsen:** None.

Poster

PSTR394

Huntington's Disease: Preclinical and Clinical Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.06/C93

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Donald A. King Fellowship
UCF OUR Grant

Title: Applying artificial intelligence and topological data analysis to neuropathological assessment in models of Huntington disease

Authors: *S. MOLDENHAUER[1], A. L. SOUTHWELL[2];
[1]Univ. of Central Florida, Orlando, FL; [2]Col. of Med., Univ. of Central Florida, Orlando, FL

Abstract: As medical treatments designed to combat neurodegeneration advance, their effects on atrophy are quantified in animal models to test validity. This is important because treatments designed to combat neuropathology are more likely to modify the disease itself, per contra to treatments designed to mask or treat symptoms. One way to quantify brain region size is MRI, which while accurate, is prohibitively expensive. Conversely, stereological volume assessment, the process of estimating the volume of 3D brain regions from 2D brain sections, is more commonly used. This method involves manually tracing cross sections of a brain region of interest, followed by application of the Cavalieri principle to estimate the volume. This approach, however, is inefficient due to the manual tracing process, and invites potential inaccuracies from individual differences in brain region boundary perception, requiring a single investigator to evaluate all brains for a particular study. By leveraging recent artificial intelligence (AI) and topological data analysis advancements, including self-attention seen in large language models, like Chat-GPT, alongside our novel down-sample and patch self-attention techniques, we have developed an AI model capable of automating and enhancing the precision of stereological volume assessment. For our project, we used Q175FDN and a humanized mouse model of Huntington disease (HD) to create two image datasets of coronal cross sections containing

outlines of the striatum, a region that experiences significant neurodegeneration and atrophy in HD. The first dataset trained our AI, while the second serves for testing, comparing automated to manual assessments. This comparison is based on genotypic differences between HD and wildtype mice, inter-group variability, and assessment time as output measures. Our program's success in automating and enhancing stereological volume assessment, could increase efficiency of preclinical evaluation of neuropathology, allowing for a greater number of experimental therapies to be tested and facilitating drug discovery for this and other intractable neurodegenerative diseases.

Disclosures: S. Moldenhauer: None. **A.L. Southwell:** None.

Poster

PSTR394

Huntington's Disease: Preclinical and Clinical Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.07/C94

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH grant R01NS040068
NIH grant U01NS103475
NIH grant U01NS105509
CHDI Foundation Grant A3917
CHDI Foundation Grant A6266

Title: Cross-sectional analysis of white matter integrity in Huntington's disease using 3T diffusion tensor imaging

Authors: *J. CLEMSEN[1], A. M. KEY[2], M. S. RUDRUD[2], B. T. BAKER[1,3], H. J. JOHNSON[4], J. S. PAULSEN[2], H. J. BOCKHOLT[1,3];
[1]TReNDS Ctr., Atlanta, GA; [2]Univ. of Wisconsin, Madison, Madison, WI; [3]Georgia State University, Atlanta, GA; [4]Univ. of Iowa, Iowa City, IA

Abstract: Background: Huntington's Disease (HD) is marked by significant structural changes in the brain, including severe white matter deterioration[4]. This cross-sectional study utilizes 3T Diffusion Tensor Imaging (DTI) to explore white matter integrity in HD patients, focusing on the correlation between 4 groups of disease severity and diffusion metrics such as Fractional Anisotropy (FA) and FreeWater.
Methods: Employing 3T DTI, we quantitatively[2,3] assessed FA and FreeWater across various white matter regions in a cohort of HD patients[1] compared to family controls. This method enabled focused analysis of white matter microstructural integrity and its association with clinical severity indicators.
Results and Discussion:
The left entorhinal area produced significant T-test differences between the low and medium

groups (p = 0.0281), and between the low and high groups (p = 0.00016). Similarly, the right inferior parietal area showcased significant differences between the low and medium groups (p = 0.0089). Less so, a significant difference was noted in the right insula area when comparing the low group with the control group (p = 0.0435). In addition, the left medial orbitofrontal area demonstrated a significant difference between the low and high groups (p = 0.0142). As expected, the comparison between the medium and high groups left pars orbitalis area was significantly larger in the medium group compared to the high group (p = 0.0438), as highlighted in the positive t-test statistic. Finally, the left entorhinal area showed a significant reduction in the control vs. high comparison (p = 0.00018).

Conclusion:

The analysis successfully identifies significant differences in brain region metrics among different comparative groups. The consistency of significant results, particularly in the left entorhinal area across different comparisons, underscores the sensitivity of this region to group variability. These findings provide a solid foundation for further research to explore the underlying causes and implications of these differences in brain structure, potentially aiding in understanding the physiological and clinical significance of these variations.

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Disclosures: J. Clemsen: None. **A.M. Key:** None. **M.S. Rudrud:** None. **B.T. Baker:** None. **H.J. Johnson:** None. **J.S. Paulsen:** None. **H.J. Bockholt:** None.

Poster

PSTR394

Huntington’s Disease: Preclinical and Clinical Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.08/C95

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: William N. and Bernice E. Bumpus Early Career Investigator’s Innovation Award
William N. and Bernice E. Bumpus Post-doctoral Fellowship
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European Molecular Biology Organization (EMBO, ALTF 1171-2013
Human Frontier Science Program (HFSP, LT000888/2014-L)
R35GM142917

Title: Age-dependent alterations in the human striatum identify a PPP1R15B-miR-196a node as a modifier of Huntington's disease phenotypes

Authors: *V. A. CHURCH[1], A. KRZYZOSIAK[4], B. MIAO[5], S. CHEN[6], J.-S. KWON[6], B. ZHANG[6], S. DAHIYA, MD[7], M. B. VICTOR[2], A. BERTOLOTTI[8], A. S. YOO[3];
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Abstract: Polyglutamine expansion in Huntingtin (*HTT*) causes its aggregation and progressive loss of striatal neurons in Huntington's disease (HD). HD is a mostly adult-onset neurodegenerative disorder with no disease-modifying therapies. Here we found that human striatal aging is associated with a global upregulation of genes involved in translation, including the translation and proteostasis regulator PPP1R15B (R15B). We used the R15B inhibitor Raphin1 to investigate if the age-associated changes could modify HD pathology. R15B inhibition rescued early learning and late motor deficits in HD^{YAC128} mice. In striatal medium spiny neurons directly reprogrammed from fibroblasts of symptomatic HD patients (HD-MSNs), Raphin1 reduced the formation of mutant HTT aggregates and neuronal death. Genetic knockdown of R15B also protected HD-MSNs from neurodegeneration whereas its overexpression exacerbated disease phenotypes. Moreover, both human striatum and reprogrammed MSNs exhibited age-dependent decline of miR-196a, a microRNA that directly targets non-conserved sites in human R15B 3'UTR and overexpressing miR-196a lowered mutant HTT aggregation. This work identifies age-dependent alterations in miR-196a and its target R15B and demonstrates the therapeutic potential of reversing these changes in diverse models and readouts of HD. We propose miR-196a and R15B as disease-modifying targets in HD.

Disclosures: V.A. Church: None. **A. Krzyzosiak:** None. **B. Miao:** None. **S. Chen:** None. **J. Kwon:** None. **B. Zhang:** None. **S. Dahiya, MD:** None. **M.B. Victor:** None. **A. Bertolotti:** None. **A.S. Yoo:** None.

Poster

PSTR394

Huntington's Disease: Preclinical and Clinical Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.09/C96

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Disruption of mutant, polyQ expanded huntingtin aggregation by modulation of UBE2K, a novel ubiquitin-proteasome drug target.

Authors: G. MAOR, D. B. OLIVER, J. RANJAN, D. B. CHIMMANAMADA, *V. K. VISHNUDAS, S. GESTA, K. M. ROSEN;
BPGbio, Framingham, MA

Abstract: Huntington's disease (HD) is a uniformly fatal, autosomal dominant, neurodegenerative disorder caused by a triplet repeat expansion of glutamine (polyQ) codons (CAG) in the N-terminal coding exon (exon 1) of the Huntingtin (Htt) gene. The expression of mutant Htt is associated with the accumulation of intracellular aggregates containing the mutant protein and leads to the degeneration of the basal ganglia with progressive loss of striatal medium spiny neurons as well as the loss of cortical and other neurons. The mutant form of Htt has been shown to impact several different cellular functions that could negatively affect neuronal survival. Additionally, debate continues as to whether it can be degraded via either proteasomal or lysosomal-based mechanisms, and whether these different pathways can be manipulated to aid in the removal of the mutant protein and its aggregates. Prior research had identified a specific ubiquitin E2 conjugating enzyme, UBE2K (or Hip2), that interacted with the N-terminal region of Htt. This interaction occurred in a manner that was not influenced by whether it was the mutant or non-mutant form of Htt (Kalchman et al., 1996). Here, we have generated stably transfected SH-SY5Y neuroblastoma cell lines expressing human Htt Exon 1 constructs with either Q23 or Q73 polyQ repeats. The SH-SY5Y cells expressing the Q73 mutant isoform developed prominent intracellular aggregates. To better study the impact of UBE2K on mutant Htt aggregation, an additional population of Htt Exon 1 expressing clones were generated that stably expressed UBE2K shRNA and achieved stable knockdowns of UBE2K in excess of 90%. The genetic reduction of UBE2K led to a dramatic decrease in muHtt aggregates in Q73 expressing cells as examined by immunofluorescence microscopy. Small molecule tool compounds that modulate the activity of UBE2K are also presently being tested for their effects in this system. Modulating UBE2K and the ubiquitin-proteasome pathway protein may provide a potential new path for therapeutic development in HD.

Disclosures: G. Maor: A. Employment/Salary (full or part-time):; BPGbio Inc. **D.B. Oliver:** A. Employment/Salary (full or part-time):; BPGbio Inc. **J. Ranjan:** A. Employment/Salary (full or part-time):; BPGbio Inc. **D.B. Chimmanamada:** 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.; BPGbio, Inc. **V.K. Vishnudas:** A. Employment/Salary (full or part-time):; BPGbio Inc. **S. Gesta:** A. Employment/Salary (full or part-time):; BPGbio Inc. **K.M. Rosen:** A. Employment/Salary (full or part-time):; BPGbio Inc..

Poster

PSTR394

Huntington's Disease: Preclinical and Clinical Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.10/C97

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Taube Philanthropies
Jean Perkins Foundation

Title: Modulating TrkB and TrkC neurotrophin receptors prevents microglia-mediated corticostriatal synapse loss in a mouse model of Huntington's Disease

Authors: *D. A. SIMMONS[1], T. CHEN[1], N. P. BELICHENKO[1], F. M. LONGO[1,2];
[1]Neurol. and Neurolog. Sci., Stanford Univ. Sch. of Med., Stanford, CA; [2]Wu Tsai Neurosciences Institute, Stanford, CA

Abstract: Huntington's Disease (HD) is a neurodegenerative disorder characterized by motor, cognitive, and psychiatric disturbances. It is caused by a mutation in the *HTT* gene encoding a mutant huntingtin (mHtt) protein leading to selective degeneration of striatal medium spiny neurons (MSNs). A critical contributor to MSN death is mHtt-induced disruption of neurotrophin (NT) signaling, mainly via brain-derived neurotrophic factor (BDNF) and NT-3, which bind to TrkB and TrkC receptors, respectively. These NTs are potent promoters of MSN health and survival and TrkB/TrkC are present on MSNs and at their synapses. Thus, targeting TrkB/TrkC may be an effective HD therapeutic strategy. Accordingly, we previously showed that systemic administration of a TrkB/TrkC ligand improved NT signaling and reduced MSN degeneration and neuroinflammation. Here, we aim to determine if a derivative TrkB/C ligand, PTXBD10-2, with oral bioavailability, can also reduce HD phenotypes. We treated early symptomatic male BACHD and wild-type (WT) mice with PTXBD10-2 (50 mg/kg, oral gavage) or vehicle (veh) for 3 mo starting at 6 mo of age. We performed behavior assays, golgi staining for spines, and immunostaining for synaptic (VGLUT1, PSD-95) and microglial (IBA1, CD68) markers. We found that modulating TrkB/TrkC with PTXBD10-2 prevented the decline in the density of MSN dendritic spines and VGLUT1, a presynaptic corticostriatal synapse marker. Striatal microglial activation and phagocytotic state were elevated in BACHD-veh mice, versus WT-veh mice, as evidenced by changes in IBA1 morphology (soma size, total area) and increased colocalization with the lysosomal protein, CD68; PTXBD10-2 prevented these changes. A previous study showed that phagocytotic microglia excessively eliminate corticostriatal synapses in 7 mo old BACHD mice. Similarly, we show here that microglia of 9 mo old BACHD-veh mice had elevated levels of VGLUT1-CD68 colocalization compared to WTs. This colocalization was reduced with PTXBD10-2 treatment. Elevated levels of post-synaptic elements (PSD-95) were also present in BACHD-veh microglia; PTXBD10-2 prevented this increase. These ameliorative effects of PTXBD10-2 were associated with improved motor and cognitive performance and

reduced anxiety-like behavior. In all, TrkB/C modulation via PTXBD10-2 promoted MSN spine and corticostriatal synapse resilience likely by reducing excessive microglia-mediated elimination of these structures. These results provide further evidence that targeting TrkB/TrkC alleviates HD-related neurodegeneration suggesting a viable multi-target, disease-modifying therapeutic strategy for HD.

Disclosures: D.A. Simmons: None. **T. Chen:** None. **N.P. Belichenko:** None. **F.M. Longo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pharmacotropix.

Poster

PSTR394

Huntington's Disease: Preclinical and Clinical Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.11/C98

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: UCF Office of Undergraduate Research Grant
HDSA Donald A. King Fellowship

Title: Investigating interactions between maternal immune activation and Huntington disease

Authors: *S. LIPKIN[1], A. L. SOUTHWELL[2];
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Abstract: Huntington disease (HD) is classified as an adult-onset disease, despite the presence of the mutant huntingtin protein (mHTT) throughout life. While overt symptoms do not present until mid-life, HTT is crucial for neurodevelopment, and recent studies in HD model mice have revealed changes in neonatal brain development, suggesting a developmental component to neuronal susceptibility in HD. It is therefore unsurprising that insults in the perinatal period (e.g. hypoxia, birth trauma, congenital infection) as well as neurodevelopmental disorders are associated with a 4-6 year earlier median age of diagnosis than predicted by CAG-repeat length. Incidences of neurodevelopmental disorders, including autism spectrum disorders (ASD), obsessive compulsive behavior (OCB), and schizophrenia, have historically increased following viral pandemics. This was first seen following the 1918 Spanish influenza pandemic, due to a phenomenon called maternal immune activation (MIA), in which an immune response in a mother during gestation interferes with fetal brain development. This is particularly salient following the recent SARS-CoV-2 pandemic. Children exposed to COVID-19 in utero are more likely to have received a neurodevelopmental disorder diagnosis within the first 12 months of life, and this effect may have detrimental effects on the HD community. This study aims to elucidate the interactions between MIA and HD pathology, focusing on behavioral and molecular mechanisms. In mice, polyinosinic polycytidylic acid (Poly(I:C)) viral mimic has been used to initiate an immune response in pregnant dams; the offspring of these dams present with

behavioral and neuropathological abnormalities consistent with human neurodevelopmental disorders. By unraveling the interplay between MIA, the HD mutation, and COVID-19-related environmental factors, we aim to provide novel insights into disease mechanisms and potential therapeutic targets. Ultimately, this research holds promise for informing clinical management strategies and improving outcomes for HD patients and their families in the face of evolving global health challenges.

Disclosures: S. Lipkin: None. **A.L. Southwell:** None.

Poster

PSTR394

Huntington's Disease: Preclinical and Clinical Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.12/C99

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: R01AG071512
R21AG073684-01
Johns Hopkins Catalyst Award
19PABH134580006

Title: Modulation of transsulfuration pathway abrogates neurodegeneration in Huntington's disease

Authors: *S. J. TRIPATHI[1], S. CHAKRABORTY[1], J. I. SBODIO[2], M. FILIPOVIC[3], S. H. SNYDER[2], A. A. PIEPER[4,5,6,7,8], B. D. PAUL[1,9,10,11];
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Abstract: Huntington's disease (HD) is a neurodegenerative disorder caused by CAG repeat expansion in the huntingtin gene (*HTT*) and characterized symptomatically by choreiform movements, mood changes, and cognitive impairments. Our previous studies have shown a role for dysregulation of the transsulfuration pathway in HD pathogenesis. For instance, a compromised transsulfuration pathway due to reduced cystathionine gamma-lyase (CSE)

expression in HD was correlated with reduced hydrogen sulfide (H_2S) levels, cysteine production, and motor deficits. Hydrogen sulfide is a gasotransmitter that signals via a posttranslational modification termed sulfhydration, and it is unknown whether modulation of sulfhydration or normalizing H_2S level with an activator of transsulfuration pathway confers neuroprotection in HD. Here, we show that activation of the transsulfuration pathway in a cellular model of HD and R6/2 mice modulates integrated stress response, mitigates oxidative stress, reduces mutant HTT protein aggregation, prevents neuronal cell death, enhances sulfhydration, prevents HD-induced behavior deficits and expands life span. Taken together, our study suggests activating transsulfuration pathways might be a putative therapeutic approach to neurodegeneration in HD.

Disclosures: S.J. Tripathi: None. **S. Chakraborty:** None. **J.I. Sbodio:** None. **M. Filipovic:** None. **S.H. Snyder:** None. **A.A. Pieper:** None. **B.D. Paul:** None.

Poster

PSTR394

Huntington's Disease: Preclinical and Clinical Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.13/C100

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: PhD Funding via BBSRC (South West Biosciences Doctoral Training Partnership)

Title: Analysing genetic modifiers of Mutant Huntingtin using the Drosophila compound eye, as an in vivo test tube.

Authors: *R. M. SPICER[1], M. LELOS[1], A. ROSSER[1,2], M. TAYLOR[3,4];
[1]Sch. of Biosci., Cardiff Univ., Cardiff, United Kingdom; [2]School of Medicine, Cardiff University, Cardiff, United Kingdom; [3]Cardiff Univ., Cardiff, United Kingdom; [4]School of Biosciences, Cardiff University, Cardiff, United Kingdom

Abstract: Our objective is to determine whether the transcription factors FoxP1 and Mef2C influence mutant Huntingtin (mHTT)-associated pathogenesis. Degeneration of striatal Medium Spiny Neurons (MSNs) is a major pathological hallmark of the genetically inherited, Huntington's Disease (HD) for which there is no disease-modifying treatment. mHTT protein has been shown to have interacting partners, via protein-protein and genetic interactions, that have not been seen with wild-type Huntingtin (wtHTT) and are potentially pathogenic. We have previously identified FoxP1 and Mef2C as two of the most highly up-regulated genes during mouse striatal development (Precious et al., 2016). FoxP1 has also previously been shown to interact with mHTT, and its loss is associated with impaired memory and learning (Titus et al., 2017; Wang et al., 2022) and we found that striatal knock-down of mouse Mef2C results in reduced MSN number (Ali et al., in preparation). Lastly, a chromosomal deficiency containing

the single Mef2 *Drosophila* gene partially rescues a ‘rough eye’ degenerative phenotype (Kaltenbach et al., 2007).
In this study, the *Drosophila melanogaster* compound eye was used as an *in viv*o test tube. We utilised the Gal4/UAS system to analyse mHTT-induced neural degeneration and test the effect of manipulating FoxP1 and Mef2 expression. We used light microscopy and confocal imaging, to visualise and quantify photoreceptor neuron (PR) degeneration associated with gene manipulation. We found that whole-eye and pan-neuronal over-expression of FoxP1 significantly suppressed mHTT-induced PR degeneration (ANOVA, $P<0.01$) and increased *Drosophila* survival compared to the HD fly model, alone. In contrast, we found that down-regulation of endogenous Mef2 significantly suppressed mHTT-induced PR degeneration (ANOVA, $P<0.01$) and increased survival.
Our ongoing studies in *Drosophila* indicate that delaying expression of mHTT until adulthood results in minor PR degeneration, whilst expression during development leads to significant mHTT-induced PR degeneration. This suggests that mHTT expression during development may with lead to production of neurons that are vulnerable to degeneration in later life, or neurons that do not fully form in the first place. We are exploring the idea that the FoxP1 and Mef2 genes may be exerting their effects over different developmental periods. In this context, we have already found that FoxP1 protein expression, which is comparable in young HD and wild-type mice, is significantly down-regulated at later timepoints in the HD mice.

Disclosures: R.M. Spicer: None. **M. Lelos:** None. **A. Rosser:** None. **M. Taylor:** None.

Poster

PSTR394

Huntington’s Disease: Preclinical and Clinical Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.14/C101

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Testing novel small molecule compounds in C. elegans models of Huntington's Disease

Authors: M. BERKO[1], T. J. ANNNULIS, Jr.[1], C. SATCHELL[3], M. DEVINE[2], S. KRUEGER[1], *A. KALLARACKAL[4];
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Abstract: Huntington’s Disease (HD) is a neurodegenerative disorder characterized by progressive loss of motor and cognitive function. HD is caused by the expansion of polyglutamine repeats in the HD gene, with the number of repeats correlating with disease severity. *C. elegans* are a useful model organism for studying mechanism of neurodegenerative disorders and for screening potential therapeutics. We identified locomotion defects and visualized protein aggregates in four different *C. elegans* polyQ transgenic strains. Additionally,

we have developed a set of candidate small molecule therapeutics that work by targeting the nucleic acids and preventing the formation of toxic protein. Our preliminary data shows that the compounds show promise for decreasing aggregate number and blocking locomotion defects in the *C. elegans* polyQ strains.

Disclosures: M. Berko: None. **T.J. Annnulis:** None. **C. Satchell:** None. **M. Devine:** None. **S. Krueger:** None. **A. Kallarackal:** None.

Poster

PSTR394

Huntington's Disease: Preclinical and Clinical Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.15/C102

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: CIHR Project Grant PJT-178043

Title: Automated monitoring to assess naturalistic behavior and skilled motor learning in a mouse model of Huntington Disease

Authors: *D. RAMANDI[1], T. L. FONG[3], T. MURPHY[1], L. A. RAYMOND[2];
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[3]Neurosci., Univ. of British Colombia, Vancouver, BC, Canada

Abstract: Automated assessment of behavior is becoming a valuable tool in neuroscience, particularly in studying group-housed mice. This allows for observing naturalistic behaviors in a stress-reduced environment, enhancing the understanding of motor learning processes.
This study utilized group-housed mice, either wild-type or zQ175 Huntington Disease (HD) models. Each mouse was tagged with an RFID chip for activity tracking and task performance assessment. The mice were housed in groups of 2-4 in spacious conventional rat cages, equipped with an overhead camera and a grid of RFID readers for continuous tracking of movements. An adjoining chamber enabled mice to perform a skilled lever-pulling task to obtain water, their sole hydration source. Data was collected via a Raspberry Pi, with offline analysis using a computer vision model, You Only Look Once (YOLO), to extract and track a bounding box around the mice, and DeepLabCut for pose estimation. We further trained the YOLO model to detect mice with more than 18,000 frames, across various cages and settings to provide utility to the community for mouse detection.
The system efficiently identified a range of behaviors and social interactions. Mice rapidly learned the lever-pulling task, reaching a performance plateau within ten days. Preliminary data shows that behavior clustering can be done on individual mice using only the segmented animal video without keypoint detection. We extracted features using a pre-trained ResNet model from the segmented animals, which was then used as input to various clustering algorithms. This method is faster and more efficient than traditional keypoint labeling and pose estimation, as it

requires less manual labeling. The segment tracking approach provides more comprehensive animal information and is less prone to jitter and noise in predictions. To date, data from one cage has been fully analyzed, involving two WT and one HD mice, with ongoing data acquisition in two additional cages (a total of 5 WT, 3 HD mice). Each cage records continuous video and behavior data 24/7, covering over two months to capture both pre-manifest and early stages of HD progression. Preliminary results indicate that HD mice perform worse in the lever-pulling motor learning task and exhibit fewer high-intensity bouts with trials more dispersed throughout their dark cycle.
In conclusion, we have developed a robust tool for studying spontaneous behaviors and task-dependent motor learning. This holds significant potential for understanding and characterizing disorders with a motor phenotype, such as HD, contributing valuable insights into neuroscientific research.

Disclosures: D. Ramandi: None. **T.L. Fong:** None. **T. Murphy:** None. **L.A. Raymond:** None.

Poster

PSTR395

Neuroinflammation and Neurotoxicity

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.01/C103

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Neurochemical Changes in the Early Postnatal Stage of Offspring from Female Mice Stressed During Gestation

Authors: *D. RAMÍREZ ORTEGA[1], J. CHÁVEZ[2], D. F. GONZALEZ ESQUIVEL[3], G. R. ROLDAN[4], B. PINEDA OLVERA[5], A. SALAZAR[6], I. FLORES[6], G. PEREZ DE LA CRUZ[7], V. PEREZ DE LA CRUZ[8];
[1]Inst. Nacional De Neurologia y Neurocirugia "Manuel Velasco", México, Distrito Federal, Mexico; [2]Univ. Nacional Autónoma De México, CDMX, Mexico; [3]INNN, MEXICO CITY, Mexico; [4]Natl. Autonomous Univ. of Mexico, Mexico City, Mexico; [5]NEUROINMUNOLOGIA, INSTITUTO NACIONAL DE NEUROLOGIA Y NEUROCIRUGIA, MEXICO CIY, Mexico; [6]Inst. Nacional de Neurología y Neurocirugía, Mexico City, Mexico; [7]Dept. de Matemáticas, Facultad de Ciencias, Univ. Nacional Autónoma de México (UNAM), Mexico City, Mexico; [8]Lab. de Neurobioquímica y Conducta,, Inst. Nacional De Neurologia Y Neurocirugia, Mexico City, Mexico

Abstract: Substantial evidence has demonstrated that prenatal stress (PS) can lead to neuropsychiatric disorders in humans, including depression, schizophrenia, and autism. Cognitive dysfunctions associated with prenatal stress, however, the molecular mechanisms underlying these effects remain poorly understood. In this context, kynurenic acid (KYNA), a tryptophan metabolite, has been implicated in cognitive dysfunction across various experimental

models. KYNA is known to modulate glutamatergic, dopaminergic, and cholinergic transmission, influencing cognitive processes. This study aims to determine the neurochemical alterations in early postnatal days after prenatal stress. Prenatal stress was induced using a predator scent; pregnant mice were exposed to cat urine from days 14 to 19 of gestation for 60 minutes daily. After birth, brains were collected immediately (P0) and 10 days postnatal (P10) to evaluate brain levels of glutamate, glutamine, and kynurenic acid by chromatography. In the prenatal stress group, it was observed that glutamate levels decreased at P0, while at P10, they tended to be higher compared to the control group. Regarding glutamine, levels of this neurotransmitter decreased at both time points evaluated in the prenatal stress group. Interestingly, brain KYNA levels decreased at P0 and were higher than those in the control group at P10. These findings suggest that the alterations in neurotransmitter levels could be associated with the cognitive alterations observed in adulthood in this prenatal stress model.

Disclosures: D. Ramírez Ortega: None. **J. Chávez:** None. **D.F. Gonzalez esquivel:** None. **G.R. Roldan:** None. **B. Pineda olvera:** None. **A. Salazar:** None. **I. Flores:** None. **G. Perez de la Cruz:** None. **V. Perez De La Cruz:** None.

Poster

PSTR395

Neuroinflammation and Neurotoxicity

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.02/C104

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: VA New Jersey Airborne Hazards and Burn Pits Center of Excellence FY2023-001
DOD CDMRP GW210060
Veterans Affairs ORD I01BX005015
Veterans Affairs ORD IK6 BX006188
Veterans Bio-Medical Research Institute

Title: Model toxic airborne particulate matter exposure alters gene expression in the brain

Authors: A. L. SHAIKH[1,2], K. E. MURRAY[1,2], A. E. MAUSOOF[1], L. E. WOLD[3], ***B. A. CITRON**[1,2,4];
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Abstract: Airborne hazards can produce significant consequences in a variety of tissues. Particulate matter exposure is particularly relevant for service personnel, Veterans, and others with proximities to burn pits, exhaust, and other smoke related hazards. Greater insight into the

mechanisms by which these exposures could affect the health of neurons in the CNS is needed. We modeled the insult by incorporating two important deployment components, airborne hazard exposure and stress. The airborne hazard was accomplished by exposing mice to air enriched in particulate matter (2.5 µm) vs. filtered air for 6 hours/day, 5 days/week over three weeks. Introduction of a retired male breeder mouse for 2 hours/day for the first 5 days of the exposure period and 3 random days in the last two weeks provided a social defeat stress. Brain samples were collected 1 day after the end of the insult period for examination of mRNA levels by RNA-seq or perfused and stained for immunohistochemistry. RNA was isolated from the cortex of snap frozen tissue (n=5/group). A variety of genes that could affect neurodegeneration were dysregulated. For example, Neuronal PAS domain protein 4 (*Npas4*) was downregulated 3.5 to 4.4-fold ($p<0.0025$ for each comparison) after insults by particulate matter alone, stress alone, and by particulate matter combined with stress. *Npas4* codes for a transcription factor that is involved in DNA repair, the loss of which has been found to lead to a loss of control over neuronal inhibition. Protocadherin gamma subfamily C, 4 (*Pcdhgc4*) was also downregulated in the combined (2.9-fold), particulate matter (2.2-fold), and stress (1.9-fold) groups ($p<0.0024$ for each). *Pcdhgc4* has been implicated in the establishment and maintenance of specific neuronal connections in the brain involving processes such as hippocampal based context discrimination, and variants of this gene have been known to cause neurodevelopmental disorders. In addition, multiple genes related to mitochondrial function and oxidative phosphorylation were significantly downregulated following particulate matter inhalation. These findings suggest that particulate matter exposure may cause neurodegeneration through interference with repair processes and synapse formation, as well as oxidative phosphorylation and mitochondrial function. We hope that further investigation into these pathways might help identify therapeutic targets useful for those receiving these toxic insults.

Disclosures: A.L. Shaikh: None. **K.E. Murray:** None. **A.E. Mausoof:** None. **L.E. Wold:** None. **B.A. Citron:** None.

Poster

PSTR395

Neuroinflammation and Neurotoxicity

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.03/C105

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant EY030747
NIH Grant EY031248
NIH Grant RR027093
Research to Prevent Blindness
Vision Research Foundation of Kansas City

Title: Human progenitor cell-derived neuronal cells differentially regulate the expression of key transcription factors in response to the degree and duration of oxidative stress

Authors: *R. S. DUNCAN[1], C. W. HALL[1], P. KOULEN[2];
[1]Univ. of Missouri - Kansas City, Kansas City, MO; [2]Biomed. Sci. and Ophthalmology, Univ. of Missouri, Kansas City, MO

Abstract: Several transcription factors are important for the cellular response against oxidative stress as they regulate the expression of genes involved in the antioxidant response as well as survival of nerve cells. Using differentiated neuronal cells derived from neural progenitor cells, we identified the expression of Nuclear Factor Erythroid 2-Related Factor 2 (NRF2), Signal Transducer and Activator of Transcription 1 and 3 (STAT1 and STAT3), Nuclear Factor Kappa B 1 (NF-κB1), and REL Proto-Oncogene NF-KB Subunits A and B (RelA and RelB). We determined the effect of mild and moderate oxidative stress for either a brief (2 hours) or a prolonged period (24 hours) after addition of the oxidant, *tert*-butyl hydroperoxide (tBHP). NRF2 and activated (phosphorylated) NRF2 (pNRF2) were expressed in the soma and nucleus of differentiated neuronal cells. Both forms of NRF2 were upregulated after brief and prolonged exposure to tBHP, but only pNRF2 was increased in the nucleus after tBHP exposure, particularly after a brief exposure. STAT1 and STAT3 were both localized predominantly to the nucleus, but only STAT1 exhibited an increase in expression and nuclear localization in response to oxidative stress. STAT3 was decreased in response to tBHP exposure, and oxidative stress had no effect on STAT3 nuclear localization. NF-κB1, RelA and RelB were expressed in the soma, neurites and nucleus. For both RelA and NF-κB1, expression and nuclear localization were increased in response to oxidative stress. Exposure of differentiated neuronal cells to tBHP did not change expression levels nor subcellular localization of RelB. Together, these data suggest that several transcription factors that are involved in the cellular response to oxidative stress are present and differentially regulated, with respect to both expression levels and subcellular localization, in differentiated neuronal cells exposed to oxidative stress.

Disclosures: R.S. Duncan: None. **C.W. Hall:** None. **P. Koulen:** None.

Poster

PSTR395

Neuroinflammation and Neurotoxicity

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.04/C106

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Metabolic measurements of disease-relevant human iPSC-derived cells using Seahorse XF Assay technology

Authors: C. B. CARLSON[1], *A. FATHI[2], J. MA[3], S. HILCOVE[1], Y. KAM[4], N. ROMERO[4], S. SCHACHTELE[1];

[1]FUJIFILM Cell. Dynamics, Madison, WI; [2]FUJIFILM Cell. Dynamics, Middleton, WI; [3]FUJIFILM Cell. Dynamics, madison, WI; [4]Agilent, Lexington, MA

Abstract: Terminally differentiated cell types generated from human induced pluripotent stem cells (iPSC) offer an important source of specialized material that is not often readily available. Furthermore, iPSC technology enables “disease-in-a-dish” studies though the differentiation of such cell types from both patient-derived and control iPSC lines. A revealing feature of human iPSC-derived cell types is their basal metabolism, and a key application is to detect discrete changes in their cellular bioenergetics based on disease state, cell activation, or toxicity. The metabolic profile of various iPSC-derived cell types and the corresponding disease models (mutation vs. isogenic control) was determined by measuring the oxygen consumption rates (OCR) on the Seahorse XF Pro Analyzer (Agilent). Seahorse protocols were developed using commercially available, cryopreserved iPSC-derived neural cell types (iCell products) from FUJIFILM Cellular Dynamics. Seahorse XF Cell Mito Stress Test Kit was used to comprehensively evaluate mitochondrial respiration and bioenergetics. All cells were thawed/cultured according to manufacturer’s instructions with media and supplements provided. Assay variables were cell density, extracellular matrix, assay media, day of assay, and [FCCP]. OCR and spare capacity were normalized to cell number by nuclei count from high content image analysis. Results include Seahorse data comparing iCell Microglia (wild-type; WT) with TREM2 mutants (AD-relevant hetero- and homozygous TREM2 functional knockouts), where WT microglia consistently displayed a 1.5X higher spare capacity. iCell Induced Excitatory Neurons, WT and progranulin mutation (GRN R493X) cell lines, demonstrated higher OCR profiles when compared to iCell GlutaNeurons and iCell GABANeurons. Additionally, respiration was the highest for the GRN R493X neurons, which correlates with higher neuronal spike activity recorded on microelectrode array (MEA). Finally, iCell DopaNeurons generated from Michael J. Fox Foundation patient-derived iPSC lines harboring PD-relevant mutations (LRRK2 G2019S or GBA N370S) and the corrected isogenic controls were compared. A high cell density (125K cells/well) was needed to achieve robust OCR signal and the GBA N370S line had the largest spare capacity. Seahorse XF cell analysis technology provides sensitive label-free measurements that are useful in detecting changes in mitochondrial function between different human iPSC-derived neural cell types, as well as between wild-type and disease-mutation containing cells. The combination of these two technologies is a powerful tool for disease modeling.

Disclosures: C.B. Carlson: A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc., Madison, WI. **A. Fathi:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc., Madison, WI. **J. Ma:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc., Madison, WI. **S. Hilcove:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc., Madison, WI. **Y. Kam:** A. Employment/Salary (full or part-time):; Agilent Technologies, Inc., San Diego, CA. **N. Romero:** A. Employment/Salary (full or part-time):; Agilent Technologies, Inc., San Diego, CA. **S. Schachtele:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics, Inc., Madison, WI.

Poster

PSTR395

Neuroinflammation and Neurotoxicity

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.05/C107

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: 2022R1C1C101018

Title: Neurotoxin mediated neuronal dysfunction regulated by lysosomal homeostasis.

Authors: *J. WIE;
The Catholic Univ. of Korea,Col. of Med.,, Seoul, Korea, Republic of

Abstract: Neurotoxins encompass diverse groups of drugs that have detrimental effects on the neuronal system, leading to cognitive and neurological impairments. These toxins can disrupt intracellular signaling and homeostasis. Lysosomes are crucial organelles responsible for waste disposal and the supply of essential materials. The connection between lysosomal homeostasis and neurological dysfunction remains poorly understood. In this study, we investigated how to alleviate neurodegeneration through the management of lysosomal function. Some neurotoxins are known to affect lysosomal ion influx or efflux. After treating N2A cells with hydroperoxide, a known neurotoxin, we observed a decrease in cell proliferation. When blocking the lysosomal ion exchange, it protected N2A cell viability against the damaging effects of hydroperoxide. For intracellular signaling, hydroperoxide increased AKT phosphorylation, but this effect was diminished after regulating lysosomal ion exchange. Hydroperoxide also increased the level of p-raptor protein, a key component of mTORC1, which plays a fundamental role in lysosomes. However, this change did not affect the total raptor protein level. When we induced starvation in the cells and added hydroperoxide, cell viability decreased, and this was accompanied by an increase in AKT phosphorylation and p-raptor levels, but those were attenuated by lysosome regulation. LC3, a specific marker for autophagy, was accelerated in response to hydroperoxide treatment but was also disrupted by the control of lysosomal ion exchange. Thus, the regulation of lysosomal activity can prevent neurodegeneration and impact intracellular signaling pathways affected by hydroperoxide.

Disclosures: J. Wie: None.

Poster

PSTR395

Neuroinflammation and Neurotoxicity

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.06/C108

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Benefits of early in vitro screening for seizure liability in problem solving and decision making

Authors: K. ROCKLEY, M. MORTON, *R. ROBERTS;
Apconix, Macclesfield, United Kingdom

Abstract: Seizure liability remains a significant cause of attrition throughout drug development both in pre-clinical and clinical studies. This emphasizes the need for improved methodologies to detect seizure liability prior to in vivo toxicology studies, ideally with reduced reliance on animals and better translation to humans. Much like the Comprehensive in vitro Proarrhythmia Assay (CiPA), which is now widely accepted for early assessment of cardiovascular safety, we have developed an approach utilizing hiPSC-neuronal cell microelectrode array (MEA) and ion channel screening for early seizure prediction. In our MEA assay, seizurogenic compounds were identified correctly with high predictivity, and correlations were observed between the in vitro and clinical exposures for many therapies known to cause seizure. We have used these assays in the early phase of nonclinical testing, and successfully derisked and prioritized a chemical series. For example, after testing a number of compounds, one was identified with low seizure risk compared to the others in the series - this compound had distinct structural features. In another study of compounds undergoing nonclinical testing, exposures that caused no CNS signs or convulsions in rats, aligned with the results of the MEA study. Conversely, where convulsions were reported in rats, seizurogenic responses were present in the MEA study at comparable concentrations.
Since these studies use human derived cells, they can be used to determine the human relevance of seizures observed in nonclinical studies. For example, nonclinical testing of a compound caused convulsions only in dogs. Testing a range of metabolites in the MEA assay revealed only the dog-specific metabolite caused the seizurogenic phenotype. In addition, screening this metabolite against a panel of ion channel targets revealed a hit, providing mechanistic insight with the opportunity to redesign the compound to eliminate the liability.
Collectively, these studies demonstrate the utility of this approach for early seizure prediction to provide mechanistic information and early de-risking, supporting optimal drug design using human in vitro models.

Disclosures: K. Rockley: A. Employment/Salary (full or part-time):; ApconiX. **M. Morton:** A. Employment/Salary (full or part-time):; ApconiX. **R. Roberts:** A. Employment/Salary (full or part-time):; ApconiX, University of Birmingham UK.

Poster

PSTR395

Neuroinflammation and Neurotoxicity

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.07/C109

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant R01 NS065808
NIH grant R01 NS127403
Legacy of Angels Foundation
European Leukodystrophy Association

Title: Galactosylceramidase deficiency in adult oligodendrocytes transforms the presentation of experimental allergic encephalomyelitis from chronic to acute, hastening its progression

Authors: *N. SALDIVIA, D. ZELADA, J. WHITEHAIR, G. J. HELLER, M. I. GIVOGRI, E. R. BONGARZONE;
Univ. of Illinois at Chicago, Chicago, IL

Abstract: Galactosyl-ceramidase (GALC) is an lysosomal enzyme crucial for proper myelination of the mammalian nervous system during early postnatal development. However, the physiological implications of GALC in the adult brain remain elusive. In this work, we generated mice with conditional GALC deficiency restricted to post-mitotic PLP+ oligodendrocytes. Exposure of these animals these to chronic experimental allergic encephalomyelitis (EAE), the primary model for multiple sclerosis, led to a lethal sensitization. Loss of function of GALC in mature oligodendrocytes enhanced demyelination, gliosis, inflammation, and impaired remyelination in EAE mice. This acute and fulminant phenotype contrasted with the mild non-lethal phenotype of GALC-ablated mice without EAE challenge, which developed adult-onset motor deficits and modest astrogliosis and microgliosis. Mechanistically, the combination of GALC ablation in oligodendrocytes and EAE led to a substantial increase in apoptotic cell death. Further typification confirmed that the cells undergoing apoptosis were chiefly oligodendrocytes and microglia and to a much lesser extent neurons, astrocytes, and oligodendrocytes progenitor cells. Additionally, we identified an impaired fusion of lysosomes and autophagosomes with accumulation of myelin debris following a TFEB-dependent increase in the lysosomal autophagosome flux. These findings underscore the consequences of GALC deficiency, influenced by cell context, inflammation, and developmental timing. We conclude that GALC expression in adult oligodendrocytes is essential for maintaining long-term central myelination and reducing susceptibility to further demyelinating insults

Disclosures: N. Saldivia: None. **D. Zelada:** None. **J. Whitehair:** None. **G.J. Heller:** None. **M.I. Givogri:** None. **E.R. Bongarzone:** None.

Poster

PSTR395

Neuroinflammation and Neurotoxicity

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.08/C110

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: National Eye Institute (EY031248)

Title: Neurodegeneration of retinal ganglion cells in a novel in vitro model of glaucoma

Authors: *A. TESHOME[1], K. E. KADOR[3], P. KOULEN[2];
[2]Biomed. Sci. and Ophthalmology, [1]Univ. of Missouri - Kansas City, Kansas City, MO;
[3]Ophthalmology, Univ. of Missouri-Kansas City, Kansas City, MO

Abstract: Retinal ganglion cell (RGC) death is a significant cause of visual impairment and blindness in eye diseases, such as glaucoma. Preceding RGC death is the process of neurite degeneration in which dendrites and axons undergo morphological changes, leading to a loss of their function and structural integrity. Since RGCs, similar to other neurons of the central nervous system, lack the capacity to regenerate, identifying the causes of RGC death is essential for the development of neuroprotective strategies to prevent and treat vision loss. In glaucoma, changes in the optic nerve, the nerve fiber layer and the ganglion cell layer have been linked to risk factors such as increased intraocular pressure, impaired synaptic activity, inflammation, and oxidative damage. Previous studies have utilized beta III tubulin immunoreactivity as a phenotypic marker for RGCs to quantify their number and characterize their morphology *in vitro*. Here, we utilized *tert*-butyl hydrogen peroxide (tBHP) to induce sublethal oxidative damage to murine RGCs and to model the cellular environment of the glaucomatous retina. We measured RGCs neurite outgrowth, changes in the subcellular morphology of RGC neurites and categorization of RGC degeneration phenotypes to identify changes in RGCs morphology. Primary RGCs were isolated from mouse retinas by enzymatic tissue digestion, mechanical trituration followed by a multi-step immuno-panning approach to isolate a purified RGC fraction. Purified RGCs were seeded and grown in cell culture media and then treated with tBHP for 24 hours. Neurite outgrowth was examined with immunocytochemistry, confocal microscopy imaging, and quantitative image analysis using the ImageJ software program. Our initial findings suggest that oxidative stress affects the subcellular morphology of RGC. Morphometry can be used to analyze the impact of external factors such as chronic disease, traumatic injury or genetic mutations on structural properties of RGCs that in turn affect RGC function.

Disclosures: A. Teshome: None. **K.E. Kador:** None. **P. Koulen:** None.

Poster

PSTR395

Neuroinflammation and Neurotoxicity

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.09/C111

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: VR 2021-03149

Title: Probing Amyloid Formation in Living Brain Tissue at Nanoscale: Super resolution Optical Photothermal Infrared Microspectroscopy to Image amyloid Structures in Their Native Environment

Authors: *O. KLEMENTIEVA;
Lund Univ., Lund, Sweden

Abstract: Spatiotemporal alterations in the chemical and structural makeup of biomolecules play an essential role in the onset and progression of various diseases, including Alzheimer's Disease. Early structural changes at the submicron level often occur well before disease symptoms can be recognized and before morphological changes can be detected using conventional tissue-level methodologies such as spatial proteomics, histology, or immunohistochemical staining. Consequently, there is a critical need for structure-sensitive techniques. Here, I present an approach capable of spatiotemporal chemical imaging of amyloid structures at submicron resolution within their native environment.
Using a recently established technique, the Medical Microspectroscopy Group from Lund University conducted groundbreaking experiments that enabled the monitoring of amyloids in the process of formation, proliferation, and cellular damage directly within living tissues.
To assess structural changes with sub-micron precision, we employed optical photothermal infrared (O-PTIR) microspectroscopy, a technique sensitive to amyloid structures. By applying O-PTIR to freshly extracted brain tissue from APP/PS1 mice, we documented structural changes in functioning brain tissue, observing the appearance of newly formed amyloids spatially and temporally colocalized with lipid damage. Achieving time-resolved submicron in situ imaging of amyloid structures marks a significant technological advancement that opens new avenues for in-depth molecular analysis of amyloid formation within their natural environment, thus facilitating an understanding of why amyloids begin to form, accumulate, and damage tissue

Disclosures: O. Klementieva: None.

Poster

PSTR395

Neuroinflammation and Neurotoxicity

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.10/C112

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NS130722
AG080917

Title: Comparative analysis of adult exposure to low dose of legacy and replacement PFASchemicals (PFOA and GenX) using cortical neuron culture derived from hiPSCs

Authors: *H. ZHAO[1], J. XIE[2], C. YUAN[2];
[1]Chem. Engin., Purdue Univ., West Lafayette, IN; [2]Purdue Univ., West Lafayette, IN

Abstract: Per- and polyfluoroalkyl substances (PFAS) are a group of chemicals abundant in the environment due to their uses in consumer products and industrial applications. Given the escalating health risks linked to PFAS exposure, the global prohibition of perfluorooctanoic acid (PFOA) has been enacted, prompting the emergence of its substitute, hexafluoropropylene oxide dimer acid (GenX). Exposure to trace amounts of the legacy PFAS compound PFOA has been correlated with neurological disease risks, yet the neurotoxicity of its replacement GenX remains understudied. In this work, we compared the effect of low dose (0.4 ppb) exposure of PFOA and GenX on cortical neuron culture derived from hiPSCs after 2 days of exposure and after 7 days relaxation. We observed persistent changes in neuron network complexity, neuronal activity, resilience towards secondary stressors targeting mitochondria, lysosome and tau protein aggregation. Significantly, our results indicate distinct disruptions in various subcellular compartments induced by PFOA and GenX exposure. Specifically, we observed persistent mitochondrial damage resulting from PFOA exposure and alterations in autolysosomes triggered by GenX exposure. Gene expression analysis derived from RNA-seq data further substantiates our observations. This study provides a comparative analysis of the neurotoxic effects of a legacy PFAS chemical and its substitute, unveiling perturbations in diverse subcellular compartments within cortical neuron cultures elicited by exposure to PFOA and GenX.

Disclosures: H. Zhao: None. **J. Xie:** None. **C. Yuan:** None.

Poster

PSTR395

Neuroinflammation and Neurotoxicity

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.11/C113

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NS130722
NIH AG080917

Title: Developmental Pb exposure increases AD risk via altered intracellular Ca2+ homeostasis in hiPSC-derived cortical neurons

Authors: *J. XIE, H. ZHAO, C. YUAN;
Chem. Engin., Purdue Univ., West Lafayette, IN

Abstract: Exposure to environmental chemicals such as lead (Pb) during vulnerable developmental periods can result in adverse health outcomes later in life. Human cohort studies have demonstrated associations between developmental Pb exposure and Alzheimer's disease (AD) onset in later life which were further corroborated by findings from animal studies. The

molecular pathway linking developmental Pb exposure and increased AD risk, however, remains elusive. In this work, we used human iPSC-derived cortical neurons as a model system to study the effects of Pb exposure on AD-like pathogenesis in human cortical neurons. We exposed neural progenitor cells derived from human iPSC to 0, 15, and 50 ppb Pb for 48 h, removed Pb-containing medium, and further differentiated them into cortical neurons. Immunofluorescence, Western blotting, RNA-sequencing, ELISA, and FRET reporter cell lines were used to determine changes in AD-like pathogenesis in differentiated cortical neurons. Exposing neural progenitor cells to low-dose Pb, mimicking a developmental exposure, can result in altered neurite morphology. Differentiated neurons exhibit altered calcium homeostasis, synaptic plasticity, and epigenetic landscape along with elevated AD-like pathogenesis markers, including phosphorylated tau, tau aggregates, and Aβ42/40. Collectively, our findings provide an evidence base for Ca dysregulation caused by developmental Pb exposure as a plausible molecular mechanism accounting for increased AD risk in populations with developmental Pb exposure.

Disclosures: J. Xie: None. **H. Zhao:** None. **C. Yuan:** None.

Poster

PSTR395

Neuroinflammation and Neurotoxicity

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.12/C114

Topic: F.04. Neuroimmunology and Neurovirology

Support: JSPS Research Fellowship for Young Scientists (23KJ0285)

Title: Autism-associated sparcl1/hevin mutant has impacts on persistent angiogenesis

Authors: *T. TAKETOMI[1], T. YASUDA[2], K. UEDA[1], R. MORITA[3], Y. SHIGETA[3,1], S. MIZUNO[4,1], S. TAKAHASHI[4,1], R. HARADA[3], F. TSURUTA[5,1];
[1]PhD Program in Human Biol., [2]Doctoral Program in Biol., [3]Ctr. for Computat. Sci., [4]Lab. Animal Resource Ctr., [5]Inst. of Life and Envrn. Sci., Univ. of Tsukuba, Tsukuba, Japan

Abstract: Autism spectrum disorder (ASD) is the most common neurodevelopmental disorder characterized by social interaction defects and repetitive behaviors. It has been reported that not only deficits in excitatory-inhibitory synaptic balance but also persistent angiogenesis occur in the brain of ASD patients. The ASD pathogenesis is known to be regulated by ASD-associated gene mutations. Genome-wide association studies have shown that mutations in the *Sparcl1* gene increase the risk of ASD. SPARCL1 is a secreted extracellular matrix protein that promotes synaptic connectivity and inhibits angiogenesis. Our previous study reported that a single amino acid substitution of Trp-647 to Arg (WR), which is associated with a familial case of ASD, exhibits abnormal protein structure, leading to low secretion levels *in vitro* (T Taketomi *et al.*, Sci Rep, 2022, https://pubmed.ncbi.nlm.nih.gov/35831437/). However, the mechanism linking the ASD-associated single amino acid substitution in SPARCL1 to ASD pathogenesis *in vivo*

remains unaddressed. In this study, we generated novel ASD-model Sparcl1 heterozygous WR knock-in mice (*Sparcl1*$^{WR/+}$) and investigated the link between amino acid substitution and ASD pathogenesis. We found that SPARCL1 WR mutant aggregates around vessels while SPARCL1 smoothly localizes the perivascular region in embryonic day 18, suggesting the SPARCL1 mutant has impacts on the vascular environment. We verified the expression level of vascular endothelial growth factor A (VEGFA) mRNA and CD34 were increased in *Sparcl1*$^{WR/+}$ mice (4-week-old) brains. Importantly, the vessel density of the medial prefrontal cortex (mPFC) in *Sparcl1*$^{WR/+}$ mice (8-week-old) is higher than that in control mice, suggesting that single amino acid substitution impairs perivascular matrix formation. On the other hand, the number of glutamatergic excitatory synapses, determined by colocalization of vesicular glutamate transporter 1 (VGluT1) and postsynaptic density protein 95 (PSD95), in the mPFC were not significantly different in *Sparcl1*$^{WR/+}$ mice (4-week-old) compared to their littermate controls, suggesting little impact on synapse formation in *Sparcl1*$^{WR/+}$ mice. Finally, we found that *Sparcl1*$^{WR/+}$ mice (8-week-old) exhibit repetitive behavior in the marble burying test. In conclusion, we show that this single amino acid substitution triggers activation of angiogenesis, resulting in abnormal vascular wiring in the mPFC of the *Sparcl1*$^{WR/+}$ mice. We also confirm that the *Sparcl1*$^{WR/+}$ exhibits ASD-like behavior. These data suggest a potential link between abnormal angiogenesis and the pathogenesis of ASD.

Disclosures: T. Taketomi: None. **T. Yasuda:** None. **K. Ueda:** None. **R. Morita:** None. **Y. Shigeta:** None. **S. Mizuno:** None. **S. Takahashi:** None. **R. Harada:** None. **F. Tsuruta:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.01/C115

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NMSS Career Transition Award TA-2105-37619
NIH R35NS116842
NIH F30HD096784
NIH T32GM007250
NIH K08EY029362
NIH F31NS124282
NIH T32NS077888
NIH ORIP S10OD024981
NIH P30CA043703
NIH P30CA014599

Title: A phenotypic screening platform for identifying chemical modulators of astrocyte reactivity

Authors: *B. L. L. CLAYTON[1], J. KRISTELL[1], K. ALLAN[1], E. COHN[1], M. KARL[2], A. JEROME[3], E. GARRISON[2], Y. MAENO-HIKICHI[1], A. STURNO[1], A. KERR[1], E. SHICK[1], J. SEPEDA[3], E. C. FREUNDT[4], A. R. SAS[3], *B. M. SEGAL[3], R. H. MILLER[2], P. TESAR[1]; [1]Genet. and Genome Sci., Case Western Reserve Univ. Sch. of Med., Cleveland, OH; [2]Dept. of Anat. and Cell Biol., George Washington Univ. Sch. of Med., Washington DC, DC; [3]Neurol., The Ohio State Univ. Wexner Med. Ctr., Columbus, OH; [4]Biol., The Univ. of Tampa, Tampa, FL

Abstract: Disease, injury and aging induce pathological reactive astrocyte states that contribute to neurodegeneration. Modulating reactive astrocytes therefore represent an attractive therapeutic strategy. Here we describe the development of an astrocyte phenotypic screening platform for identifying chemical modulators of astrocyte reactivity. Leveraging this platform for chemical screening, we identify histone deacetylase 3 (HDAC3) inhibitors as effective suppressors of pathological astrocyte reactivity. We demonstrate that HDAC3 inhibition reduces molecular and functional characteristics of reactive astrocytes in vitro. Transcriptional and chromatin mapping studies show that HDAC3 inhibition disarms pathological astrocyte gene expression and function while promoting the expression of genes associated with beneficial astrocytes. Administration of RGFP966, a small molecule HDAC3 inhibitor, blocks reactive astrocyte formation and promotes neuroprotection in vivo in mice. Collectively, these results establish a platform for discovering modulators of reactive astrocyte states, inform the mechanisms that control astrocyte reactivity and demonstrate the therapeutic benefits of modulating astrocyte reactivity for neurodegenerative diseases.

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Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.02/C116

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: USU Intramural APG-70-12215

Title: Intravenous lipid-based isoflurane emulsion injection for neuroprotection against organophosphate-induced toxicity

Authors: *J. KRISHNAN[1], J. MOFFETT[2], N. PUTHILLATHU[2], A. NAMBOODIRI[2]; [1]Anatomy, Physiol. and Genet., Uniformed Services Univ., Bethesda, MD; [2]Anatomy, Physiol. and Genet., Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD

Abstract: Organophosphate (OP) poisoning, causing thousands of annual deaths from pesticide misuse or potential terror acts, is a major global health concern. These substances, initially impacting cholinergic systems, can trigger a cascade of neurological effects, escalating to glutamatergic system involvement, status epilepticus, and enduring CNS damage. Conventional therapeutic interventions often fail to counter these chronic neurotoxic sequelae. Using the paraoxon (POX) model of OP poisoning, we assessed the neuroprotective efficacy of a novel lipid-water isoflurane emulsion (ILE) for CNS protection through intravenous delivery. Male Sprague Dawley rats, each weighing approximately 300 grams, were randomly assigned to three groups: untreated (subcutaneous POX injections, n=7), treated (POX exposure followed by intravenous ILE administration through implanted jugular cannulas 30 minutes after POX, n=8), and control (catheter implantation without further treatment, n=5). To mitigate peripheral toxicity, both the treated and untreated groups received intramuscular 2-pyridine aldoxime methyl chloride (2-PAM) plus atropine sulfate, part of the current treatment regimen for OP poisoning. We evaluated the effectiveness of ILE by measuring convulsion severity using a modified Racine scale and assessing neuronal damage using Fluoro-Jade B (FJB) staining. Following POX administration, all subjects exhibited severe convulsions (modified Racine scale levels 5-6), which moderated to levels 3-5 over 30 minutes. Introduction of ILE via jugular catheter 30 minutes post-POX led to unconsciousness and cessation of convulsions within a minute of infusion onset, contrasting with continued seizures in untreated animals. Post-infusion, ILE-treated rats showed reduced mobility yet responded to stimuli, recovering normal activity by 24 hours. In contrast, untreated rats remained very lethargic at the 24-hour time point. The cohort experienced a 35% mortality rate prior to any treatment intervention. Neuropathological assessment using FJB staining revealed significant neuronal damage in untreated rats, with 6 out of 7 showing extensive injury. ILE-treated animals demonstrated dramatically lower neuronal damage, underscoring ILE's neuroprotective efficacy. These findings highlight ILE's potential as a rapid, enduring anti-convulsant and neuroprotective agent against OP toxicity without the need for re-administration, contrasting with the transient efficacy of benzodiazepines. This data underscores ILE as a viable, single-dose adjunct treatment, potentially reshaping emergency responses to OP poisoning.

Disclosures: J. Krishnan: None. **J. Moffett:** None. **N. Puthillathu:** None. **A. Namboodiri:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.03/C117

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: 1R03NS121831
RTI IRD

Title: Novel small molecule antagonist of GPR17 promotes oligodendrocyte precursor cell differentiation

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Abstract: Multiple Sclerosis (MS) is a severe neurological disease characterized by autoimmune-mediated demyelination of neurons and oligodendrocyte damage. The resulting axonal degeneration impairs rapid nerve conduction, which leads to neurological disability if not repaired through remyelination. The remyelination process requires proliferation and maturation of oligodendrocyte precursor cells (OPCs) into myelin producing mature oligodendrocytes. However, as MS progresses, neurons lose their ability to participate in remyelination despite the presence of substantial numbers of OPCs in demyelinating regions. Current medications for MS mostly consist of immunomodulating and immunosuppressive agents that block the abnormal immune response to reduce the severity of attacks and frequency of relapses; however, they have little effect on inducing myelin repair. Therefore, strategies that promote OPC differentiation to foster development of myelinating oligodendrocytes have enormous clinical importance. GPR17 is a class A, G protein-coupled receptor, predominantly expressed in OPCs in the central nervous system. Studies with GPR17 knockout and overexpressing mice identified GPR17 as a potent negative regulator of oligodendrocyte differentiation and myelination and selective inhibition of GPR17 can be a potential therapeutic opportunity to promote endogenous remyelination. To date, very few small molecule antagonists have been reported in the literature and pharmacological studies with the existing non-selective and less potent antagonists have been challenging. We have identified several novel, selective small molecule GPR17 antagonists through systematic structural modification of Pranlukast, the non-selective CysLT1 antagonist. In vitro evaluation of our early selective lead **SN-70** (GPR17 IC_{50} = 938 nM, CysLT1 IC_{50} > 10,000 nM) in a rat OPC differentiation assay resulted in significant expansion of Olig2+ OPCs and induction of myelin basic protein (MBP) expression at 10 µM concentration, suggesting the ability of these compounds to promote oligodendrocyte differentiation and potential for myelin repair. Our continued SAR efforts have resulted in promising lead **SN-191**, which exhibits ~400 nM GPR17 antagonist potency, >5000-fold reduced potency at CysLT1 compared to Pranlukast, >20-fold preference for GPR17 over CysLT1, and negligible off-target antagonist activity over closely related CysLT2 and P2Y1 receptors. **SN-191** serves as an excellent lead for further optimization, which may lead to more potent, selective small molecule drug-like *in vivo* probes of GPR17 antagonists.

Disclosures: S. Narayanan: None. **A.M. Decker:** None. **C. Song:** None. **X. Liu:** None. **R.Q. Lu:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.04/C118

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant DA0704

Title: Drugs acting on trpm4 modulate nmda receptor-mediated ca^{2+}influx and neurotoxicity

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Abstract: Transient receptor potential melastatin-4 (TRPM4) forms a complex with *N*-methyl-D-aspartate receptors (NMDARs) that facilitates NMDAR-mediated neurotoxicity. Here we used pharmacological tools to determine how TRPM4 regulates NMDAR signaling. Brophenexin, a compound that binds to TRPM4 at the NMDAR binding interface, protected hippocampal neurons in culture from NMDA-induced death, consistent with published work. Brophenexin (10 µM) reduced NMDA-evoked whole-cell current by 87 ± 14 % with intracellular Ca^{2+} chelated to prevent TRPM4 activation. Brophenexin inhibited NMDA-evoked currents recorded in Na^{+}-free solution by 87 ± 13 %, suggesting that brophenexin and TRPM4 modulate NMDAR function. Incubating cultures in Mg^{2+}-free buffer containing tetrodotoxin, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and bicuculline for 30 min inhibited NMDA-evoked increases in $[Ca^{2+}]_i$ by a process that occluded the 51 ± 16 % inhibition produced by brophenexin in the absence of these inhibitors. Treatment with the TRPM4 antagonist 4-chloro-2-(1-naphthyloxyacetamido)benzoic acid (NBA; 10 µM) increased NMDA-evoked Ca^{2+} influx by 90 ± 15 % and blocked the inhibition produced by brophenexin. Increasing extracellular NaCl to 237 mM, a treatment that activates TRPM4, also inhibited the NMDA-evoked increase in $[Ca^{2+}]_i$ by a process that occluded the inhibition produced by brophenexin and was prevented by NBA. These results are consistent with a model in which TRPM4 interacts with NMDARs to potentiate their activity and that this interaction can be enhanced by NBA and inhibited by brophenexin.

Disclosures: J. Casby: None. **B. Gansemer:** None. **S.A. Thayer:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.05/C119

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Onset of CIPN delayed through SARM1 inhibition in human NerveSim preclinical drug-discovery platform

Authors: *C. BYRNE[1], T. RODRIGUEZ[1], M. TERRAL[1], E. SCHMIDT[1], J. L. CURLEY[1], M. J. MOORE[2], M. J. MOORE[1], M. J. MOORE[3], E. SPACK[1], C. ROUNTREE[1];

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Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is a debilitating roadblock to lifesaving medications for critically ill cancer patients. *In vitro* and animal models are poor predictors of CIPN due to incongruencies of the underlying biology. We have developed the human NerveSim (hNS) model, the first physiologically-relevant, preclinical model of myelinated peripheral nerve tissue, ideal for investigating CIPN and preventative therapeutics. The hNS combines cell culture, tissue engineering, and neuroscience to produce an *in vitro* neurobiology platform. Human iPSC-derived neuron and primary Schwann cell coculture spheroids are placed in our custom 24-well embedded electrode array culture plate and cultured for 42 days prior to a 7-day dosing period. Axons extend down the growth channel and over the electrodes; Schwann cells align with the axons and myelinate them. hNS is powered by the Intan electrophysiology (Ephys) data acquisition system allowing stimulation and recording from each electrode longitudinally. We developed advanced metrics that utilize machine learning algorithms for in-depth Ephys analysis. These metrics include velocity density index (VDI), stimulus threshold, nerve-conduction velocity, and response amplitude. Multiplexing allows other endpoints, in addition to functional Ephys analysis, including transcriptomics, western blot, PCR, image-based neurodegeneration analysis, IHC, LDH, and more. We demonstrated the ability to induce peripheral neuropathy *in vitro* with the chemotherapeutic vincristine (VinC) and to delay CIPN onset through SARM1 inhibition with compounds NB-7, DSRM-3716, and WX-02-37. First, we validated our hNS CIPN model using VinC; we established the IC50 via multiple functional and morphological metrics and also identified a CIPN transcriptional profile. Next, co-administration of VinC with the SARM1 inhibitors revealed functional and morphological protection against CIPN over the dosing period. NB-7 and WX-02-37 preserved some neuronal functionality as assessed by VDI for up to 5 days. They also showed morphological protection for the entire 7-day dosing period which implies the potential for recovery. Ephys proved to be the most sensitive of the assays by repeatedly detecting functional dysregulation before neurodegeneration analysis detected morphological dysfunction. The ability to multiplex longitudinal Ephys data with morphological, molecular biology, and next-generation sequencing data in hNS makes it a powerful platform for neurotoxicity screening and drug discovery.

Disclosures: C. Byrne: A. Employment/Salary (full or part-time):; AxoSim, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AxoSim, Inc. **T. Rodriguez:** A. Employment/Salary (full or part-time):; AxoSim, Inc. **M. Terral:** A. Employment/Salary (full or part-time):; AxoSim, Inc. **E. Schmidt:** A. Employment/Salary (full or part-time):; AxoSim, Inc. **J.L. Curley:** A. Employment/Salary (full or part-time):; AxoSim, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AxoSim, Inc. **M.J. Moore:** A. Employment/Salary (full or part-time):; Tulane University. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AxoSim, Inc. **M.J. Moore:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AxoSim, Inc. **M.J. Moore:** A. Employment/Salary (full or part-time):; Tulane University. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds);

AxoSim, Inc. **E. Spack:** 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.; AxoSim, Inc. **C. Rountree:** A. Employment/Salary (full or part-time):; AxoSim, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AxoSim, Inc..

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.06/C120

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant RO3NS095063
NIH Grant RO1NS102337

Title: Potent antioxidant and mitochondrial-protective effects of ATH434, a novel therapeutic with moderate iron-binding affinity, in HT22 and iPSC-derived neuron models of neurodegenerative diseases

Authors: *D. K. BAILEY[1], R. NIHLAWI[1], M. BRADBURY[2], D. J. KOSMAN[1];
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Abstract: Iron is essential for energy metabolism, mitochondrial function, and maintaining cellular redox potential. Excess cellular labile ferrous iron generates reactive oxygen species leading to sustained oxidative stress and eventual cell death. Multiple System Atrophy (MSA), Parkinson's disease (PD) and Friedreich's ataxia (FA) and are neurodegenerative conditions characterized by regional excess brain iron and resultant oxidative stress, leading to clinical trials of iron-binding small molecules. ATH434, a small molecule drug candidate with *moderate* ferric iron affinity (K_d 10^{-10}), reduces excess brain iron and aggregated αsynuclein, improves neuronal survival, and restores motor performance in murine PD and MSA models. ATH434 is currently in phase 2 MSA trials. Deferiprone (DFP) is a drug with high ferric iron affinity (K_d 10^{-21}) approved for treating systemic iron-overload disorders. DFP's high affinity enables reduction of toxically elevated organ iron but has potential for maladaptive pharmacological effects on iron stores in healthy cells. Although DFP's efficacy in preclinical FA and PD models led to clinical testing, trials demonstrated adverse effects consistent with high ferric iron affinity-induced cellular iron depletion. Thus, iron-related treatments may require features that allow management of cytotoxic labile iron. ATH434 has previously demonstrated restoration of neuronal mitochondrial membrane potential in a menadione-induced oxidative stress model. ATH434 also showed direct antioxidant activity in solution-based electron transfer and radical assays. DFP was ineffective in both *in vitro* and *in cellulo* assays.ATH434 has been further evaluated in

neuronal models that replicate the altered cellular oxidative status of iron-related neurodegenerative diseases. In HT22 cell line-derived neurons, ATH434 potently protected plasma membranes from menadione-induced lipid peroxidation (BODIPY staining). Extensions of these findings are being conducted in neurons differentiated from FA patient iPSCs, and dopaminergic neurons derived from healthy control iPSC which, when injured, replicate PD and MSA pathophysiology *in vitro*. ATH434 has been evaluated for protective effects on mitochondrial function, glycolytic and oxidative metabolism, iron status, oxidative stress including lipid peroxidation, and expression of disease-related proteins. Together, our results suggest that antioxidant activity is an important contributor to the efficacy of ATH434 in neurodegenerative disorders characterized by excess labile subcortical iron and oxidative stress, thus enhancing the efficacy of its moderate iron binding.

Disclosures: D.K. Bailey: F. Consulting Fees (e.g., advisory boards); Alterity Therapeutics. **R. Nihlawi:** None. **M. Bradbury:** A. Employment/Salary (full or part-time):; Alterity Therapeutics. **D.J. Kosman:** 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.; Alterity Therapeutics.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.07/C121

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: CONAHCYT CVU:1253871

Title: N-acetyl cysteine reversed cisplatin-induced memory deficits and brain kynurenic acid levels fluctuation in female rats.

Authors: *T. D. AREMU[1], V. PEREZ DE LA CRUZ[2], D. RAMÍREZ ORTEGA[3], G. R. ROLDAN[4], T. BLANCO AYALA[5], D. GONZALEZ[6];
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Abstract: N-acetyl cysteine reversed cisplatin-induced memory deficits and brain kynurenic acid levels fluctuation in female rats.Aremu Teminijesu Dorcas [a,c], Verónica Pérez de la Cruz[b@,] Daniela Ramírez Ortega[b], Tonali Blanco Ayala[b], Dinora González Esquivel[b], Gabriel Roldán-Roldán[c]a Doctorado en Ciencias Biológicas, Universidad Autónoma de Tlaxcala, Tlaxcala,

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Cisplatin (cis-diamminedichloroplatinum [II]), a very effective chemotherapy drug, has certain adverse effects that impair human brain function. Up to 75% of patients receiving chemotherapy for tumors outside of the nervous system develop chemotherapy-related cognitive impairment, commonly known as "chemobrain". Deficits in attention, learning, memory and locomotor activity are signs of chemobrain. Abnormalities in the kynurenine pathway (KP) have been suggested as one of the mechanisms by which chemobrain develops. Kynurenic acid (KYNA), a metabolite of KP, is associated with cognitive impairment in diseases or pathologies affecting the Central Nervous System (CNS). Here, we assessed the cognitive function, brain cortex KYNA levels as well as kynurenine aminotransferase II (KAT II) activity in cisplatin-treated female Wistar rats. We also explored whether the effects of cisplatin might be prevented by N-acetylcysteine (NAC), a redox modulator and KAT-II inhibitor, the enzyme that produces KYNA. Rats (56 Post Natal Day) were fed with NAC 300 mg/day for 3 days, then they were injected intraperitoneally with cisplatin 3 mg/kg while taking NAC for 5 days. Five days later, cognitive performance, indicators of oxidative stress, and KYNA levels were assessed. Impaired short-term memory was observed using the novel object recognition test (NORT) in the cisplatin group. Furthermore, cisplatin administration induces an increase in malondialdehyde (MDA), oxidized glutathione and KYNA levels in the cerebral cortex, while KATII remained unchanged compared to control group. However, when NAC was administrated in combination with cisplatin learning and memory alterations decreased, the redox environment was recovered, KATII activity decreased around 70% and KYNA levels returned to normal values. Our results are consistent with the hypothesis that cisplatin deteriorates frontal cortex-based cognitive functions and that this effect might be mediated, at least partially, by increasing KYNA levels.

Disclosures: T.D. Aremu: None. **V. Perez De La Cruz:** None. **D. Ramírez Ortega:** None. **G.R. Roldan:** None. **T. Blanco Ayala:** None. **D. Gonzalez:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.08/C122

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: CONAHCyT

Title: Neuroprotective effect of prolactin against excitotoxicity damage by glutamate in neuroblastoma SH-SY5Y cell line

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Abstract: Prolactin (PRL) is a peptide and pleiotropic hormone that has been shown to play an important role in the nervous system. Recently, PRL has been known to be a neuroprotective function against various types of neuronal damage, such as oxidative stress, neuroinflammation, and excitotoxicity induced by glutamate (Glu) and kainic acid. The aim of this study was to determine whether PRL exerts neuroprotection during a time-curse exposure against glutamate damage in differentiated SH-SY5Y cells. Experiments were conducted in an *in-vitro* model using SH-SY5Y differentiated cells. PRL pre-treatments (20 ng/ml) were administrated at 3, 6, and 12 h. Then, Glu (100 µM) was administrated by 1h. Cell survival was measured through MTT assay, and proteins related to survival function were evaluated such as PRLR, Nrf2, Synaptophysin, and NeuN. results show that PRL promoted a significant cell survival increase. The higher rate of cell survival against Glu-induced excitotoxicity correlates with the longer time of prolactin administration. In addition, we observed protein expression of PRLR, Nrf2, Synaptophysin by effect of prolactin's neuroprotection. These findings suggest that prolactin may mitigate a wide range of cellular damage. Thus, PRL could represent a valuable therapeutic strategy in treatments of neurological diseases associated with neuronal damage.**Disclosures: None**Key words: Neuroprotection, prolactin, glutamate, excitotoxicity, oxidative stress.

Disclosures: K. Almeida: None. **M. Cerbon:** None. **R. Artiguez Sanchez:** None. **O. Picazo:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.09/C123

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: CONAHCYT

Title: The effect of estradiol, prolactin and combination on SH-SY5Y cells under excitotoxicity conditions

Authors: *R. ARTIGUEZ SANCHEZ[1], K. ALMEIDA[2], O. PICAZO[3], M. CERBON[4];
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Abstract: The 17β-estradiol (E2) is the major hormone in the reproductive years of women, it is part of the estrogen family and it's involved in numerous physiological processes including the regulation and maintenance of female sexual characteristics. Prolactin (PRL) is a hormone that is known to have more than 300 physiological effects, mainly related to the production of breast milk. However, although both are linked to reproductive processes, functions in the brain such as: neurogenesis, neuronal plasticity and neuroprotection have also been attributed to it. Excitotoxicity produced by glutamate is a common alteration in many neurodegenerative diseases that results in neuronal death.
The goal of the present study was to evaluate the neuroprotective capacity individually and together of estradiol and prolactin under conditions of excitotoxicity in the SH-SY5Y neuroblastoma cell line, which is a broad in-vitro model used in research into neurodegenerative diseases.
The SH-SY5Y cell line was differentiated by the application of all-trans retinoic acid (RA). Cell viability was measured using MTT release assays. The cells were trated with 100 uM glutamate and E2, PRL and combination at different concentrations (0 to 100 nM).
The results show that the SH-SY5Y cell line has E2 and PRL receptors, and under excitotoxicity conditions these hormones promote cell survival.These findings show a possible strategy for the treatment of neurodegenerative diseases and an alternative use to hormone replacement therapies.

Disclosures: R. Artiguez Sanchez: None. **K. Almeida:** None. **O. Picazo:** None. **M. Cerbon:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.10/C124

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Citizens United for Research in Epilepsy (CURE)
NIH NS112350
NIH NS112308

Title: Inhibition of monocyte chemoattractant receptor, CCR2, alleviates cognitive impairments associated with status epilepticus

Authors: S. POURKHODADAD[1], R. J. DINGLEDINE[2], ***N. VARVEL**[1];
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Abstract: Although epilepsy is known as a disease of seizures, many epilepsy patients also suffer from seizure-associated cognitive comorbidities. These comorbidities can degrade quality of life in people with epilepsy. Neuroinflammation is an invariant feature of chronic neurologic

disease, including epilepsy, and acute insults such as status epilepticus (SE). Our previous work has demonstrated neuroinflammatory induction within 30 minutes of SE onset in the mouse hippocampus, activation of brain-resident microglia, and brain recruitment of CCR2+ monocytes. Limiting brain monocyte entry by global *Ccr2* KO or CCR2 antagonism rescues several adverse SE-induced effects including blood-brain barrier (BBB) erosion, microgliosis, and neuronal damage. Here we asked if fleeting exposure to the CCR2 antagonist offers protection against cognitive impairments associated with SE. To this end, we subjected male C57BL/6NCrl mice to pilocarpine to induce SE for one hour. Surviving mice were then randomized into vehicle or CCR2 antagonist treatment groups with drug treatments 24 and 48 hours after SE onset. Four weeks after SE, the mice were subject to a battery of behavioral tests to assess cognitive performance in the Y-maze to examine working memory, open field to investigate locomotion and anxiety-like behavior, and the novel object recognition (NOR) test to examine long-term memory. The working memory deficit in the y-maze was reversed in SE mice given the CCR2 antagonist. In an open field arena, SE mice spent significantly less time in the center anxiogenic zone than their nonSE counterparts indicating that SE provokes anxiety-like behavior. However, CCR2 antagonism was ineffective in restoring normal behavior in the open field arena. Finally, we assessed the ability of the CCR2 antagonist to improve long-term memory in the novel object recognition test. Control nonSE mice, spent a more time exploring the novel object, whereas SE mice given vehicle spent nearly equal time exploring the familiar and novel objects. In contrast, SE mice treated with the CCR2 antagonist were able to differentiate between novel and familiar objects. In sum, our findings demonstrate that CCR2 antagonism rescues SE-induced working and long-term memory deficits. These findings reinforce the pathological role of brain invading CCR2-expressing monocytes after SE and provide further evidence for the idea that targeting peripheral monocytes with fleeting CCR2 antagonism after SE could represent a novel strategy for alleviating cognitive comorbidities associated with neurologic disease.

Disclosures: S. Pourkhodadad: None. **R.J. Dingledine:** None. **N. Varvel:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.11/C125

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: PREDOCS-UB 2022 grant
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Centro Investigación Biomédica en Red. Enfermedades Neurodegenerativas (CB06/05/0024)

Title: Epigallocathequin-3-gallate as a promising neuroprotective therapy to allivates colistin induced neurotoxicity and cognitive impairment

Authors: *L. GUZMAN[1,7,8,2], A. PARCERISAS[9,10], E. SÁNCHEZ-LÓPEZ[3,8,4], A. CANO[11,3,8,4], M. H. BUENROSTRO-JAUREGUI[7], Y. CAJAL[3,4], F. RABANAL[5], M. BARENYS[1,6], A. CAMINS[1,2,8,12], M. ETTCHETO[1,2,8,12];
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Abstract: The appearance of drug-resistant bacteria has made necessary the reintroduction of effective antibiotics with a narrow therapeutic window like colistin. It is known that colistin is nephrotoxic and causes alterations in the peripheral nervous system by increasing the reactive oxygen species. Moreover, recent studies suggested that it may also affect at central level (CNS) while the mechanism of toxicity remains unclear. Hence, this study aims to investigate the effects of colistin in the CNS and evaluate 3 different strategies based on changes in chemical structure, new formulations, or a combination with natural antioxidant compounds to protect against colistin induced toxicity. To select the best strategy, the neuro- and systemic toxicity of zebrafish embryos exposed to 10 colistin analogs, to colistin nanoparticles, and to 5 different natural antioxidant compounds was evaluated. As the pre-exposure to antioxidant compounds seemed to be the most promising strategy, to accurately select the best of those 5 compounds the viability of primary cortical neurons pre-exposed to the same antioxidants was assessed. Then, to better extrapolate to a clinically relevant scenario an *in vivo* study with the best candidate was performed. 7 weeks old C57BL/6 female mice were administrated intraperitoneally with epigallocatechin-3-gallate (EGCG), a potent antioxidant of green tea, at a dose of 40 mg/kg for one week, followed by a 14-day administration with colistin (9 mg/kg 8h apart). Then, eight behavioral tests were performed, and brains were preserved for different molecular analysis. Moreover, hippocampal primary neurons were used to study in detail the molecular mechanism of colistin in synapsis. From all the strategies tested, only the pre-exposure of EGCG demonstrated significant protection against colistin-induced neurotoxicity in primary neurons and in zebrafish embryos. Moreover, our results demonstrated that colistin treatment induced anxiety-, depression-, and cognition-related deficits in mice. In addition, it caused a significant decrease in DCX positive neurons ($p< 0.01$, n=5), a reduction of dendritic spines *in vivo* and *in vitro* ($p<0.05$, n=5) and an increase of astrogliosis (DG: $p< 0.01$, n=5; CA1: $p<0.1$, n=5). By contrast, when a pre-administration with EGCG was carried out, a reduction in behavioral alterations and neuroinflammation, together with a prevention of dendritic spine loss was observed. To sum it up, we have demonstrated that a 14-days treatment with colistin causes alterations in the CNS. Furthermore, EGCG pretreatment could be a promising therapy to alleviate colistin-induced toxicity and increase its therapeutic window.

Disclosures: L. Guzman: None. **A. Parcerisas:** None. **E. Sánchez-López:** None. **A. Cano:** None. **M.H. Buenrostro-jauregui:** None. **Y. Cajal:** None. **F. Rabanal:** None. **M. Barenys:** None. **A. Camins:** None. **M. Ettcheto:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.12/C126

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Memorial University
OFI- Vitamin Research Fund
Beatrice Hunter Cancer Research Institute

Title: Dietary methionine restriction as a therapeutic strategy for Chemotherapy-induced cognitive impairment

Authors: *D. RIWA[1], M. HIRASAWA[2];
[1]Mem. Univ. of Newfoundland, St. John's, NL, Canada; [2]Div. of Biomed. Sci., Mem. Univ., St John's, NL, Canada

Abstract: Introduction: Chemotherapy-induced cognitive impairment, also known as chemobrain, is a common side effect of cancer chemotherapy. Chemobrain is characterized by impairment in memory, learning, attention, and executive function, thus adversely affecting the quality of life of cancer patients and survivors. 5-Fluorouracil (5FU) is a common chemotherapy drug for many cancers and is known to induce chemobrain. Chemobrain can persist for up to 20 years after cessation of chemotherapy, but currently, there is no evidence-based therapeutic option for chemobrain. As a dietary intervention, Methionine (Met) restriction has been shown to increase neurogenesis in mouse hippocampus and decrease neuroinflammation and oxidative stress, resulting in improved cognitive functions. Using a mouse model, this study tests the hypothesis that dietary Met restriction prevents synaptic impairment in chemobrain. **Methods**: 8-10 weeks old male C57BL/6 mice were fed with either a normal Met diet (0.86% Met) or a low Met diet (0.12% Met) for three weeks prior to and during four weekly intraperitoneal injections of 60 mg/Kg 5FU or saline. At 7 days post-injection, field EPSP was recorded ex-vivo at the CA3-CA1 synapse. Input-output relationship and paired pulse ratio (PPR) were analyzed to assess basal synaptic function, while long-term potentiation (LTP) was induced by 100Hz, 100 pulse afferent stimulation. To determine the effect of 5FU and Met restriction on synaptic structure, Golgi-Cox and immunohistochemical detection of synaptic markers are performed. **Results**: In mice fed with normal Met diet, basal fEPSP was decreased without altering PPR, a measure of synaptic release probability, in 5FU-treated group compared to saline controls. Conversely, 5FU treatment did not impair fEPSP in mice fed with a low Met diet as compared to

their saline controls. Additionally, LTP was impaired in 5FU mice on normal Met, which was not observed in 5FU mice on low Met diet. No significant dietary effect on basal fEPSP and LTP was observed between saline-treated mice fed with either normal or low Met diet. A structural analysis indicates that 5FU causes synapse loss in the CA1 hippocampus; whether low Met diet limits this loss is currently under investigation. **Conclusion:** Our results indicate that a low Met diet may ameliorate 5FU-induced impairment in hippocampal synaptic transmission and plasticity. Together with its known anti-cancer effect, dietary Met restriction is a promising candidate as an adjunct cancer treatment that can also limit chemobrain.

Disclosures: D. Riwa: None. **M. Hirasawa:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.13/C127

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: I. Parra was supported by a scholarship from CONACYT-Mexico (1024175).
VIEP-BUAP (2023–2024)

Title: Peroxisome proliferator-activated receptors: A Possible Anti Neuroinflammatory Role on probiotics effects

Authors: *I. PARRA[1], A. CARRASCO CARBALLO[1], J. L. GONGORA-ALFARO[2], J. AGUILERA[3], L. MARTINEZ MENDIETA[1];
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Abstract: Author: Irving Parra, Alan Carrasco Carballo, Isabel Martínez García, José Aguilera, Liliana Martínez Mendieta. It is well established that the gut-brain axis is a bidirectional communication between the intestine and the brain. The gut-brain axis is regulated at the neuronal, endocrine, and immunological levels that could be influenced by the microbiota. Microbiota-gut-brain axis components communicate in a complex and multidirectional way to maintain homeostasis and this interaction contributes to the regulation of key events in the development of the immune and central nervous systems, metabolism, and behavior. However, dysregulation of bacterial composition plays a role in the disruption of homeostasis that results in inflammatory processes in the gut and brain systems. Currently, some strategies have been used to efficiently reestablish the microbiota, such as the consumption of probiotics. There is a wide variety of microorganisms with probiotic capacity, although the most studied probiotic genera are Lactobacillus and Bifidobacterium generally the species L. rhamnosus GG and B. lactis. It is

under debate how signals from the microbiota and probiotics affect organisms and, even more so, how they reach the brain. However, we propose that one of the communication routes is through the metabolites that these microorganisms produce. That is: based on the idea that the microbiota and probiotics produce metabolites during their life cycle that would act as drugs and, therefore, could be absorbed, distributed, have biological targets, be metabolized, and eliminated. With this, the objective was set to study, through computational analysis, this pathway of action mechanism of the two most studied probiotics and explore the possibility of finding new ligands and molecular targets that explain the mechanism of action of the probiotics. We collected metabolites that *L. rhamnosus* GG and B. *lactis* produce to choose a possible protein modulated by the metabolites and proposed it as a target of study in the mechanism of action of probiotics. Data related with the metabolism of the probiotics were collected using the PubMed web page and BioCyc Database Collection platform. Our results indicate that PPAR mechanism is one of the key protein to modulate some antiinflammatory effect of probiotics.

Disclosures: I. Parra: None. **A. Carrasco Carballo:** None. **J.L. Gongora-Alfaro:** None. **J. Aguilera:** None. **L. Martinez Mendieta:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.14/C128

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Contribution of the Sigma 1 receptor as a neuroprotector against damage in a preclinical model of chronic kidney disease

Authors: *Y. K. GUTIERREZ-MERCADO;
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Abstract: The central nervous system (CNS) and the peripheral (PNS) is one of the organs that are most affected by chronic kidney disease (CKD), along with cardiovascular diseases (CVD); suffers various damages due to the accumulation of toxins in the blood, hypoxia due to low hemoglobin levels or strokes, among other causes, which generates a wide variety of pathologies, such as uremic encephalopathy, cognitive-behavioral disorders, among other serious consequences that can lead to death.One of the molecules that could be involved is the sigma 1 receptor (S1R), which is a chaperone protein present in the membrane of the endoplasmic reticulum adjacent to the mitochondria. In CKD, the S1R antagonist has an antifibrotic effect, which could indirectly protect the CNS. Likewise, S1R agonist drugs inhibit the formation of reactive oxygen species; it has also been observed that they increase anti-inflammatory cytokines and inhibit several inflammatory ones. There is an important need to develop pharmacological strategies focused on reducing chronic damage in patients with neurological disorders caused by

CKD, which is why this project seeks to clarify how the S1R influences in neuroprotection in a preclinical model of chronic kidney disease.For this, a model of chronic kidney damage was carried out through unilateral ureteral obstruction (UUO) in female and male C57BL/6 mice, which were divided into 5 groups, the Sham group, the group with UUO and physiological saline solution, the group of OUU and PRE 084, the group of OUU (0.5 mg/Kg) and NE100 (0.5 mg/Kg) and the group of OUU and haloperidol (2 mg/Kg), and each of these groups, in turn divided into subgroup of females and males, were sacrificed 21 days after surgery and different organs were taken (kidney, heart, brain). These organs were fixed and immunohistochemistry was performed for the identification of the S1R.As previous results, the UUO and haloperidol group showed a lower amount of fibrosis in the kidney and heart, also with Masson's trichrome staining, a lower amount of fibrosis is shown, which suggests that haloperidol shows an improvement, over all in female mice. In the nervous system, the prefrontal cortex, hippocampus and nuclei of the thalamus and hypothalamus were analyzed, where a lower expression of the S1R was observed in mice subjected to haloperidol, unlike S1R agonist drugs. It remains to be done. various techniques that make it easier for us to observe damage to the nervous system, such as astrocyte reactivity in different areas of the brain, as well as markers of cell death to identify if there is any difference between the various study groups, as well as sexual differences, as observed with the kidney and the heart.

Disclosures: Y.K. Gutierrez-Mercado: None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.15/C129

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Istituto Superiore di sanità grant#ISS20-5cf22604a04d

Title: Dipyridamole ameliorates recognition memory in Niemann Pick C1 mice and increases hippocampal calbindin expression

Authors: L. GADDINI[1], V. CHIODI[1], A. MATTEUCCI[1], Z. BOUSSADIA[1], S. EDDARKAOUI[2], *D. BLUM[2], C. RAGGI[1], R. DI BENEDETTO[1], P. POPOLI[1], **A. FERRANTE**[1];
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Abstract: NPC1 is a genetic disorder characterized by the progressive loss of Pukinje neurons in the cerebellum and by a synaptic dysfunction of the hippocampus, both contributing to the devastating neurological symptoms characteristic of the disease (motor impairment and cognitive deficit). Since miglustat is the only drug approved for the treatment of neurological symptoms, searching for new disease-modifying drugs is highly warranted. We previously demonstrated that

the clinically-approved drug dipyridamole (DIP), able to enhance adenosine signaling through the inhibition of its transporter ENT1 and the augmentation of its extracellular levels, was effective in ameliorating pathological hallmarks of fibroblasts derived from NPC1 patients. The objective of the present study is to verify if DIP could be effective also in vivo, in NPC1^{nih} mice (hereafter indicated as NPC1).Male and female WT and NPC1 mice were intraperitoneally administered with DIP at 30mg/kg or with vehicle (PBS:PEG400, 1:1) in a pre-symptomatic stage of disease (post-natal day 28, PND28); the effect of the drug was evaluated on cardinal symptoms of the disease: motor impairments (by using the Erasmus Ladder at PND42 and 62), recognition memory deficits (by using the Novel Object Recognition test at PND52), brain pathology (cerebellar Purkinje neurons loss, gliosis and AD-like pathology at PND62) as well as lipid accumulation in peripheral organs (by HP-TLC of liver and spleen at PND62).The results demonstrated that DIP was ineffective towards motor impairments in mice, Tau and APP-related brain changes and peripheral lipid accumulation, while it completely rescued the reduced motivation of NPC1 mice to walk onto the Erasmus Ladder (measured by the percentage of back-steps performed on the ladder; N=14-16; p=0.01, Two-way Repeated Measure (RM) ANOVA) and their deficit in recognition memory (measured by the discrimination index; N=14-16; p=0.0034, One-way ANOVA). Interestingly, DIP completely restored the reduced levels of calbindin, whose expression is known to correlate with recognition memory performance, observed in the hippocampus of NPC1 *vs.* WT mice (N=6; p=0.0415, One-way ANOVA).Overall, our data indicated that DIP is able to restore hippocampal function in NPC1 mice but it did not impact on cerebellar-dependent motor impairments as well as on AD-like pathology in the brain and on lipid accumulation in peripheral organs. Transcriptomic and proteomic analysis are in progress in an attempt to determine signaling pathways responsible for the observed effect of DIP in NPC1.

Disclosures: L. Gaddini: None. **V. Chiodi:** None. **A. Matteucci:** None. **Z. Boussadia:** None. **S. Eddarkaoui:** None. **D. Blum:** None. **C. Raggi:** None. **R. di benedetto:** None. **P. Popoli:** None. **A. Ferrante:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.16/C130

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant EY033047
NIH Grant GM121198

Title: Protective effects of nicotinamide in a mouse model of glaucoma DBA/2 studied by second-harmonic generation microscopy

Authors: *H. LIM;
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Abstract: Glaucoma is a blinding disease where the retinal ganglion cells and their axons degenerate. Degradation of axonal microtubules is thought to play a critical role in the pathogenesis, but the mechanism is unknown. Here we investigate whether microtubule disruption in glaucoma can be alleviated by metabolic rescue. The morphology and integrity of microtubules of the retinal nerve fibers were evaluated by second-harmonic generation microscopy in a mouse model of glaucoma, DBA/2, which received a dietary supplement of nicotinamide to reduce metabolic stress. It was compared with control DBA/2, which did not receive nicotinamide, and non-glaucomatous DBA/2-*Gpnmb*+. We found that morphology but not microtubules are significantly protected by nicotinamide. Furthermore, from co-registered images of second-harmonic generation and immunofluorescence, it was determined that microtubule deficit was not due to a shortage of tubulins. Microtubule deficit colocalized with the sectors in which the retinal ganglion cells were disconnected from the brain, indicating that microtubule disruption is associated with axonal transport deficit in glaucoma. Together, our data suggests significant role axonal microtubules play in glaucomatous degeneration, offering a new opportunity for neuroprotection.

Disclosures: H. Lim: None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.17/C131

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: FBC Grant R34302
GRSC Grant R36209

Title: Neuroprotection of retinal ganglion cells in experimental glaucoma using a novel gene therapy construct

Authors: *A. J. JAMET, B. CHAUHAN;
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Abstract: Purpose: The current available treatments for glaucoma, an age-related eye disease characterised by progressive retinal ganglion cell (RGC) degeneration, only consider the main modifiable risk factor, intraocular pressure (IOP). Recently, as an adjunct to lowering IOP, there has been a shift towards new gene therapy approaches with recombinant adeno-associated viral (AAV) vectors designed to target and boost RGC survival, by expressing brain derived neurotrophic factor (BDNF), an agent essential for the survival of RGCs that is altered in

glaucoma. The results have shown limited benefit to RGCs as the efficiency of BDNF alone is time limited due to the downregulation of the receptor for BDNF, tropomyosin-related receptor kinase-B (TrkB). We investigated a novel gene therapy approach to express not only BDNF but also TrkB, to generate a sustained neuroprotective benefit for RGCs in experimental glaucoma (EG). Methods: This research was conducted in Thy1-YFP-H mice (which express YFP in 0.2% of RGCs and which can be used to monitor dendritic arbour alterations) injected with AAV2-SYN1-TrkB-2A-BDNF. The mice were subsequently sacrificed at 3 weeks and processed for immunohistochemistry (IHC). The retinal whole mounts were stained for anti-TrkB, and anti-BDNF, as well as anti-pAkt and anti-pERK1/2, anti-apoptotic markers known to be upregulated with BDNF. The IHC was also performed in the non-injected contralateral control eyes of the mice. The whole mounts were stained for RGCs with the RGC-specific marker, RNA Binding Protein with Multiple Splicing (RBPMS), to determine BDNF and TrkB's hit rate to YFP+ RGCs. Results: There was a substantial upregulation of mBDNF and its receptor, TrkB, correlated to an upregulation of pAkt and pERK1/2, in the treated eyes compared to the untreated contralateral (Fig. 1). Hit rate with the vector showed that 89% and 86% of the YFP+ RGCs were labelled with BDNF and TrkB, respectively (Fig. 2). Conclusion: The injection of the novel construct created a neuroprotective environment for RGCs in the Thy1-YFP-H mouse strain.

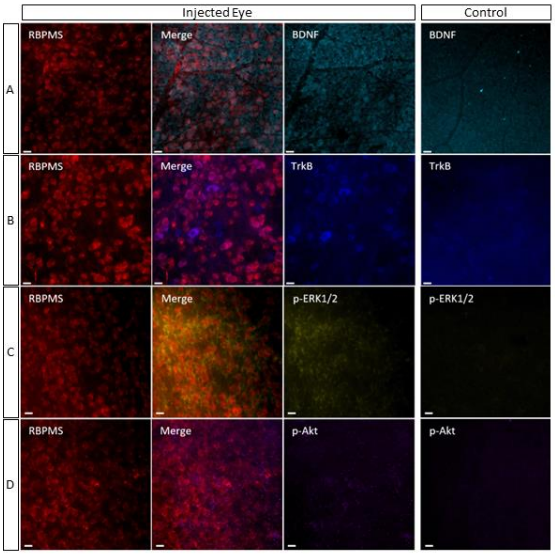

Fig. 1. Upregulation of the injected versus the non-injected contralateral control eye of a Thy1-YFP-H mouse 3-weeks post single injection of the novel AAV2 SYN1-TrkB-2A-mBDNF gene therapy construct. **A-B.** Immunohistochemistry for BDNF and TrkB, **C-D.** Immunohistochemistry for p-ERK1/2 and p-Akt. All markers were colocalized to RGCs (RBPMS and Merge panels; n=8). Scale bar = 10 µm.

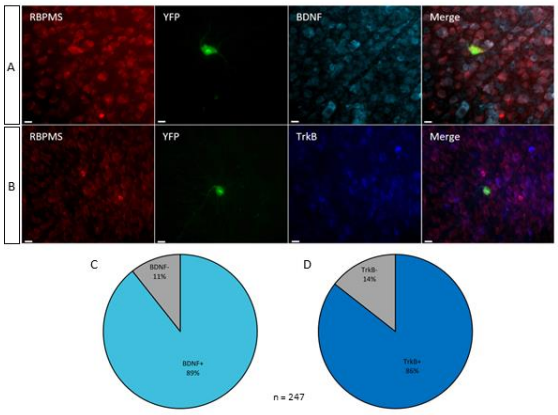

Fig. 2. Transduction of YFP+ RGCs with an intravitreal injection of the novel gene therapy construct AAV2 SYN1-TrkB-2A-mBDNF vector in Thy1-YFP-H mice. Retinal wholemounts 3 weeks after injection showing colocalization between a YFP+ RGC and BDNF (**A**) TrkB (**B**). RGCs were labelled with an RGC-specific marker (RBPMS). **C-D.** Hit rate of the vector showed that around 89% and 86%, respectively, of the YFP+ RGCs are labelled with BDNF and TrkB (**A-B**, n = 4). Scale bar = 10 µm.

Disclosures: A.J. Jamet: None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.18/C132

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NEI R01 EY012543
NIH NEI R01 EY032136
NIH NEI P30 EY002687
The Knights Templar Eye Foundation Pediatric Ophthalmology Career-Starter Research Grant
VitreoRetinal Surgery Foundation Research Fellowship
Research to Prevent Blindness

Title: Gene augmentation therapy for CRX-associated inherited retinal diseases

Authors: *C. SUN;
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Abstract: Cone-rod homeobox (CRX) is a homeobox transcription factor that is primarily expressed in vertebrate photoreceptors. CRX regulates downstream gene expression in photoreceptors, which is essential to photoreceptor functional development and maintenance. Mutations in human *CRX* are associated with blindness-causing inherited retinal diseases including Leber congenital amaurosis (LCA), cone-rod dystrophy (CRD), and retinitis pigmentosa (RP); treatment is unavailable. These mutations are located within the coding regions for the two functional domains of CRX: the homeodomain (HD) that mediates DNA binding activity and the carboxy terminus (C-terminal) that mediates transcriptional regulation. Mouse models have been generated to study mutation-mediated pathogenesis. Loss-of-function $Crx^{-/-}$ mutation caused LCA with undifferentiated photoreceptors. Hyperactive $Crx^{E80A/+}$ missense mutation in HD coding region resulted in precocious rod differentiation but perturbed cone differentiation, causing CRD phenotypes. Antimorphic $Crx^{E168d2/+}$ frameshift mutation in C-terminal coding region impaired photoreceptor survival and visual functions especially in cones, also causing CRD phenotypes. This study aimed to test if gene augmentation therapy is an effective strategy to CRX-associated inherited retinal diseases. A transgenic mouse model of doxycycline-inducible *Tet-On-hCRX* was generated to express ectopic CRX in photoreceptors. The therapeutics of gene augmentation therapy was evaluated by photoreceptor morphology and visual functions in treated mutants. As a result, *Tet-On-hCRX* promoted opsin expression in $Crx^{-/-}$ retina as well as prolonged photoreceptor survival in $Crx^{E80A/+}$ and $Crx^{E168d2/+}$ retinas. *Tet-On-hCRX* also improved electroretinogram (ERG) responses in treated retinas as compared to untreated counterparts. Moreover, the therapeutics of gene augmentation therapy can be improved with pharmacological supplementations. Two types of supplementations were applied to *Tet-On-hCRX* treated mutants. Histone deacetylase (HDAC) inhibitors further enhanced photoreceptor gene expression in $Crx^{E80A/+}$ and $Crx^{E168d2/+}$ retinas. Antioxidants treatment palliatively extended the photoreceptor survival till late adulthood in $Crx^{E168d2/+}$ retina. In conclusion, the translational potential of gene augmentation therapy for CRX-associated inherited retinal diseases is evident in our research.

Disclosures: C. Sun: None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.19/C133

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Perceive Biotherapeutics
NIH Grant R01EY029342

Title: A novel compact bidirectional promoter for adeno-associated virus-based expression in retinal ganglion cells

Authors: M. A. CHEW[1], A. K. PATEL[1], M. P. AVILA[1], P.-W. ZHANG[2], N. L. HOANG[1], M. K. PHAM[1], D. J. ZACK[2], *D. S. WELSBIE[1];
[1]Ophthalmology, The Univ. of California, San Diego, La Jolla, CA; [2]Johns Hopkins Univ., Baltimore, MD

Abstract: Gene therapy with adeno-associated virus (AAV) has been used successfully in the eye to replace expression of a single gene, as was done with RPE65 and Leber congenital amaurosis. However, complex neurodegenerative diseases like glaucoma will likely require simultaneous targeting of multiple pathways. Given the 4.7 kilobase (kb) packaging capacity of AAV, it has been a challenge to express multiple therapeutic genes along with the necessary regulatory elements like promoters. To identify a small promoter capable of simultaneous outward expression of two transgenes, we bioinformatically ranked the bidirectionality of human endogenous promoters. The top 20 were tested for their ability to drive the expression of green fluorescent protein (GFP) and mScarlet in HEK293T cells. The top five were further tested in immunopanned primary mouse retinal ganglion cells (RGCs) and resulted in the identification of a single promoter capable of robust bidirectional expression. Naturally, this 235 base pair (bp) promoter is situated on the short arm of chromosome 16, but could be truncated to only 185 bp with minimal loss of activity. We showed that the promoter could also be used to drive bidirectional transgene expression in human stem cell-derived RGCs. To test whether the promoter supported bidirectional expression in the context of AAV gene therapy, we developed an AAV vector using the promoter to express GFP and mScarlet. AAV2 particles were produced by serial affinity purification and ultracentrifugation, titered with digital droplet PCR (ddPCR) and injected intravitreally into 3-month-old C57Bl6/J mice at 10^9 viral genomes (vg)/eye. Retinas were harvested at various timepoints and flatmount stained for RGC markers. Bidirectional expression was seen at the latest timepoint of six months, with comparable intensity to that seen with the CMV enhancer / chicken beta actin promoter / rabbit globin exon/intron (CAG) promoter. Notably, while others have shown that some strong promoters can produce retinal toxicity, no toxicity was seen with this bidirectional promoter. Together, these results establish that an endogenous human regulatory region can be used for robust bidirectional gene expression in AAV while only consuming ~0.2 kb of AAV's packaging capacity.

Disclosures: M.A. Chew: None. **A.K. Patel:** F. Consulting Fees (e.g., advisory boards); Perceive Biotherapeutics. **M.P. Avila:** None. **P. Zhang:** None. **N.L. Hoang:** None. **M.K. Pham:** None. **D.J. Zack:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Perceive Biotherapeutics. **D.S. Welsbie:** 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.; Perceive Biotherapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Perceive Biotherapeutics. F. Consulting Fees (e.g., advisory boards); Perceive Biotherapeutics.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.20/C134

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Tetrahydrocurcumin, A Curcumin metabolite shows Neuroprotective Properties: A Potential Therapeutic Metabolite for Neurodegenerative pathologies

Authors: *S. PATHAK[1], K. LIU[1], M. DHANASEKARAN[2];
[1]Auburn Univ. - Harrison Col. of Pharm., Auburn, AL; [2]Harrison Col. of Pharm., Auburn Univ., Auburn, AL

Abstract: One of the most advantageous natural compounds utilized worldwide is *curcumin*, often known as *curcuma longa* or turmeric, because of its strong pharmacodynamic qualities with minimal side effects. *Curcumin* has been utilized both prophylactically and therapeutically as a nutraceutical and dietary supplement. Additionally, it has also been used for cosmetic purposes. The low bioavailability of Curcumin, on the other hand, has long minimized its use in healthcare. As a result, research on curcumin's metabolites has recently gained prominence, and several studies have been conducted to improve curcumin utilization. Curcumin or curcuminoid metabolites also exhibit biological activity that is equivalent to or superior to that of its precursor, according to past and current studies. The current study aimed to establish novel neuroprotective activities of Tetrahydrocurcumin (a key curcumin metabolite). To elucidate and validate the neuroprotective properties of Tetrahydrocurcumin along with potential neuroprotective mechanisms, both *in-silico* and *in-vitro* studies were carried out. The effect of tetrahydrocurcumin on the viability of hippocampus and dopaminergic neurons was demonstrated using HT-22 (hippocampal neurons) and N27 (dopaminergic neurons). Additionally, to study the neuroprotective mechanisms, markers of oxidative stress, mitochondrial function, inflammation, and apoptosis were examined. Tetrahydrocurcumin demonstrated significant neuroprotection on both hippocampal and dopaminergic neurons. The

neuroprotection was attributed to its antioxidant and anti-apoptotic actions. Furthermore, RNA sequencing was performed to validate the neuroprotective effects. Thus, Tetrahydrocurcumin can be an impactful therapeutic natural bioactive metabolite for preventing, reducing the rate of progression, and treating neurodegenerative pathologies.
Keywords: Tetrahydrocurcumin; Oxidative stress; Mitochondrial function; Inflammation; Apoptosis; RNA sequencing; HT-22 cells; N27 cells; Neuroprotection

Disclosures: S. Pathak: None. **K. Liu:** None. **M. Dhanasekaran:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.21/C135

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Graduate Council Fellowship, University of Alabama

Title: Fucoxanthin as a potential regulator of mitochondrial permeability transition during excitotoxicity

Authors: *K. FERDOUS[1], H.-A. PARK[2];
[1]Human Nutr., The Univ. of Alabama, Tuscaloosa, AL; [2]Univ. of Alabama, Tuscaloosa, AL

Abstract: Excitotoxicity triggers neuronal death associated with the opening of the mitochondrial permeability transition pore (mPTP) during brain disorders such as cerebral ischemia. Previous research has reported that the F_1F_o ATP synthase forms mPTP, which results in the loss of mitochondrial membrane potential and the inefficient operation of oxidative phosphorylation, ultimately leading to cell death. Therefore, it is important to find a strategy to regulate the F_1F_o ATP synthase and prevent this mPTP-mediated neuronal loss. Fucoxanthin is a carotenoid found predominantly in brown seaweed that has been shown to protect mitochondria in various brain disease models. Here, we hypothesized that fucoxanthin inhibits excitotoxicity-induced neuronal death by preventing the opening of the mPTP. Rat primary hippocampal neurons were treated with fucoxanthin, glutamate, or their combination. After 24 h incubation, viable and dead cells were labeled with calcein and propidium iodide, respectively. Fucoxanthin treatment attenuated neuronal death induced by glutamate. Furthermore, mitochondrial membrane potential and superoxide anion levels were examined using tetramethylrhodamine methyl ester (TMRM) and MitoSOX probes, respectively. Neurons treated with fucoxanthin prevented the loss of mitochondrial membrane potential and the production of mitochondrial superoxide under glutamate treatment. The F_1F_o ATP synthase has been previously shown to disassemble during excitotoxicity. Our immunocytochemical analysis consistently revealed that glutamate-challenged neurons decreased the co-localization of the β and c-subunit. However, treatment with fucoxanthin prevented the dissociation of these subunits. The findings presented

here indicate that fucoxanthin has potential as a neuroprotectant against excitotoxicity by regulating the mPTP. Further studies exploring the interactions between fucoxanthin and the F_1F_o ATP synthase and the direct role of fucoxanthin on mPTP opening are necessary to understand the underlying mechanism of excitotoxicity-mediated neuronal death.

Disclosures: K. Ferdous: None. **H. Park:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.22/C136

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Mitacs
Kapoose Creek Bio
Ontario Graduate Fellowship

Title: Exploring the mechanism of action of neurotrophic and neuroprotective fungal natural products

Authors: *D. KUKJE ZADA[1], E. BROWN[1,2];
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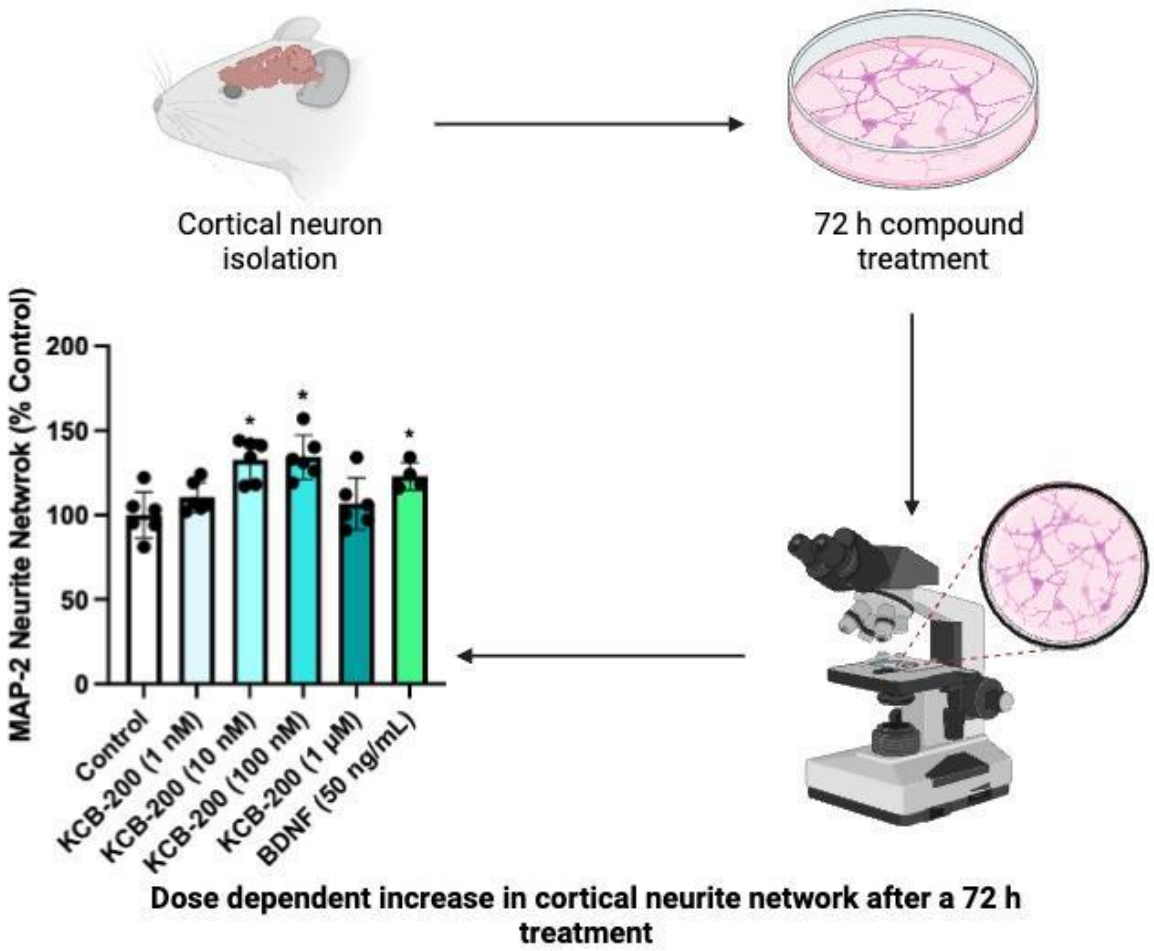
Abstract: Natural product-based drug discovery offers diverse and structurally complex scaffolds that can provide unique leads. Nature has proven to be a rich reservoir for bioactive compounds, leading to numerous advances in human health, for example, the discovery of penicillin from *Penicillium* species. While natural products are better known for their antibacterial, antifungal, and anticancer activity, we are interested to explore more subtle activities, for example, compounds that might address the gap in treatment for neurodegenerative diseases. Neurodegenerative diseases, such as Alzheimer's and Parkinson's, are an emerging public health concern with an estimated 50 million people worldwide currently affected. We have been investigating the activity and mechanism of a series of chemically-related fungal natural products. After a 72 h treatment of primary cortical neuronal cultures, these compounds stimulated neurite outgrowth and displayed neuroprotective effects at nM concentrations. We hypothesize that these compounds work through a neurotrophic mechanism and on-going work aims to test this hypothesis.



Disclosures: D. Kukje Zada: None. **E. Brown:** A. Employment/Salary (full or part-time):; Kapoose Creek Bio, Vancouver, BC.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.23/C137

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: MITACS
Kapoose Creek Bio, Vancouver, BC, Canada

Title: Fungal-derived small molecule promotes neuritogenesis and synaptogenesis in vitro: potential therapeutic implications for neurological disorders

Authors: *T. BHANDO[1], E. BROWN[2,3], *T. BHANDO[2];
[1]Biochem. and Biomed. Sci., [2]McMaster Univ., Hamilton, ON, Canada; [3]Kapoose Creek Bio, Vancouver, ON, Canada

Abstract: Neurological and neuropsychiatric disorders encompass a wide array of conditions that profoundly impact millions globally, affecting both physical and mental health. These include neurodegenerative disorders like Alzheimer's and Parkinson's disease, neurotrauma such as spinal cord injuries, and neuropsychiatric conditions like Major Depressive Disorder (MDD). Central to many of these conditions is the dysregulation of neurotrophins—nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF)—which are crucial for neuronal survival, development, and synaptic connectivity. Neurotrophins and their receptors present a promising therapeutic avenue for treatment of a broad spectrum of neurological and neuropsychiatric disorders. However, traditional approaches using exogenous neurotrophins face challenges such as instability, poor permeability across the blood-brain barrier (BBB), and side effects. This has spurred interest in small molecules that can enhance endogenous neurotrophin production or modulate receptor signaling. We present our research on a small molecule, KCB100, which exhibited strong neurotrophic and neuritogenic effects *in vitro*. KCB100 displayed enhanced neuroprotection and neurite outgrowth in a dose-dependent manner in the concentration range 10 nM-1 µM in various neuronal models, including hippocampal and dorsal root ganglion (DRG) sensory neurons. Importantly, KCB100 promoted synaptogenesis and neuroplasticity in mature neurons, offering potential therapeutic benefits for cognitive and functional recovery. Additionally, KCB100 showed potential for addressing mitochondrial dysfunction, a common feature in these disorders, by enhancing the number of active mitochondria in mature neurons. Further, KCB100 exhibited permeability in the *in vitro* BBB Parallel artificial membrane permeability assay (PAMPA) assay, indicating its potential to reach brain tissue effectively. These results highlight the potential of KCB100 as a versatile neurotherapeutic agent. Future studies are underway to elucidate its molecular mechanism of action and assessing its efficacy *in vivo*, aiming to provide a new avenue for the treatment of these complex disorders.

Disclosures: T. Bhando: None. **E. Brown:** A. Employment/Salary (full or part-time):; Kapoose Creek Bio, Vancouver, BC, Canada. **T. Bhando:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.24/C138

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Neuroprotective mechanisms of transcranial magnetic stimulation in rats subjected to the amygdaloid kindling model

Authors: *G. GONZALEZ-GARIBAY[1], L. MARIN-CASTAÑEDA[1], C. MARTÍNEZ ZAMORA[1], A. LOPEZ, Sr.[1], S. VIDAL[1], H. M. ROMO-PARRA[2], M. RUBIO OSORNIO[1], C. R. OSORNIO[1];
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Abstract: Epilepsy is a highly prevalent neurological condition characterized by repeated, unprovoked seizures, affecting approximately 70 million people worldwide. Despite the vast array of therapeutic options, one-third of patients remain unresponsive to conventional treatments. Repetitive Transcranial Magnetic Stimulation (rTMS) has emerged as a new potential non-invasive treatment for epilepsy. This study aims to elucidate the neuroprotective effects that Repetitive Transcranial Magnetic Stimulation confers on apoptosis secondary to epilepsy pathophysiology. For this experiment, a group of twenty-five male Wistar rats was divided into five groups: Control, Sham/Kindling, Kindling, Kindling/TMS, and Sham/TMS. After the experiment was completed, rats were sacrificed, and brain tissue was collected for caspase-3 and Bcl-2 quantification in the cerebellum, hippocampus, amygdala, and cortex. Total protein quantification was performed with Western blot and immunohistochemistry techniques. Biostatistical analysis was performed using SPSS software, and quantification of Bcl-2 and caspase-3 was conducted with the help of ImageJ software. Rats subjected to real Transcranial Magnetic Stimulation treatment showed a 75% decrease in postdischarge activity compared to the Sham/TMS group. Statistical analysis revealed a considerable and statistically significant increase in the protective factor Bcl-2 ($p < 0.0003$) in the groups receiving repetitive transcranial magnetic stimulation. The increase in Bcl-2 and the decrease of Caspase-3 levels in the cerebellum, hippocampus, amygdala, and cortex suggest apoptosis mitigation. This compelling evidence calls for further investigations into the intricate mechanisms underlying rTMS's ability to mitigate apoptosis, alleviate glutamate excitotoxicity, and reduce postdischarge duration within epileptogenic zones.

Disclosures: G. Gonzalez-Garibay: None. **L. Marin-Castañeda:** None. **C. Martínez Zamora:** None. **A. Lopez:** None. **S. Vidal:** None. **H.M. Romo-Parra:** None. **M. Rubio Osornio:** None. **C.R. Osornio:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.25/C139

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Wireless dual-channel passive neural stimulator for electroceutical pain management

Authors: *J. YU, C. KOH, H. JUNG;
Dept. of Neurosurg., Yonsei Med. Gamma Knife Ctr., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: The conventional approach to pain alleviation relies on deep brain stimulation systems, where an implantable pulse generator (IPG) powered by a battery delivers electrical pulses via an electrode to target neurons. However, the reliance on batteries results in bulky devices with limitations in implantation options. Attempts to solve these issues including external battery removal devices, yet they still require complex circuitries with numerous active components. Recently, passive neural stimulators without batteries or active components have emerged, promising reduced size and complexity. In this study, we propose a dual-channel passive stimulator utilizing a single-tapped coil, operating wirelessly through inductive links between the skull and dura of the rat. The experiment design incorporates a compact device for full implantation between the skull and dura of rats. Stimulus parameters, such as current amplitude and repetition rate, are optimized to alleviate pain effectively. The dual-channel stimulator utilizes two carrier frequencies for modulation and inductive delivery. Tuning capacitors and tapping inductors are employed for frequency selectivity, ensuring stimuli are delivered precisely. A three-layered coil design maximizes inductance while minimizing parasitic capacitance. The system's performance is validated through simulations and measurements, ensuring resonance and efficiency. Output voltage monitoring demonstrates selective stimulation of motor cortex regions. Animal behavior studies confirm the stimulator's efficacy in pain alleviation, validating its potential for in vivo applications. By strategically tapping the coil, it resonates at two frequencies (1.5 MHz and 11.5 MHz), enabling channel selection based on input carrier frequency. Using this miniaturized stimulator, measuring 9 x 1.3 x 3.4 mm^3 and weighing 0.451 g, we achieve high link efficiency and offers effective pain modulation through motor cortex electrical stimulation in a chronic pain rat model. Before stimulation, the pain threshold was measured at 0.59 ± 0.20 g, and following the stimulation, it increased to 12.23 ± 2.9 g (*$p<0.05$). This research represents a significant advancement in the development of compact and efficient neural stimulators for chronic pain management, offering potential benefits over conventional battery-powered systems.

Disclosures: J. Yu: None. **C. Koh:** None. **H. Jung:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.26/C140

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: RS-2022-00141392

Title: Ultrasoft neural probe for deep brain stimulation to treat Parkinson's disease

Authors: *M. SEUNG HYUN[1], W. MUN[1], Y. KWON[2], C. KOH[1], J.-U. PARK[2], H. JUNG[1]; [1]Dept. of Neurosurg., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [2]Dept. of Materials Sci. and Engin., Yonsei Univ., Seoul, Korea, Republic of

Abstract: Introduction Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta, leading to a reduction in striatal dopamine. This loss causes various motor symptoms that worsen over time. Traditional therapies often lose their effectiveness, necessitating the exploration of new treatment modalities. Deep brain stimulation (DBS), involving the implantation of microelectrodes deep within the brain to regulate abnormal neural activities, is a clinical approach used to alleviate the symptoms of PD. However, the stiff metal electrodes currently in use can cause physical damage and glial scarring. This study investigates the use of Gallium-Indium eutectic (EGAIn) based liquid metal electrodes, which are ultrasoft and closely mimic the mechanical properties of brain tissue, such as Young's modulus. These properties potentially enable more effective and longer-lasting neuromodulation, as demonstrated in a rat model of Parkinson's disease.
Method The PD model was established by administering desipramine intraperitoneally, followed by injecting 6-OHDA into the Substantia Nigra (AP: -5.0mm, ML: +2.0mm, DV: -7.5mm). On the 16th postoperative day, soft liquid metal electrodes were implanted into the subthalamic nucleus (STN) at coordinates AP: -3.7mm, ML: +2.5mm, DV: -8.2mm. Stimulation was conducted for one hour daily over five consecutive days using the STG 4004. The effectiveness of the treatment was assessed using a rotarod and a rotameter to measure motor symptom levels.
Result DBS with soft liquid metal electrodes improved motor functions in PD models as seen in rotarod and rotameter tests. In the rotarod test, the control group scored 157.6 ± 42.42 seconds, significantly higher than the PD group's 30.63 ± 14.09 seconds (*$p<0.05$). After five days of DBS, the PD group's performance increased to 124.5 ± 21.23 seconds (*$p<0.05$). In the rotation test, the PD group's involuntary rotations significantly reduced from 41.33 ± 12.41 seconds to 2.66 ± 2.66 seconds after stimulation (**$p<0.01$), demonstrating a substantial decrease in motor symptoms.
Conclusion This study introduces a novel electrode system for DBS in Parkinson's disease. Using a PD animal model, it was shown that DBS with liquid metal electrodes effectively addresses motor symptoms of the disease. Liquid metal electrodes thus represent a promising alternative in the advancement of treatment technologies for Parkinson's disease.

Disclosures: M. Seung Hyun: None. **W. mun:** None. **Y. Kwon:** None. **C. Koh:** None. **J. Park:** None. **H. Jung:** None.

Poster

PSTR396

Neuroprotective Mechanisms: Preclinical Therapeutic Discovery

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.27/C141

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: RS-2022-00141392

Title: A biocompatible brain-interfaced electrode using laser-assisted structuring Graphene film with liquid crystal polymer

Authors: *C. KOH[1], H. JUNG[2];
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Abstract: Introduction: Biological electrodes can selectively record neuronal signals or detect biomolecules and stimulate specific regions to replace or recover damaged sensory or motor neurons. Graphene is one of the materials commonly used in the research of biological electrodes due to its high electrical conductivity, mechanical strength, and biocompatibility. However, obtaining graphene can be time-consuming or require complex methods, making it challenging to produce in large quantities. Recently, laser-induced graphene (LIG) obtained by applying a laser to polyimide has gained significant attention. LIG-based biological electrodes offer the advantage of easily obtaining desired patterns using flexible polymers as substrates. We have developed brain interface biological electrodes by patterning LIG on liquid crystal polymer (LCP), one of the biocompatible polymers, demonstrating the potential for next-generation biological electrodes. Methods: We formed LIG by applying a 2.25 W, 450 nm UV laser to LCP films and manufactured the LIG/LCP biological electrodes for brain interfaces. To evaluate the developed LIG/LCP biological electrodes for brain interfaces, we conducted cortical electrical stimulation and recording on anesthetized Sprague-Dawley rats. Results: The electrical stimulation was conducted through the LIG/LCP electrodes, placed on the rat's motor cortex, and the current was applied to induce movements in the rat's contralateral hind limb. The displacement of toes and heels relative to the rest positions, as temporally aligned by the onset of stimulation, demonstrates an increasing magnitude of movements induced by stronger stimulation. Additionally, electrodes were placed on the rat's barrel cortex to measure cortical signals induced by whiskers touching. Recorded signals exhibited typical slow-up-down dynamics with oscillations associated with a sleep-like state of rats induced by urethane anesthesia, and touch-induced activities during stimulated period 20-30s with high-frequency oscillations from the channels at the corresponding whisker area were observed. Conclusion: With their high biocompatibility and versatility, LIG electrodes hold immense potential across various fields. As brain interfaces, they are poised to revolutionize biomedical areas thanks to their superior electrochemical performance compared to conventional electrodes. This potential excites the scientific community and opens up new horizons for advancements in medical technology and treatment methodologies, inspiring a future where LIG electrodes play a pivotal role in improving human health.

Disclosures: C. Koh: None. **H. Jung:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.01/C142

Topic: C.09. Stroke

Support: Canadian Institutes for Health Research
Heart and Stroke Foundation of Canada
Motyl Graduate Studentships in Cardiac Sciences
David Lawson Graduate Scholarship

Title: Futile Recanalization in Ischemic Stroke: Insights, Mechanisms, and Therapeutic Strategies

Authors: *S. ABBASI-HABASHI[1], Y. MA[2], G. JICKLING[3], I. R. WINSHIP[4];
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Abstract: Acute ischemic stroke presents a significant healthcare challenge and despite treatment advances, many patients still have devastating outcomes. A key therapeutic goal is to restore blood flow to, or reperfuse, ischemic brain tissue. Yet, even with successful vessel recanalization, complete reperfusion of brain tissue and positive outcomes are not always attained, resulting in "futile recanalization." We hypothesize that collateral and microcirculatory failure contribute to this phenomenon. Here, we employed a transient filament-induced middle cerebral artery occlusion (MCAo, 60 min occlusion) in mice to mimic large vessel occlusion observed in humans. High-resolution *in-vivo* imaging of collaterals and capillary blood flow enabled us to quantitatively analyze collateral and microvascular failure post-MCAo. Two-photon laser scanning microscopy data reveal a progressive decline in cerebral collateral perfusion, correlated with infarct size and blood biomarkers. Our findings suggest that inadequate collateral flow before recanalization may contribute to futile recanalization. Moreover, despite complete recanalization of the MCA, large areas of the capillary bed do not reperfuse, indicating microcirculatory dysfunction. Neutrophil accumulation in ischemic capillaries and improved flow post-neutrophil depletion suggest neutrophil adhesion plays a role in microcirculatory failure. Enhancing collateral flow and modulating neutrophil activity could prevent futile recanalization and improve stroke outcomes. Defining biomarkers associated with poor outcomes and developing novel interventions targeting neutrophil activation could enhance recanalization therapies. Here, we probed blood biomarkers associated with enhanced neutrophil stalls and tested novel pharmacotherapies that target phosphodiesterase (PDE) signaling as a stroke treatment. Ibudilast, a PDE inhibitor, regulates inflammation and enhances brain blood flow, was tested as an agent to reduce ischemic injury and improve recanalization outcomes. Our initial data indicated that the administration of Ibudilast enhanced blood flow, alleviated capillary stalls, and mitigated neutrophil adhesion in our mouse model, ultimately resulting in improved microvascular flow, reduced infarct size, and enhanced stroke outcomes. Blood biomarker assays and RNA measurements were conducted in mice treated with either vehicle or Ibudilast. The analysis of inflammatory markers and investigation of neutrophil gene expression

profiles associated with PDE signaling activation will provide valuable insights into biomarkers of and treatments for futile recanalization.

Disclosures: S. Abbasi-Habashi: None. **Y. Ma:** None. **G. Jickling:** None. **I.R. Winship:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.02/C143

Topic: C.09. Stroke

Title: Accelerated brain ageing after stroke: a marker for secondary post-stroke degeneration and its relevance for motor outcome

Authors: *R. TAKYI[1], S. CHARRON[2], M. A. MAIER[4], J.-C. BARON[5], C. ROSSO[6], C. DEBACKER[3], P. G. LINDBERG[7];
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Abstract: Brain age, distinct from chronological age, has been shown to be increased post-stroke and may be related to poor sensorimotor outcomes. High brain age in stroke might be attributed to early life conditions and lifestyle factors, as well as to pathophysiological processes occurring post-stroke. However, longitudinal studies tracking brain age after stroke are lacking. We hypothesized that brain ageing would accelerate over the first months post-stroke, reflecting secondary grey and white matter degeneration, and that this would relate to upper limb sensorimotor outcome. We retrospectively analysed T1-weighted MRI and clinical motor function at two time-points: at 3 weeks (T1, baseline) and 3-6 months (T2) post-stroke. Brain age was computed using a validated Gaussian regression model and the difference to chronological age was calculated (brain age gap). Three lesion masking approaches were compared to control for lesion effects on brain age. Voxel-based morphometry was used to investigate brain structures undergoing secondary post-stroke degeneration. Voxel-based lesion symptom mapping was used to identify lesion location related to accelerated brain age. 114 patients with first-ever stroke and arm/hand hemiparesis were included from three studies. Brain age gap was

0.48±1.30 years at 3 weeks and 4.09±1.44 years at 3-6 months post-stroke. ANOVA showed a significant main effect of time post-stroke ($F(1, 113)=36.681$, $P<0.001$) and masking method ($F(1.692, 191.193)=28.477$, $P<0.001$): brain age gap decreased successively from lesion masking, over no masking, to lesioned hemisphere masking. Lesion to internal capsule, and corona radiata predicted accelerated brain ageing. Patients with increasing brain age gap had significantly reduced grey matter volume and white matter volume, distant to the stroke lesion confirming secondary degeneration. Change of brain age gap, but not chronological age, was associated with motor outcomes in the sub-acute to chronic phase: upper limb Fugl-Meyer Assessment scores ($B=-1.403$, $P=0.001$), maximum grip strength ($B=-0.024$, $P=0.001$), and index-thumb dexterity assessment ($B=-0.018$, $P=0.004$). Change in brain age gap was, however, not a significant outcome predictor when baseline motor scores were included. These findings demonstrate accelerated brain ageing in non-lesioned neural tissue most pronounced in the ipsilesional hemisphere in the months following stroke. This secondary neurodegeneration was negatively related to motor outcome. Brain age may be a valid whole-brain probe of individual secondary post-stroke degeneration, relevant for recovery prediction and for neural plasticity targets.

Disclosures: R. Takyi: None. **S. Charron:** None. **M.A. Maier:** None. **J. Baron:** None. **C. Rosso:** None. **C. Debacker:** None. **P.G. Lindberg:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.03/C144

Topic: C.09. Stroke

Support: Canadian Institutes of Health Research (CIHR) / Project Grant PJT-166176 (PI LAB)

Title: Characterizing interneuronal activation in the primary motor cortex after stroke

Authors: *A. RAJENDRAN[1], R. DENYER[2], C. RUBINO[3], L. A. BOYD[4];
[1]Neurosci., Univ. of British Columbia, Vancouver, BC, Canada; [2]Inst. of Neurosciences, Univ. catholique de Louvain, Brussels, Belgium; [3]Rehabil. Sci., Univ. of British Columbia, Vancouver, BC, Canada; [4]Physical Therapy, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Interneuronal networks in the primary motor cortex (M1) are critical in planning, preparation, and execution of movement. Distinct interneuronal networks in M1 can be indexed by analyzing the latency of motor evoked potentials (MEP) generated by directional transcranial magnetic stimulation (TMS). Stimulation in the anterior-posterior (AP) direction results in longer latency MEPs as compared to stimulation in the posterior-anterior (PA) direction. Furthermore, activation of the corticospinal tract (CST) can be measured with pulses in the lateral-medial

(LM) direction. Variance in MEP response latency after stimulation is attributed to differences in synaptic distance between interneurons and CST neurons.
The objectives of the current study were to: 1) characterize the effect of stroke on the latency of PA and AP elicited MEPs, and 2) assess whether the degree of change in MEP latency relates to changes in arm function after stroke. Single pulse TMS was delivered over contralesional and ipsilesional M1 in the LM, PA, and AP directions in 21 individuals with chronic (> 6 months) stroke (n=21, 5 female, mean age = 66.90 ± 13.43). Network onset times were indexed by subtracting mean MEP onset times in the LM direction from the AP and PA directions. The Wolf-Motor Function test (WMFT) quantified arm motor function in the upper limbs.
Results from a linear mixed effects regression demonstrated a significant interaction between hemisphere and TMS stimulation direction on onset time. Estimated marginal means calculations revealed that AP-LM onset latency was significantly higher than PA-LM in both hemispheres (Contralesional hemisphere: $\mu = -1.87 \pm 0.29$, $p<0.0001$; ipsilesional hemisphere: $\mu = -1.03 \pm 0.29$, $p = 0.0053$). Importantly, AP-LM onset latency was significantly higher in the contralesional hemisphere as compared to the ipsilesional hemisphere ($\mu = -1.18 \pm 0.43$, $p<0.045$). Furthermore, linear regression results showed that 21% of variation in the degree of arm function in the impaired arm as compared to the less impaired, was related to differences in AP-LM onset latency between hemispheres (F (1,18) = 4.74, $p = 0.043$).
Findings suggest that AP-LM onset latency, reflective of polysynaptic activation of the CST, is altered post-stroke and longer onset times in the AP direction are related to better motor function. These data uncover a previously unknown relationship between interneuron pools in the human motor cortex and motor function after stroke.

Disclosures: A. Rajendran: None. **R. Denyer:** None. **C. Rubino:** None. **L.A. Boyd:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.04/C145

Topic: C.09. Stroke

Support: NIH Grant NS132778-01
NIH Grant NS132778-01S1

Title: Lrp1 regulates the expression of cxcr4 in neural stem cells to modulate migration to ischemic stroke lesions

Authors: *D. LOZANO[1], K. DIETERT[2], M. WANG[2], S. SPRAGUE[3], P. REED[3], E. KOKOVAY[1], N. L. SAYRE[3];
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Abstract: Strokes are the fifth leading cause of death and the primary cause of long-term disability within the United States. Remedies for stroke patients are limited, however, after injury, the brain has an endogenous repair response in the form of neural stem cells (NSCs) which can be utilized to enhance post-stroke healing. After a stroke, the ischemic lesion secretes several signaling factors including stromal-derived factor 1 (SDF1), which drives chemokine receptor CXCR4-mediated migration of NSCs to the stroke injury. We have previously shown that low-density lipoprotein receptor-related protein 1 (LRP1) was a novel regulator for CXCR4 in adult NSCs, as knockout caused reduced migration toward stroke lesions *in vivo* and SDF1 gradients *in vitro.* We aim to investigate the mechanism by which LRP1 regulates CXCR4 in NSCs to facilitate migration to the site of injury during an ischemic stroke. LRP1 is a multifaceted receptor that can function through various pathways including endocytosis and trafficking, co-transcriptional regulation, and signal mediation. We hypothesize that LRP1 mediates CXCR4 expression through transcriptional regulation, which we will evaluate via testing the expression of LRP1 domain-specific constructs in NSCs *in vitro* to determine if any rescue the expression of CXCR4. Data generated will mechanistically explore the relationship LRP1 has with CXCR4 to provide a novel understanding of NSC migration in the context of ischemic stroke and in cellular migration in general.

Disclosures: D. Lozano: None. **K. Dietert:** None. **M. Wang:** None. **S. Sprague:** None. **P. Reed:** None. **E. Kokovay:** None. **N.L. Sayre:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.05/C146

Topic: C.09. Stroke

Support: Veterans Affairs Grant

Title: Functional connectivity changes in areas associated with reaching behavior following subcortical stroke.

Authors: *G. HADDADSHARGH[1,2,3,4], J. MAK[5,2,6,3], A. BOOS[1,2,6], F. LIU[1,6], X. FANG[7,2,6], G. F. WITTENBERG[7,2,6,3,8];
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Abstract: Stroke often leaves survivors with severe motor impairments due to disrupted neural pathways necessary for motor control. While brain plasticity can theoretically improve motor

function post-stroke, recovery is typically incomplete, and there is a need for more effective interventions and optimization of rehabilitation methods. Resting-state functional magnetic resonance imaging(rsfMRI) is a powerful tool to measure brain connectivity, offers insights into the brain's adaptation post-stroke and guides the development of targeted rehabilitation strategies. Some of these strategies include target stimulation of brain areas with potential for more integration into motor control circuitry.In this study, we focus on the functional connectivity changes in key brain regions associated with movement planning and reaching, including the bilateral dorsal premotor cortex (PMd), ventral premotor cortex (PMv), posterior parietal cortex (PPC), and the primary motor cortex (M1). We investigated functional connectivity in 30 subjects (20 healthy controls, 10 with internal capsule stroke) using 3T Siemens Prisma MRI. Data were acquired for two 9-minute sessions with eyes open and preprocessed using the FMRIB Software Library (FSL). Regions of interest (ROIs) were defined using 7 mm spheres centered on individual anatomical locations of these key regions to extract time series signals. Connectivity measures, including Pearson correlation, partial correlation, and mutual information, quantified connections within and between the ROIs. Differences were statistically evaluated with the Wilcoxon rank-sum test and adjusted for multiple comparisons using the False Discovery Rate (FDR) method, set at $p < 0.05$. Controls showed uniform, strong connectivity across all examined regions. Stroke survivors showed significant interhemispheric connectivity reductions between bilateral PPC, PMv, and M1. Within each hemisphere, connectivity between PMd - M1 and PMv - M1 was notably lower in stroke patients, affecting interhemispheric and intrahemispheric connections. Despite the pattern of overall connectivity decreases, there was evidence of slight increases, in some connection possibly reflecting post-stroke plasticity, some of which could underly recovery. These findings underscore the alterations in neural networks important for motor function, explaining persistent deficits in stroke survivors. This study supports the potential of rsfMRI to inform customized rehabilitation strategies based on individual neural recovery profiles.

Disclosures: G. Haddadshargh: None. **J. Mak:** None. **A. Boos:** None. **F. Liu:** None. **X. Fang:** None. **G.F. Wittenberg:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.06/C147

Topic: C.09. Stroke

Support: Medical Research Council, UK

Title: Blood-brain barrier junctional protein as potential biomarker in differentiating ischaemic stroke patients from stroke mimics.

Authors: P. KAKKAR[1], M. ALMUSINED[1], T. KAKKAR[2], T. MUNYOMBWE[1], L. MAKAWA[3], K. KAIN[4], A. HASSAN[3], ***S. SAHA**[1];
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Abstract: Stroke is the second leading cause of death and the third leading cause of disability worldwide. Stroke is diagnosed by neurological scores combined with brain imaging assessments. Despite advances in the field of brain imaging, potential limitations such as lengthy procedures, costly equipment and image analysis variations have hampered their efficient use in stroke diagnosis. A biomarker test to discriminate between stroke and stroke mimic will substantially impact stroke management and treatment throughout the patient care pathways. In preclinical ischaemic stroke model we previously observed that glial fibrillary acidic protein (GFAP), an astrocytes specific protein and the blood-brain barrier tight junctional protein, zonula occludens-1 (ZO-1) expressions in the hippocampus of stroke mice were decreased significantly compared to sham-operated mice. Increased level of GFAP and ZO1 in blood serum was observed in ischaemic mice compared to sham-operated mice suggesting that this protein can be a potential biomarker for determining ischaemic stroke. The present study examines several circulating biomarkers including ZO1 in acute stroke patients compared to stroke mimics.
This study recruited 66 patients with ischaemic stroke and 24 stroke mimics presenting to Leeds Teaching Hospitals NHS Trust, UK within 2 days of the event. The levels of potential stroke biomarkers e.g., GFAP, neuron-specific enolase (NSE), and ZO-1 in blood serum obtained from stroke patients and stroke mimics were measured by enzyme-linked immunosorbent assay (ELISA). Biomarker levels in stroke patients and stroke mimics were compared using the Mann–Whitney U test. Multivariable logistic regression was used to evaluate the role of blood biomarkers in combination with the National Institutes of Health Stroke Scale (NIHSS) to differentiate between ischaemic stroke patients and stroke mimics.
The results showed a significant difference in circulating GFAP (mean difference = 0.240, 95% CI: 0.079-0.400; $p<0.0001$) and ZO-1(mean difference = 1.449, 95% CI: 1.003-1.895; $p<0.0001$) but not NSE (mean difference = 1.539, 95% CI: 1.516-4.596; $p=0.07$) in stroke patients compared to stroke mimics. The results also suggest that a combination of ZO-1 with NIHSS gives a higher diagnostic accuracy of 89.41 (95% CI: 80.85-95.04) in differentiating ischaemic stroke patients from stroke mimics as compared to NIHSS only (Accuracy: 47.06, 95% CI: 36.13-58.19).In conclusion, our results suggest that a combination of NIHSS and ZO-1 level provides a better prediction model to differentiate ischaemic stroke patients from stroke mimics.

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Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.07/C148

Topic: C.09. Stroke

Title: Beyond One Size Fits All: Optimizing Brain Registration Techniques in Perinatal Stroke

Authors: *G. MILLER[1], C. STEEBY[1], K. ALHADID[2], C. CHUI[2], A. SULLIVAN[2], P. L. MUSOLINO[2], A. L. COHEN[3];
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Abstract: Studying the relationship between behavioral outcomes and lesion connectivity is a key approach in understanding brain networks. An important step in this process is registration: aligning patients' brains to a standard space to allow accurate group comparisons. This step, however, can present a challenge in patients with lesions and associated extra-lesional brain distortion, such as in chronic perinatal stroke. Here, we compare the performance of different registration software packages in registering chronic perinatal stroke.
Data from 11 patients with perinatal stroke were registered to an MNI template using three algorithms (ANTs, FNIRT, EasyReg) with their default lesion compensation techniques (e.g., cost-function masking). To compare methods, we segmented the warped patient brains, as well as the MNI template, with FreeSurfer-based ROIs then calculated the dice similarity scores between them. We also calculated similarity scores between warped and manually drawn lesion masks. We then used these scores to conduct two-way ANOVAs examining the effect of method and lesion size. We also examined ways to further improve the algorithms, including the addition of brain grafting, and compared software versions and transform types.
We found a significant main effect of method ($F(2, 719)=13.924$, $p<0.00001$), where FNIRT performed worse than ANTs and EasyReg, and lesion size ($F(2,719)=54.222$, $p<0.00001$), where larger lesions caused poorer registration. Interestingly, when comparing the resultant lesion masks to those hand-drawn by trained researchers, there was no significant effect of method or lesion size. Given its superior performance compared to FNIRT and flexibility compared to EasyReg, ANTs was selected for optimization. Additionally, the regular version of ANTs (1-2 hour runtime), compared to the "Quick" version (5-15 minute runtime), resulted in significantly better registrations ($t(10)=5.444$, $p=0.0003$). Within ANTs, the use of b-spline normalization did not lead to significant improvements.
In conclusion, the registration of chronic perinatal stroke imaging to a standard atlas remains a challenge; however, for brains with larger lesions, EasyReg and regular ANTs appeared to perform better than FNIRT. Nonetheless, without further optimization, none of the current best-in-class software packages produce a reliable registration without manual correction. However, the ever-increasing accessibility of new methods, like deep learning, show promise for future improvement.

Disclosures: G. Miller: None. **C. Steeby:** None. **K. Alhadid:** None. **C. Chui:** None. **A. Sullivan:** None. **P.L. Musolino:** None. **A.L. Cohen:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.08/C149

Topic: C.09. Stroke

Support: RF1NS119872
Woodnext Foundation

Title: Intraperitoneal IGF1 treatment repairs the gut epithelium in the acute phase of stroke and attenuates cognitive behaviors and secondary neurodegeneration in the chronic phase in middle-aged female rats

Authors: *K. A. PICKLE[1], F. SOHRABJI[2], Y. EL-HAKIM[3];
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Abstract: Our previous studies showed that intracerebroventricular (ICV) infusion of IGF1 was neuroprotective in the acute phase of stroke but did not reduce peripheral inflammation or improve cognitive function in the chronic phase. Due to the severe effect of stroke on gut dysmorphology, and evidence that IGF-1 has beneficial effects on the gut epithelium, we investigated whether intraperitoneal (IP) injection of IGF1 would be more effective in attenuating stroke-induced cognitive impairment. Acylic female rats (9-11 mos) were subjected to endothelin-1 induced MCAo or sham operation. Animals received IP injections of IGF1 (IP-IGF1) at 4h and 24h post MCAo or ICV infusions of IGF-1. Controls received vehicle injections/infusions. Animals were tested on the Barnes Maze test and Novel Object recognition test at 30 days after stroke. Thereafter animals were terminated and their brains assessed for volumetric changes. MCAo impaired performance on both NORT and the Barnes maze, however, this impairment was attenuated in the IP-IGF1 treated animals but not in ICV-IGF1 treated groups. Volumetric analysis in the chronic phase showed that the volume of the ischemic hemisphere was significantly reduced compared to the non-ischemic hemisphere in the ICV-Vehicle and ICV-IGF1 groups. Similarly, both groups had enlarged ventricles in the ischemic hemisphere. No differences were observed in the width of the corpus callosum. In the IP-study, the volume of the ischemic hemisphere was not significantly different from the non-ischemic hemisphere in either group, however, ventricular enlargement was seen in the IP-vehicle group, but not in the IP-IGF1 or sham groups. Moreover, the width of the midline corpus callosum was reduced in the IP-Vehicle group, but not the Sham or IP-IGF1 group. These data show that improved cognitive performance in the IP-IGF1 is associated with preservation of the gray and white matter structures, suggesting that systemic IGF1 may be a better therapeutic option for long term cognitive behaviors after stroke. Since IP-IGF1 reduces peripheral inflammation, it is likely that brain infiltration of peripheral cytokines is reduced, thus preserving the brain from significant atrophy. Overall, targeting peripheral structures such as the gut presents a novel approach to enhancing brain health in aging females.

Disclosures: K.A. Pickle: None. **F. Sohrabji:** None. **Y. El-Hakim:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

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Program #/Poster #: PSTR397.09/C150

Topic: C.09. Stroke

Support: Simons Foundation Bridge to Independence Award

Title: Perinatal Strokes Associated with Autism Spectrum Disorder Impact Distinct Brain Networks Compared to Non-Autistic Peers

Authors: *C. J. STEEBY[1], N. SHEIKHI[1], S. TRIPATHY[1], G. N. MILLER[1], C. WU[1], A. L. COHEN[2], L. LEHMAN[1];
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Abstract: Recent research from our institution suggested that children with venous, rather than arterial, perinatal stroke are three times likelier to develop autism spectrum disorder (ASD). Here, we analyze the same patient cohort to identify whether this finding is explained by differences in affected brain networks.
Data from 40 patients with perinatal stroke were identified from a retrospective registry, half with an ASD diagnosis and half without. Groups were matched on sex, stroke subtype, and lesion size. Lesions were manually segmented, then registered to a standard template. We performed a voxel-wise lesion symptom mapping (VLSM) analysis to identify any relationship between lesion location and ASD diagnosis. Next, we performed a lesion network mapping (LNM) analysis where we generated maps of functional connectivity for each lesion by leveraging resting state data from 1000 healthy nine-year olds. We then statistically compared patterns of lesion connectivity between groups controlling for stroke subtype and lesion size. Finally, we conducted a logistic regression to assess the ability of connectivity to identified ROIs to predict ASD diagnosis.
VLSM analysis identified a significant association between ASD diagnosis and lesions intersecting voxels in the left temporoparietal junction (lTPJ), implicated in theory of mind, when controlling for stroke subtype (FDR, $p<0.05$). LNM analysis identified distinct functional connectivity in ASD in this same region, extending through the middle temporal gyrus to the anterior temporal lobe, in the cerebellum, and in the ventromedial prefrontal and posterior cingulate cortex, regions associated with the default mode network and social cognition (FDR, $p<0.05$).Our logistic regression model explained 28.3% of the variance in diagnosis, where lesions associated with ASD were more correlated with connectivity to the lTPJ ($p=0.007$). When controlling for ASD, LNM analysis by stroke subtype found a significant difference in

connectivity to the left superior temporal lobe, part of the middle cerebral artery territory (FDR, $p<0.05$).
These findings demonstrate that lesions in and/or functionally connected to the lTPJ are associated with ASD diagnosis, regardless of stroke subtype. This suggests the observation that children with venous perinatal strokes are more likely to develop autism is driven by distinct and biologically meaningful differences in affected brain networks, such as those implicated in theory of mind and the default mode network. These findings support that a specific neuroanatomical substrate injury may underlie the development of ASD.

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Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.10/C151

Topic: C.09. Stroke

Title: Bilateral Prefrontal Cortex Activation During Sensorimotor Conditions in People With Subacute Stroke - An Exploratory fNIRS Study

Authors: *S. LUAN, S. KOHLI, S. PETERS;
Western Univ., London, ON, Canada

Abstract: Following a stroke, damage to the prefrontal cortex (PFC) can disrupt the neural circuits involved in motor control and sensorimotor integration, which can contribute to impairments in ankle sensorimotor functions, and other areas. In this study, we aim to use functional near-infrared spectroscopy (fNIRS) to explore whether PFC activation differs during various sensorimotor conditions, namely active and passive dorsiflexion/plantarflexion and light touch somatosensory stimulation (SS) of the paretic ankle in people with subacute stroke. Our secondary objectives are to evaluate differences between hemispheres and the relationship between interhemispheric asymmetry and functional outcomes. To measure cortical activity, we placed the fNIRS devices over the bilateral PFC in 9 participants. Hemodynamic responses were collected during three conditions: active and passive dorsiflexion/plantarflexion and SS. Sensorimotor function and interhemispheric asymmetry was assessed using Fugl-Meyer Lower Extremity Assessment (FMLE) and laterality index (LI), respectively. Our results showed no statistically significant differences in PFC activation between conditions, nor between hemispheres. However, there was a statistically significant relationship between interhemispheric asymmetry for SS for deoxygenated hemoglobin and the sensory portion of the FMLE ($p=0.01$). Our results highlight that various ankle sensorimotor functions may not generate different levels of PFC activation in a single hemisphere. However, the balance of activation between the

hemispheres from somatosensory input may be related to clinical sensorimotor function. We believe if PFC activation asymmetry is related to clinical function, it could then potentially be a useful neuroimaging predictor of recovery. Future stroke research should include consideration of somatosensory function.

Disclosures: S. Luan: None. **S. Kohli:** None. **S. Peters:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.11/C152

Topic: C.09. Stroke

Title: Exploring Fate of Myelin After Subarachnoid Hemorrhage

Authors: A. S. REGNIER-GOLANOV[1], N. HASSAN[1], H. CHUONG[1], R. CHANDRASEKARAN[1], N. KVIRKVELIA[1], E. V. GOLANOV[1], ***G. BRITZ**[2]; [1]Neurosurg., The Methodist Hosp. - Res. Inst., Houston, TX; [2]The Methodist Hosp. - Res. Inst., Houston, TX

Abstract: Subarachnoid hemorrhage (SAH) survivors have seen improved survival rates, but they often face long-term cognitive impairments. Up to 95% of SAH survivors experience learning and memory deficits, hindering their return to work. Currently, the mechanisms behind these cognitive impairments remain unclear. Our laboratory used transcriptomics analysis of the hippocampus in a mouse model replicating SAH-induced cognitive damage observed in humans. We identified a downregulation of genes related to myelin and axons. In this study, we aimed to validate these findings using fluorescent hybridization in situ (FISH) at 24 hours. Naïve brains were processed as per the manufacturer's instructions, whereas SHAM and SAH brains were further processed for immunohistochemistry. We conducted RNAscope FISH for *Mbp* (Myelin basic protein) and *Mog* (myelin oligodendrocyte glycoprotein) in naïve mice and performed *Mbp* followed by PDGFRα (pre-myelinating oligodendrocyte marker) immunostaining in SAH and Sham mice on frozen brain tissue. SAH was induced by endovascular perforation of the circle of Willis, while Sham mice underwent identical surgery without perforation. In the cortex (Cx) of naïve mice, *Mbp* messenger staining appeared perinuclear and filament-like, while in the hippocampus (Hpc), it was mostly perinuclear. Of note, Allen Brain atlas doesn’t indicate differences in staining in Cx and Hpc, and others reported that *Mbp* localized in the end terminal of the axonns, near assembly sites. Colocalization of *Mbp* and *Mog* was observed in naïve mice. Notably, *Mbp* and PDGFRα colocalization was detected only in SAH mice. Quantitative analysis revealed a decrease in *Mbp* intensity , confirming our RNAseq data. However, quality control showed reduced *Mbp* intensity in Sham FISH and Sham FISH + IHC compared to naive, indicating variations based on brain processing. This study demonstrated differences in *Mbp*

expression following SAH, depending on brain structure and treatment. Our results underscore the unique patterns observed after SAH, emphasizing the importance of studying myelination processes in its aftermath.

Disclosures: A.S. Regnier-Golanov: None. **N. Hassan:** None. **H. Chuong:** None. **R. Chandrasekaran:** None. **N. Kvirkvelia:** None. **E.V. Golanov:** None. **G. Britz:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

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Program #/Poster #: PSTR397.12/C153

Topic: C.09. Stroke

Support: R01NS115845
NIH P41EB015922
R01NS110696

Title: Brain age is longitudinally associated with mild cognitive impairment, but not sensorimotor impairment, in subacute stroke

Authors: *O. MARIN-PARDO[1], M. H. KHAN[1,2], S. CHAKRABORTY[1], M. R. BORICH[6], M. CASTILLO[7], J. H. COLE[8], S. C. CRAMER[9], E. E. FOKAS[10], N. H. FULLMER[11], J. GUMARANG[11], L. X. HAYES[10], H. KIM[3], A. KUMAR[4], E. A. MARKS[1], E. R. ROSARIO[11], H. M. SCHAMBRA[10], N. SCHWEIGHOFER[5], G. SONG[1], M. TAGA[10], C. J. WINSTEIN[5], Z. ZHENG[11], S.-L. LIEW[1,3];
[1]Chan Div. of Occup. Sci. and Occup. Therapy, [2]Neurosci. Grad. Program, [3]Stevens Neuroimaging and Informatics Inst., [4]Dornsife Col. of Letters, Arts and Sci., [5]Div. of Biokinesiology and Physical Therapy, USC, Los Angeles, CA; [6]Rehabil. Med., Emory Univ., Atlanta, GA; [7]Col. of Hlth. and Human Develop., California State University, Fullerton, Fullerton, CA; [8]Dept. of Computer Sci., Univ. Col. London, London, United Kingdom; [9]Dept. of Neurol., UCLA, Los Angeles, CA; [10]Langone Dept. of Neurol., New York Univ., New York, NY; [11]Res. Inst., Casa Colina Hosp. and Centers for Healthcare, Pomona, CA

Abstract: Brain age, a proxy of global brain atrophy estimated from structural neuroimaging, is associated with sensorimotor function in chronic stroke. However, the relationship between brain age, cognitive, and sensorimotor outcomes after stroke in early subacute stroke (<3 months after onset) is not fully understood. The goal of this work is to investigate associations between stroke survivors' brain predicted age difference (brain-PAD, quantified as a person's predicted brain age minus their chronological age) and measurements of cognitive and motor impairment in subacute stroke. We hypothesized that brain-PAD would be negatively correlated with cognitive scores, such that older-appearing brains would be associated with more severe cognitive impairment. We also hypothesized that cognitive impairment would be correlated with motor

impairment. We conducted a longitudinal study using a multisite dataset of 3D T1-weighted brain structural MRIs and clinical tests for mild cognitive impairment (Montreal Cognitive Assessment [MoCA]) and sensorimotor impairment (Fugl-Meyer Upper Extremity Assessment [FMUE]) to investigate these questions. Data were obtained at baseline (within 25.5±8.7 days post-stroke) and within 3 months after onset. Brain-PAD was calculated from 187 neuroanatomical features using a publicly available machine-learning model (PyBrainAge). Robust mixed-effects linear models were used to investigate associations between brain-PAD, MoCA, and FMUE scores, including intracranial volume, lesion volume, age, and sex as fixed effects and site as random effect. Finally, we used a logistic regression model to predict mild cognitive impairment (MoCA<23 points) at 3 months from baseline brain-PAD. We examined 29 participants from two cohorts. We found negative correlations between brain-PAD and MoCA scores at baseline (β=-0.40, p=0.025) and at 3 months (β=-0.28, p=0.004). However, cognitive impairment was not correlated with motor impairment either at baseline (β=-0.18, p=0.808) or at 3 months (β=0.03, p=0.961). Finally, brain-PAD at baseline predicted mild cognitive impairment (MoCA<23) at 3 months (β=0.24, p=0.016). Overall, our results suggest that the amount of cognitive impairment is associated with brain aging but not with motor impairment up to 3 months after stroke, and that older-appearing brains at baseline predict mild cognitive impairment (MoCA<23) 3 months after stroke. Future research with a diverse sample and longer time scales may allow us to better understand relationships between brain aging, cognitive function, and sensorimotor outcomes and contribute to the development of precision rehabilitation after stroke.

Disclosures: O. Marin-Pardo: None. **M.H. Khan:** None. **S. Chakraborty:** None. **M.R. Borich:** None. **M. Castillo:** None. **J.H. Cole:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BrainKey, Claritas HealthTech PTE. F. Consulting Fees (e.g., advisory boards); BrainKey, Claritas HealthTech PTE. **S.C. Cramer:** None. **E.E. Fokas:** None. **N.H. Fullmer:** None. **J. Gumarang:** None. **L.X. Hayes:** None. **H. Kim:** None. **A. Kumar:** None. **E.A. Marks:** None. **E.R. Rosario:** None. **H.M. Schambra:** None. **N. Schweighofer:** None. **G. Song:** None. **M. Taga:** None. **C.J. Winstein:** None. **Z. Zheng:** None. **S. Liew:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.13/C154

Topic: C.09. Stroke

Title: Gpt-4 using history and physical localizes stroke lesions

Authors: *W. LYTTON[1,2], J.-H. LEE[1], E. CHOI[3], R. A. MCDOUGAL[4];
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Abstract: A standard Generative Pre-trained Transformer (GPT-4) used published patient History and Physicals (H&Ps) to perform as a virtual neurologist to locate strokes in cerebral hemisphere, brainstem, cerebellum, or spinal cord. Results were compared with ground-truth imaging to determine accuracy. Prompting was developed using two techniques: Zero-shot Chain-of-Thought prompting to provide a logical framework: "Let's work this out step-by-step ..."; and Text Classification to provide choices of particular locations. We presented 46 cases, with each presented 3 times to check for consistency. We used verbatim text from journal H&Ps, after prior review to make sure that no direct localization information or clue was present in the text itself. Localization accuracy was analyzed for specificity, sensitivity, precision, and F1-score. Agreement with imaging was as follows: 0.87, 0.74, 0.75, 0.74 for sidedness and 0.94, 0.85, 0.84, 0.85 for general region. Most localization failures were due to inadequacies of the input -- published case reports are necessarily abbreviated and therefore incomplete, particularly with regard to lack of pertinent negatives. Interestingly, this lack of detail also led to a confabulation error where GPT-4 falsely attributed a symptom of gait instability in a single trial (of the 3 for that case) in a patient presenting with dysarthria and nystagmus. This then led to the incorrect localization of cerebellum in this brainstem stroke -- again just in that single trial. In addition to confabulation, the system also showed errors due to inadequate knowledge: particularly wrong-side error for hemiplegia due to spinal cord lesions presumably due to faulty knowledge of location of the pyramidal decussation. Overall, the performance of GPT-4 was impressive, given that it was not given any extra training (fine tuning) or augmented prompting for neuroanatomy or stroke syndromes. Future explorations will involve the use of more complete H&P, preferably through access to electronic medical record; and use of retrieval augmented generation (RAG) or other augmented prompts which provide additional general background for context. We will also explore the use of extended conversational prompts to lead to a conclusion, including conversation between a digital twin patient and a GPT-4 neurologist to improve on the single-prompt technique.

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Poster

PSTR397

Stroke: Imaging and Assessment

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Topic: C.09. Stroke

Support: CIHR Project Grant MOV 451579
CIHR Fellowship Award 491607
Alberta Innovates Fellowship Award

Title: Impaired visuomotor adaptation at early stages after stroke

Authors: *E. T. KOCH[1], C. EAMON[2], R. STONE[3], R. T. MOORE[5], S. P. DUKELOW[1], T. CLUFF[4];
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Abstract: One in four adults over age 25 are expected to experience a stroke in their lifetime. About 75 percent of stroke survivors have upper limb impairments that disrupt their ability to perform essential motor skills such as feeding and grooming, and negatively affect quality of life. The ability to regain motor skills after stroke relies in part on visuomotor adaptation, the process of adapting to changes in the body, environment, or task using visual feedback. In everyday life, examples of visuomotor adaptation include using a mirror for grooming and using a mouse to control an on-screen cursor. Due to the importance of visuomotor adaptation to activities of daily living and stroke rehabilitation, it is crucial that we gain a better understanding of how this form of adaptation is affected after stroke. In the laboratory, robotics can be used to examine visuomotor adaptation by disrupting the relationship between motion of a participant's occluded arm and a feedback cursor displayed in their workspace. Here, we used the Kinarm exoskeleton robot to examine visuomotor adaptation during reaching in early subacute stroke patients and healthy age-matched controls. Our inclusion criteria for stroke participants were clinical stroke, <4 weeks post-stroke, motor and/or sensory deficits, and corrected vision >20/50. Exclusion criteria were previous stroke, visuospatial neglect, and severe cognitive/other neurological issues. Previously, our group has shown that subacute to chronic stroke survivors have deficits in a single target visuomotor rotation task . In the current work, the task was modified to include three targets. After a baseline period of 24 reaches between targets located 10 cm apart, a rotation was applied to the on-screen cursor causing it to move 30 degrees medial to the motion of the subject's arm during the adaptation phase (108 reaches). The adaptation phase was followed by a wash-out phase (18 reaches) where the perturbation was removed unexpectedly. Our outcome measures were amount of initial adaptation, amount of final adaptation, number of trials to adapt, and amount of washout. We found that participants with stroke were able to adapt to some extent but showed impairments in all outcome measures compared to controls. Overall, this work extends our findings from a single-target task to a multi-target task. Importantly, it reveals that some stroke participants display impairments in visuomotor adaptation, and the profile of impairments varies between individuals. Future work characterizing impairments in visuomotor adaptation and how they relate to other stroke-related impairments may aid therapists in planning and delivering care to stroke survivors.

Disclosures: E.T. Koch: None. **C. Eamon:** None. **R. Stone:** None. **R.T. Moore:** None. **S.P. Dukelow:** None. **T. Cluff:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.15/C156

Topic: C.09. Stroke

Support: CIHR Project Grant MOV 451579

Title: Profiling impairments in the adaptation of reaching movements using visual or proprioceptive feedback during early stroke recovery

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Abstract: Stroke is a leading cause of disability and leaves around 70% of survivors with impairments in moving their arm(s). Many participate in rehabilitation to relearn movements that are essential for daily life. Therapists often try a range of strategies to determine how best to promote recovery, such as providing visual or proprioceptive cues to help patients improve their movements. This approach assumes that motor learning is possible after stroke, and that it can be leveraged to promote motor recovery. Previous work has shown that stroke can cause widespread impairments in the ability to learn from visual or proprioceptive feedback. Here, we questioned whether stroke survivors display selective impairments in the ability to learn from either of these forms of feedback. Stroke survivors in early recovery from supratentorial, unilateral stroke (median days post-stroke = 14.5; range: 3-26days) and healthy controls performed planar reaching movements with their arm supported against gravity in a Kinarm exoskeleton robot. Controls reached with their dominant arm and stroke survivors reached with the arm that was more affected by stroke. The robot was paired with a visual display that projected a virtual feedback cursor and targets into the participant's workspace. Participants performed a visuomotor adaptation task to examine their ability to learn from visual feedback and a force adaptation task to examine their ability to learn from proprioceptive feedback. The visuomotor adaptation task rotated the motion of the cursor by 30° to create a discrepancy between the participant's movements and the motion of the cursor displayed in their workspace. The force adaptation task applied position-dependent forces that displaced the participant's hand perpendicular to the direction of their reach. Both tasks required participants to adapt their movements to reach directly to the targets. We quantified adaptation by measuring initial angular deviations of the hand in the visuomotor adaptation task and peak lateral deviations in the force adaptation task. Participants were considered impaired if their average adaptation in the last 18 of 108 total adaptation trials was below 95% of the range of control data for each task. We observed several impairment profiles, with some survivors displaying selective impairments in adapting to visual or proprioceptive feedback, while others were impaired in both or neither task. The findings reveal a range of impairments in learning from visual and/or proprioceptive feedback in early stroke recovery. In the long term, profiling impairments may help therapists deliver more personalized rehabilitation based on individual learning needs.

Disclosures: C. Eamon: None. **R.T. Moore:** None. **S.P. Dukelow:** None. **T. Cluff:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.16/Web Only

Topic: C.09. Stroke

Title: Covid-19 & stroke: Preliminary results from a retrospective cohort study at a comprehensive stroke center

Authors: Z. AHLFINGER[1], C. BETTS[1], C. STAHELI[1], *B. KIRMANI[2,3];
[1]Texas A & M Sch. of Med., College Station, TX; [2]Texas A&M Univ. Syst. Hlth. Sci. Ctr., College Station, TX; [3]CHI St Joseph Health, College Station, TX

Abstract: COVID-19 is primarily a respiratory infection. As the pandemic progressed, extrapulmonary manifestations of the infection were also appreciated. Recent literature shows that COVID-19 leads to a hypercoagulable state. This suggests that COVID-19 patients may be at increased risk for stroke. The aim of this study is to assess a comprehensive stroke center's experience with regards to risk factors, prognosis, hospital course, and outcome. This is a retrospective study to review the records of COVID-19 positive patients from January 1st, 2020 through February 28th, 2023 who experienced a stroke within 2 months of a COVID-19 diagnosis. Patient data was acquired through electronic medical records from CHI St. Joseph in Bryan, TX. Approval of this study was given by the CHI Institutional Review Board. 95 patients were identified according to inclusion criteria. The patients included 50 females and 45 males with a median age of 69 years old. Of these patients, 57 were white, 19 black, and 19 hispanic. 63 ischemic strokes, 25 TIAs, and 7 hemorrhagic strokes were diagnosed. Ischemic and hemorrhagic strokes as well as transient ischemic attacks (TIAs) seemed to increase in incidence during the pandemic with an incidence in our population of 2.6%. Of the 95, 68 (71.6%) of the strokes were identified within 1 week of a COVID diagnosis. Notably, a high number of strokes (23 out of the 95) were identified between December 2021 and February 2022 when the omicron variant was most prevalent. This is increased from the only 14 found during that same 3 month timeframe the year before. Hypertension (76 patients), dyslipidemia (44 patients), and type 2 diabetes mellitus (33 patients) were the most common comorbidities and patients had a median of 3 total comorbidities. Of the 78 patients evaluated after the first available vaccine; 23 had received at least one shot, 22 had received no shot, and 33 had an unknown status. 43 patients were discharged home, 15 to a skilled nursing facility, 16 to inpatient rehabilitation, 14 expired, 6 to hospice care, and 1 was transferred to another hospital. Of 79 patients evaluated at discharge, the median Modified Rankin Scale was determined to be 4 correlating with moderately severe disability. Stroke is seen in COVID-19 infection and has a poor prognosis.

Disclosures: Z. Ahlfinger: None. **C. Betts:** None. **C. Staheli:** None. **B. Kirmani:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

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Program #/Poster #: PSTR397.17/C157

Topic: C.09. Stroke

Support: RS-2023-00208884
KMDF-RS-2022-00140478

Title: Associations between altered resting-state intra- and inter-network functional connectivity in subacute stroke patients

Authors: *J. LEE[1], **H. KIM**[1], Y.-H. KIM[2];
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Abstract: Brain networks might demonstrate various dynamics during recovery after stroke onset. Recovery of interhemispheric interaction and balance and the occurrence of global network reorganization have also been reported during stroke recovery. This study aimed to investigate additional dynamics of brain networks during recovery after stroke onset. We focused on associations between changes in intra- and inter-network functional connectivity, especially the inter-network change originating from each intra-network connectivity change. Eighty-three subacute ischemic stroke patients participated. All patients underwent resting-state functional MRI (rs-fMRI) and motor function assessments at two weeks and three months after stroke onset using the Fugl-Meyer assessment upper extremity (FMA-UE) score. Large-scale brain networks, including 12 resting-state networks, were extracted using the rs-fMRI. In subgroup analysis, participants were classified into good or poor recovery subgroups based on whether the FMA-UE score improved by 13 points. The associations between changes in intra- and inter-network functional connectivity was investigated using multiple linear regression. The difference in the associations between good and poor recovery groups was investigated using ANCOVA. Alteration of intra-network connectivity of 12 resting-state networks was associated with alteration of inter-network connectivity. In particular, there was a difference between good and poor recovery groups in the associations between the alterations of intra-network connectivity of each of the 12 networks and the inter-network connectivity originating from each intra-network. The inter-network connectivity associated with the change in each intra-network accounted for 49.2% of the total network in the good recovery group and 31.1% in the poor recovery group. Inter-networks associated with altered each intra-network that showed the difference between groups were oriented in cingulo-opercular, parieto-occipital, fronto-parietal, salience, and default networks, respectively. These results demonstrated that change in inter-network connectivity

with change in intra-network is more active in the good recovery group than the poor recovery group. The associated network connectivity, including cognition-related networks, showed differences in the two motor recovery groups. This study suggests the importance of adopting a plasticity-oriented perspective focused on changes in intra- and inter-network connectivity throughout the entire brain rather than solely considering the motor-related area and network for predicting motor function recovery.

Disclosures: J. Lee: None. **H. Kim:** None. **Y. Kim:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.18/C158

Topic: C.09. Stroke

Support: RFAG042189
AARF-21-849749

Title: Cognitive impairment due to ischemic stroke in female rats associated with secondary neurodegeneration which is attenuated by microRNA-20a-3p treatment.

Authors: *Z. AKBARI[1,2], D. SAMPATH[3], B. GOPALAKRISHNAN[4], F. SOHRABJI[5]; [1]texas A&M Col. of Med., Bryan, TX; [2]Texas A&M University System Health Scien Neuroscience and Experimental Therapeutics, Bryan, TX; [3]Dept. of Neurosci. and Exptl. Therapeut., Texas A&M Univ. Syst. Hlth. Scien Neurosci. and Exptl. Therapeut., Bryan, TX; [4]Texas A&M Univ. Syst. Hlth. Scien Neurosci. and Exptl. Therapeut., Bryan, TX; [5]Neurosci. and Exptl. Therapeut., Texas A&M Univ. Syst. Hlth. Scien Neurosci. and Exptl. Therapeut., Bryan, TX

Abstract: Introduction: Our studies show that iv injections of the small non-coding RNA, mir20a-3p, is neuroprotective for stroke in the acute phase and also attenuates long term cognitive decline in middle-aged female rats. Cognitive deterioration resulting from vascular diseases, such as stroke, is associated with secondary neurodegeneration in regions distal from the initial infarction. This study evaluated changes in white matter tracts and gray matter volume to characterize pathological tissue changes in the postmortem brains of rats with stroke.
Methodology: Middle-aged female subjects underwent ischemic stroke induction with vasoconstrictor endothelin 1, administered adjacent to the left middle cerebral artery (MCAo). Following stroke induction, mir-20a-3p mimic (n=10) or scrambled oligo (n=8) was intravenously administered at 4-hour, 24-hour, and 70-day intervals post-stroke. Throughout the 100-day post-stroke period, animals underwent periodic evaluation of cognitive function using both the cued fear conditioning test and the novel object recognition test (NORT). Following perfusion fixation of the brains, postmortem tissues were prepared for histological analysis and

quantification of white matter tracts with Weil myelin stain (Neuroscience Associates Inc, TN). **Results:** The stroke-induced impairment observed in both the cued fear conditioning test and the NORT was mitigated by treatment with mir-20a-3p (Sampath et al., 2023). Sections stained with Weil myelin revealed a decrease in volume of the corpus callosum, internal capsule and the anterior commissure of the ischemic hemisphere compared to the non-ischemic hemisphere in MCAo animals treated with the vehicle (scrambled oligo). However, sham-operated and MCAo animals treated with Mir-20a-3p showed no discrepancies in volume between hemispheres in either tract. Lateral ventricular volume was also assessed,. The MCAo+scrambled group exhibited volume asymmetry in the ventricles, with ventricular enlargement in the ischemic hemisphere as compared to the non-ischemic hemisphere. No hemispheric differences were seen in the ventricular volume of Sham and MCAo+mir20a-3p treated animals. **Conclusion:** Changes in cognitive function caused by stroke as indicated by remote fear memory retrieval and NORT performance, were associated with a loss of density of key forebrain white matter pathways, as well as an expansion in ventricular size, which is indicative of cellular loss. The impairments caused by stroke were alleviated in animals treated with Mir-20-3p, suggesting that microRNA treatment mitigated secondary neurodegeneration.

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Poster

PSTR397

Stroke: Imaging and Assessment

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.19/C159

Topic: C.09. Stroke

Support: NIH/NINDS grant RF1NS117486

Title: Investigating sex differences in glucose metabolism in a mouse model of Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts & Leukoencephalopathy using [^{18}F]-FDG-PET

Authors: *L. LETICA[1], S.-H. CHOI[1], L. DUBBERLEY[1], C. LAYTON[1], R. DYER[1], D. SZCZUPAK[1], J. PARK[1], D. J. SCHAEFFER[2], A. C. SILVA[2];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is a prevalent inherited small vessel disease characterized by mutations to the Notch3 gene, such as Notch3C456R that disrupt small vessels, causing lacunar infarcts and stroke. While the vascular components of the CADASIL mouse model have been extensively studied, the potential links between Notch3 mutations and brain glucose metabolism are not fully understood. In this study, we are investigating sex differences in glucose

metabolism in a Notch3C456R CADASIL mouse model.We administered 18F-FDG to awake mice via a tail vein injection, with an average dose of 18.5 MBq. The mice were fasted for a minimum of 2 hours before 18F-FDG administration. After an awake 30-minute 18F-FDG circulation period, we imaged the mice using a Bruker Si78 PET/CT instrument. The resulting static datasets were analyzed for standard uptake values (SUV) and corrected for glucose levels (SUVglc). We employed statistical tests such as unpaired t-test and 1- & 2-way ANOVA using GraphPad Prism 9 to analyze the data.12-month-old female Notch3C456R mice had significantly decreased body weight compared to males. Baseline blood glucose levels for 12-month-old Notch3C456R female mice were significantly reduced compared to age-matched wild-type (WT) females and Notch3C456R males. Regional analysis of SUV values indicates that 12-month-old Notch3C456R female mice have decreased SUV values in 8 out of 14 ROIs compared to age-matched Notch3C456R male mice. Whole brain analysis shows a statistically significant decrease in SUVglc values for Notch3C456R females compared to males (p-value < 0.01). Furthermore, regional analysis of 12-month SUVglc values indicated a statistically significant reduction in Notch3C456R females compared to age-matched Notch3C456R males in the following regions: Striatum, Cortex, Hippocampus, Thalamus, Cerebellum, Basal Forebrain Septum, Hypothalamus, Amygdala, Brainstem, Central Gray Matter, Olfactory Bulb, Midbrain, Inferior & Superior Colliculi.The statistically significant decrease in SUV and SUVglc values in 12-month-old female Notch3C456R mice compared to age-matched Notch3C456R male mice is indicative of sex differences in 18F-FDG ligand uptake and cerebral glucose metabolism in Notch3C456R mice. The higher severity in females versus males is opposed to reported sex differences in CADASIL patients [Jia et al. Neuroimage Clin 2023; Jiménez-Sánchez et al. Frontiers in Neurology 2021; Gunda et al. Stroke 2012; Opherk et al. BRAIN 2004] and warrants further investigation.

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Poster

PSTR397

Stroke: Imaging and Assessment

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: C.09. Stroke

Support: NIH Grant 5R01HD095187-05

Title: Quantification of the lower boundary of the Box and Block Test by hand opening measure in moderate to severe stroke survivors

Authors: *A. T. CHANG[1], J. Z. SHAO[1], A. LI[2], J. DROGOS[1], C. CARMONA[1], R. ARCEO[1], J. YAO[1];

[1]Physical Therapy & Human Movement Sci., Northwestern Univ., Chicago, IL; [2]Univ. of Illinois Chicago, Chicago, IL

Abstract: Introduction: The Box and Block Test (BBT) is a well validated and recommended clinical outcome measure (OM) for assessing hand and arm function post-stroke. In a previous study[1] of 64 individuals with stroke with moderate to severe arm impairment, the BBT demonstrated significant sensitivity to change, yet it also exhibited the largest floor effect compared to five other validated OMs. Furthermore, the range of hand and arm function wherein the BBT exhibits inaccuracy in evaluating the recovery of functional ability in individuals with stroke is unclear. To address this gap, we conducted a repeated weekly BBT assessment alongside a quantitative hand opening assessment, both with and without volitional arm elevation, to delineate the lower threshold of the BBT's effective range as an OM.
Methods: Twelve individuals with stroke and moderate to severe arm/hand impairment (Fugl-Meyer Assessment-Upper Extremity score: 10-40 out of 66) participated in an 8-week arm/hand intervention, including a total of 24 sessions. Every week, we assessed the BBT and participants' maximum hand opening area (HOA) quantitatively using the trakSTAR 3D tracking device (NDI, Canada). The HOA was measured both with the arm resting stationary on a table (supported) and while volitionally holding the arm above the table without support (unsupported).
Results:HOA in both the supported and unsupported condition showed significant correlation with BBT score ($p<.0001$). Correlation was stronger in the unsupported condition ($R=.821$) than in the supported condition ($R=.671$). Across all measures, the unsupported HOA was in the range of 3.5-38.7 cm^2, and BBT results ranged from 0-16. Additionally, the association between BBT and HOA was not significant when the unsupported HOA was less than 17.90$cm^{2,}$ reflecting that the BBT's lower boundary may begin at 17.90cm^2.
Significance: Taken together, the data indicate the existence of a minimum threshold of hand and arm function beneath which the BBT is not sensitive to improvements, where quantitative measures such as HOA can provide a more accurate representation of recovery. It's important to note that our study has a small sample size which could have influenced the results. Future work will further evaluate the correlation between HOA and BBT, as well as other outcome measures, and will include a larger sample size.

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Poster

PSTR397

Stroke: Imaging and Assessment

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Topic: C.09. Stroke

Support: NIH National Institute On Aging R01AG072756
NIH Eunice Kennedy Shriver National Institute of Child Health and Human Development F32HD105458
NIH Eunice Kennedy Shriver National Institute of Child Health and Human Development R01HD095975

Title: Cortical structural correlates of balance and perceptual function in individuals post-stroke

Authors: *C. LAFOLLETTE[1], J. L. MIRDAMADI[2], T. M. KESAR[2], M. R. BORICH[2]; [2]Rehabil. Med., [1]Emory Univ., Atlanta, GA

Abstract: Changes in cortical thickness after stroke have been well established in several motor and non-motor regions distant from the lesion. There is growing interest in characterizing cortical thickness for predicting motor recovery, though most research has focused on upper limb motor function during seated tasks. The structural basis underlying deficits in whole-body functional behaviors that require complex multisensory integration and may contribute to increased falls risk is unclear. We hypothesized that reduced cortical thickness in ipsilesional multisensory regions rather than primary motor or sensory regions would be associated with lower balance and perceptual function. 38 individuals with chronic stroke (>6 months) completed a T1-weighted structural MRI scan and a clinical balance assessment using the miniBEST. A subset of individuals (n=16) also completed a whole-body motion perception assessment using a reactive balance paradigm, in which they discriminated the direction of whole-body motion arising from standing balance perturbations. 20 older adults without stroke also completed a T1 scan to examine the effects of aging vs stroke. Cortical thickness was quantified and extracted using FreeSurfer. Regions of interest included: superior frontal gyrus, supramarginal gyrus, precentral gyrus, postcentral gyrus, and mean cortical thickness for each hemisphere. After removing individuals with unsuccessful segmentation due to large territory cortical lesions (n=8), we found that individuals with stroke had decreased thickness in both ipsilesional and contralesional hemispheres compared to controls. Between individuals with stroke, those with both lower balance and lower perceptual function had decreased cortical thickness in precentral gyrus (balance: r=0.38, p=0.033; perception: r=-0.58, p=0.02) and supramarginal gyrus (balance: r=0.47, p=0.01; perception: r=-0.65, p=0.01) in the ipsilesional but not the contralesional hemisphere. Balance and perception also had distinct cortical associations: mean thickness was associated with balance (r= 0.42, p=0.02) but not perception while superior frontal gyrus thickness was associated with perception (r=-0.57, p 0.03) but not balance. Our findings suggest the potential utility of structural indices of post-stroke cortical status as a biomarker of altered whole-body motion perception and balance function. Greater understanding of the overlapping and distinct neuroanatomical correlates of perception and balance deficits post-stroke is needed for guiding prognostic algorithms and personalized rehabilitation to increase balance function and decrease falls risk.

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Poster

PSTR397

Stroke: Imaging and Assessment

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Topic: C.09. Stroke

Support: Taichung Veterans General Hospital (TCVGH) Collaborative Project (NHRI-TCVGH-112)
National Health Research Institutes (NHRI, NP-111-PP-08)

Title: Mmp-9 upregulation may predict hemorrhagic transformation after endovascular thrombectomy

Authors: *W.-H. CHOU;
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Abstract: Hemorrhagic transformation (HT) is a serious complication after endovascular thrombectomy (EVT) for patients with acute ischemic stroke (AIS). We analyzed the plasma levels of MMP-9 before and after EVT and assessed the temporal changes of MMP-9 that may be associated with, and therefore predict, HT after EVT. We enrolled 30 AIS patients who received EVT, and 16 (53.3%) developed HT. The levels of MMP-9 in plasma collected from the arteries of AIS patients before and immediately after EVT were measured using ELISA. The percent change in MMP-9 after EVT (after/before) was calculated and compared between patients with and without HT. The median age of the AIS patients was 70 years, and 13 patients (43.3%) were men. The median National Institutes of Health Stroke Scale (NIHSS) scores of patients with HT were 18 on admission and 18 after EVT. The median NIHSS scores of patients without HT were 17 on admission and 11 after EVT. Patients with HT demonstrated significantly greater percentage increases in arterial MMP-9 levels after EVT. Patients with AIS who developed HT had significantly increased arterial MMP-9 levels after EVT, suggesting that the upregulation of MMP-9 following EVT could serve as a predictive biomarker for HT.

Disclosures: W. Chou: None.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.23/D3

Topic: C.09. Stroke

Support: NIH T32 NS077889

Title: Establishing salient rewards for operant touchscreen tasks in aged female mice

Authors: *K. M. COTTER[1], T. A. UJAS[1], M. K. COLSON[1], P. YANEV[2], E. WINFORD[1], J. TURCHAN-CHOLEWO[2], A. M. STOWE[3];
[1]Neurosci., Univ. of Kentucky, Lexington, KY; [2]Neurol., Univ. of Kentucky, Lexington, KY; [3]Neurol., Univ. of Kentucky Med. Ctr., Lexington, KY

Abstract: Background: Training animal models to perform behavioral tasks requires a reward, which traditionally is 7μL of strawberry milkshake (Ensure®) for the operant touchscreen tasks. To maintain motivation using this reward usually requires prior food restriction which is detrimental to stroke recovery (PMID:27449604). For this experiment, we used a 2 μL peanut oil (PO) reward that is a salient reward for aged animals in the operant forelimb reach task (PMID:36715943), and the traditional strawberry milkshake (SM) reward. Neither cohort underwent food restriction prior to behavioral testing. Aim: We hypothesized that there would not be a significant difference in touchscreen task performance between the PO and SM cohorts. Based on our prior studies, we also hypothesized that B cell depletion would result in more severe post-stroke cognitive deficits in the AUTO task in aged animals (PMID:32051245), with decline correlating with the loss of neuroprotective B cells in the hippocampus and prefrontal cortex. To test long-term effects, however, we need to establish this method in aged mice. Methods: Female aged (11-27 months, total n=40; PO n=27, SM n=13) hCD20tamCRE(+)/fBDNF(+/+) mice and hCD20tamCRE(-)/fBDNF(+/+) littermate controls, were first trained on the Initial Touch phase of the Paired Associate Learning (PAL) task to confirm motivation for the specified reward. Measures for saliency were # of trials/session, and time to reward collection. Mice were then trained on Autoshaping (AUTO). Primary AUTO measures are # of trials/session, # of approaches, approach difference, and approach latency. Analysis of histology on the brain to localize B cells to the hippocampus and the prefrontal cortex and a replicate SM group are ongoing. Results: For the PAL task, 0/27 PO mice completed 25 trials/30 min session on day 1, with mice averaging 11.14 trials/session over 5 days. However, 8/13 SM mice reached PAL criterion on day 1, with mice averaging 22.46 trials/session over 5 days (unpaired t-test, $p<0.001$). Unlike AUTO tasks using SM in young (3-6 mos.) male and female mice that reached criterion (40 trials/60 min) typically on day 1 (PMID:36564387), aged mice with a PO reward averaged 23.58 trials/60 min session, while SM aged mice averaged 38.53 trials/60 min session (unpaired t-test, $p<0.0001$). Conclusions: From the pre-stroke PAL and AUTO data, we can conclude that SM is a more salient reward than PO, even without prior food restriction, as the aged mice complete more trials per session and a shorter time to reward collection (unpaired t-test, $p=0.0002$). It also shows that our aged animals can complete the task at the same level as young animals when given the same reward.

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Poster

PSTR397

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Program #/Poster #: PSTR397.24/D4

Topic: C.09. Stroke

Support: NIH R01NS100801
DoD W81XWH-22-1-0930
DoD W81XWH-20-1-0245
DoD W81XWH-16-1-0497

Title: Atf3 is a neuron-specific biomarker for spinal cord injury and ischemic stroke

Authors: J. Z. PAN[1], N. SINGHAL[2], M. S. BEATTIE[3], H. SU[4], *Z. GUAN[5];
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Abstract: Background: Although many molecules have been investigated as biomarkers for spinal cord injury (SCI) or ischemic stroke, none of them are specifically induced in central nervous system (CNS) neurons following injuries with low baseline expression. However, neuronal injury constitutes a major pathology associated with SCI or stroke and strongly correlates with neurological outcomes. Biomarkers characterized by low baseline expression and specific induction in neurons post-injury are likely to better correlate with injury severity and recovery, demonstrating higher sensitivity and specificity for CNS injuries compared to non-neuronal markers or pan-neuronal markers with constitutive expressions. Methods: In animal studies, young adult wildtype and global *Atf3* knockout mice underwent unilateral cervical 5 (C5) SCI or permanent distal middle cerebral artery occlusion (pMCAO). Gene expression was assessed using RNA-sequencing and qRT-PCR, while protein expression was detected through immunostaining. Serum ATF3 levels in animal models and clinical human samples were measured using commercially available enzyme-linked immune-sorbent assay (ELISA) kits. Results: Activating transcription factor 3 (ATF3), a molecular marker for injured dorsal root ganglion sensory neurons in the peripheral nervous system, was not expressed in spinal cord or cortex of naïve mice but was induced specifically in neurons of the spinal cord or cortex within 1 day after SCI or ischemic stroke, respectively. Additionally, ATF3 protein levels in mouse blood significantly increased 1 day after SCI or ischemic stroke. Importantly, ATF3 protein levels in human serum were elevated in clinical patients within 24 hours after SCI or ischemic stroke. Moreover, *Atf3* knockout mice, compared to the wildtype mice, exhibited worse neurological outcomes and larger damage regions after SCI or ischemic stroke, indicating that ATF3 has a neuroprotective function. Conclusion: ATF3 is an easily measurable, neuron-specific biomarker for clinical SCI and ischemic stroke, with neuroprotective properties.

Disclosures: J.Z. Pan: None. **N. Singhal:** None. **M.S. Beattie:** None. **H. Su:** None. **Z. Guan:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.25/D5

Topic: C.09. Stroke

Support: Korea Disease Control and Prevention Agency (3300-3334-300-260-00, 2013-E33017-00, 2013E-33017-01, 2013E-33017-02, 2016-E33003-00, 2016-E33003-01, 2016-E33003-02, 2019-E3202-00, 2019-E3202-01, 2019-E3202-02, 2022-11-006)

Title: Revealing the time-dependent changes in inflammatory response after ischemic stroke

Authors: *H. LEE[1], W. CHANG[2], J. LEE[3];
[1]Konkuk Univ., Seoul, Korea, Republic of; [2]Physical and Rehabil. Med., Samsung Med. Ctr., Seoul, Korea, Republic of; [3]Konkuk Univ. Med. Ctr., Seoul, Korea, Republic of

Abstract: Research using animal models has elucidated the cellular components that initiate an inflammatory response following ischemic stroke. In the same vein, studies in humans have confirmed the occurrence of a systemic inflammatory response after ischemic stroke. However, the specific timing and progression of these responses in humans remain poorly understood. Our study aims to investigate the temporal dynamics of the inflammatory response after ischemic stroke in humans.
We reviewed clinical data of patients enrolled in the Korean Stroke Cohort for Functioning and Rehabilitation between August 2012 and January 2021. Inclusion criteria of the KOSCO: 1) First-ever acute ischemic stroke or intracerebral hemorrhage with neuroimaging evidence, 2) Age ≥19 years, 3) Symptom onset within 7 days. Exclusions for this study: 1) Hospital arrival >96 hours post-stroke, 2) Ischemic lesions in bilateral hemisphere or history of craniectomy, 3) Intervention >1 week post-stroke, 4) Death or recurrent stroke within 12 months. The AHA/ASA Guidelines for the Early Management of Acute Ischemic Stroke mandate routine specific blood tests at admission to guide diagnosis and treatment, a practice followed by South Korean emergency rooms, ensuring that initial blood tests occur upon ER arrival. We divided the interval from ischemic stroke onset to hospital arrival into seven time periods: 0-3, 3-6, 6-12, 12-24, 24-48, 48-72, and ≥72 hours, classifying patients into groups based on these intervals. We identified multiple variables affecting CRP levels post-ischemic stroke—such as age, sex, premorbid conditions, functional status, BMI, stroke etiology, and initial NIHSS score—as covariates for propensity score calculation. We calculated the scores using logistic regression, random forest, and Bayesian additive regression tree models. We conducted two-stage propensity score matching for all groups. Initially, we matched two temporally adjacent groups sequentially to identify the period with the lowest CRP levels. Subsequently, we matched other time periods individually to this reference group.
We enrolled 6,495 patients with ischemic stroke and observed that CRP levels progressively

increased post-stroke, peaking between 48-72 hours. In contrast, leukocyte counts peaked earlier than this period. Other laboratory parameters, including hemoglobin and albumin levels, remained relatively stable regardless of the time elapsed since the stroke onset.
These findings are expected to lay the foundational groundwork for further research about how and how much the inflammatory response influences motor function post-stroke.

Disclosures: H. Lee: None. **W. Chang:** None. **J. Lee:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.26/D6

Topic: C.09. Stroke

Support: NIH Grant NS122808

Title: Recurrent hypoglycemia exposure increases stroke risk in aged insulin-treated diabetic male rats.

Authors: *A. REHNI, S. CHO, K. R. DAVE;
Neurol., Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Nearly 16.5 million patients with diabetes in the United States are older than 65 years and suffer from an increased risk of cardiovascular disease, stroke and associated cognitive deficits. Intensive antidiabetic therapy increases the risk of hypoglycemia in subjects with diabetes. Clinical data shows that hypoglycemia exposure increases stroke risk in subjects with diabetes[1]. Hypoglycemia exposure causes a prothrombotic effect[2]. We have previously shown that the thrombotic effect of 5-day recurrent hypoglycemia (RH) exposure (one episode / day for 5 days) last for at least 7 days post-exposure and that the twice-a-week hypoglycemia exposure for 6 weeks increases the risk of thrombosis in young insulin-treated diabetic (ITD) rats. Because of the high prevalence of diabetes in Americans aged 65 and older, we evaluated the detrimental time window of 5-day RH exposure-induced increased thrombosis (a surrogate for stroke risk) in aged male ITD rats. Next, we determined if twice-a-week frequency of RH exposure for 6 weeks increases thrombosis in aged male rats. Aged male (21±0 months old) streptozotocin diabetic rats were treated with insulin 2-3 weeks after diabetes induction. ITD rats were assigned to either hyperinsulinemic euglycemia (control) or hyperinsulinemic hypoglycemia groups. The extent of stroke risk was quantified using an in vivo model of thrombosis. Briefly, either 7 days after 5-day RH or 3 days after 6-week RH, the carotid artery was attached to the jugular vein using a shunt containing a pre-weighed suture, and blood flow was allowed for 15 minutes. The suture was withdrawn and weighed to quantify thrombosis. The clot weight in the RH-exposed aged male ITD rats (29±3 mg, n=7) was 88% higher ($p<0.01$) than in the control group (15±1 mg, n=6). The clotting time for the RH-exposed ITD rats (5.04±0.48 min, n=7) was significantly

shorter ($p<0.05$) than in the controls (6.76±0.30 min, n=6). Our results show that 5-day RH exposure increases stroke risk in aged male ITD rats when determined 7 days post-exposure. The clot weight in the aged male ITD rats exposed to RH (28±3 mg, n=6) was 66% higher ($p<0.01$) than in the control group (17±1 mg, n=7). The clotting time for the RH-exposed aged male ITD rats (4.36±0.31 min, n=6) was not significantly different from that of the controls (4.64±0.64 min, n=7). Our results indicate that the twice-a-week hypoglycemia exposure for 6 weeks produces a pro-thrombotic effect in aged male ITD rats. Next, we intend to identify the mechanism of RH exposure-induced increase in thrombosis in diabetes. References: 1) Ann.N.Y.Acad.Sci. 2018;1431(1):25-34.; 2) Diabetes Care. 2018;41(12):2625-2633.
Acknowledgement: NIH (NS122808).

Disclosures: A. Rehni: A. Employment/Salary (full or part-time):; University of Miami. **S. Cho:** A. Employment/Salary (full or part-time):; University of Miami. **K.R. Dave:** A. Employment/Salary (full or part-time):; University of Miami.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.27/D7

Topic: C.09. Stroke

Support: NINDS Grant 1064947
AHA Grant GR1065747

Title: Development of an ex-vivo stroke model by inducing oxygen and glucose deprivation on acute mouse brain slices

Authors: *M. BHUIYAN[1], A. BROOKSHIER[1], H. CHANG[2], P. LYDEN[3];
[1]Physiol. and Neurosci., [3]Neurol., [2]USC, Los Angeles, CA

Abstract: The interaction between the components of neurovascular units (NVU) including neuronal, glial and vascular cells, plays a critical role during poststroke functional recovery. However, there is no established *in vitro* model system to study NVU cellular interactions after stroke. Oxygen/glucose deprivation (OGD) in acute hippocampal slices has been an established model to evaluate the effect of ischemia in different hippocampal subregions. In the present study, we sought to develop a novel model system for studying the interactions among different components of the NVU by inducing OGD in fresh whole brain slices. OGD was applied to 400 µm thick mouse brain slices for 30, 60 or 90 min which was then followed by a reperfusion of 60 min. After reperfusion, the slices were fixed with 4% paraformaldehyde solution and immunohistochemistry was performed to assess neuronal injury (flourojade B) and cell death (propidium iodide). The model proved feasible and technically replicable. OGD resulted in uniform cell death throughout the coronal slices. In addition, increasing the time duration of

OGD resulted in an increase in neuronal damage and overall cell death. Staining with cell-type markers is underway. Although this data is from a preliminary stage, the use of propidium iodide in this novel system appeared successful. We expect that this acute brain slice OGD model will provide a new, useful, high throughput, clinically meaningful system to study ischemia and drug treatment in an environment that includes all elements of the NVU.

Disclosures: M. Bhuiyan: None. **A. Brookshier:** None. **H. Chang:** None. **P. Lyden:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.28/D8

Topic: C.09. Stroke

Support: NINDS RO1 NS075930
NINDS U24 NS130600
AHA Predoctoral Fellowship GR1065747

Title: The impact of the selective conditional knockout of endothelial cell PAR1 on cognitive function and stroke severity

Authors: *A. BROOKSHIER[1], J. LAMB[1], M. BHUIYAN[2], S. LISKA[1], H. CHANG[1], L. ECKSTEIN[1], E. WU[3], P. LYDEN[4];
[1]USC, Los Angeles, CA; [2]Physiol. and Neurosci., USC, Los Angeles, CA; [3]Johns Hopkins Univ., Baltimore, MD; [4]Neurol., USC, Los Angeles, CA

Abstract: The protease activated receptor 1 (PAR1) mediates an important signaling mechanism in the CNS that has been implicated in response to cerebral ischemia. PAR1 is expressed by multiple elements of the neurovascular unit, and depending on the mechanism of activation, it can lead to cerebroprotective or cytotoxic effects. To better understand the effect of PAR1 signaling on the responses of endothelial cells to cerebral ischemia, a novel tamoxifen inducible Cre-loxP system was developed. $PAR1^{f/f}$ and $Tie2cre^{ERT2}PAR1^{f/f}$ mice were injected intraperitoneally daily with vehicle or tamoxifen (randomly assigned and blinded) for three days (n = 10/group). The effect of endothelial cell-specific PAR1 knockout on memory and cognitive function was assessed using novel object recognition (NOR), object location recognition (OLR), and Y-maze. Corner test was used to evaluate somatosensory deficits. The middle cerebral artery was occluded (MCAo) for 60 minutes in the two groups followed by the assessment of a neural deficit score and an MRI to measure lesion size. The animals survived for two days and were then intracardially perfused with saline and 4% PFA. The brains were sectioned into 30 μm thick sections with a sliding microtome. Apoptosis was labeled with TUNEL staining. To evaluate blood-brain barrier leakage, 2 MDa dextran conjugated with fluorescein iso-thiocyanate was injected at time of surgery. Successful incorporation of the LoxP and the Cre genes was

confirmed with PCR and gene sequencing. The conditional knockout of PAR1 in endothelial cells did not have an effect on memory and cognitive function under non-stroke conditions. While there was not enough data to demonstrate a statistically significant difference in the severity of cerebral ischemia between the conditional knockout and control mice, the preliminary data suggested the knockout of PAR1 may have an impact on stroke outcomes. The novel endothelial cell-specific knockout of PAR1 mouse model will be beneficial for understanding the role of PAR1 and thrombin in the maintenance of the blood-brain barrier during cerebral ischemia and the impact it has on the severity of the injury.

Disclosures: A. Brookshier: None. **J. Lamb:** None. **M. Bhuiyan:** None. **S. Liska:** None. **H. Chang:** None. **L. Eckstein:** None. **E. Wu:** None. **P. Lyden:** None.

Poster

PSTR397

Stroke: Imaging and Assessment

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.29/D9

Topic: C.09. Stroke

Support: NINDS RO1 NS075930
NINDS U24 NS130600

Title: Temporal assessment of PAR1 reconstitution after PAR1 knockout in cue-recombinase expressing mice

Authors: *S. LISKA[1], A. BROOKSHIER[1], H. CHANG[1], M. BHUIYAN[2], P. LYDEN[3]; [1]USC, Los Angeles, CA; [2]Physiol. and Neurosci., USC, Los Angeles, CA; [3]Neurol., USC, Los Angeles, CA

Abstract: Protease-activated receptor 1 (PAR1) is a receptor for thrombin, a blood plasma enzyme that causes blood clotting via the coagulation cascade. When thrombin binds to platelet-bound PAR1, platelets are activated and cluster together; this can cause thrombosis, resulting in ischemic stroke and brain damage. PAR1 is expressed on various other cells, including neurons, astrocytes, and endothelial cells. PAR1 activation is crucial in astrocyte activation leading to neuron protection. To investigate the role of PAR1 on astrocytes during stroke, conditional knockout animals are generated with the Cre/lox system that allows precise excision of the receptor in cells of interest. Usually, animals are studied immediately after gene knockout. In brain injury research (stroke, TBI), it is important to study long-term outcomes, including MRI and behavioral assessment. A critical gap in our understanding of the Cre/lox recombination system is whether the knockout gene can reconstitute and restore the PAR1 receptor; currently, the temporal dynamics of PAR1 reconstitution are unknown. The tetracycline-inducible system is used to control the knockout of PAR1. Doxycycline (DOX) was given to 5 tet-on GFAP-Cre GFP $PAR1^{f/f}$ mice (3 females, 2 males) aged 26 weeks for 14 days (20mg/ml in 2% sucrose in

sterile water) in light-protected water bottles, refreshed with new medicated water every 3 days. After 14 days of DOX treatment, a randomly chosen mouse underwent an intracardiac perfusion with heparinized saline followed by 4% paraformaldehyde. The same procedure was completed weekly (5 wks total) for the remaining mice and control. 30µm thick sections were sliced and stained for PAR1, GFAP, NeuN, and Tie2 to label astrocytes, neurons, and endothelial cells, respectively to visualize the colocalization of the PAR1 gene in the cell types. We confirmed successful placement of the trans genes using PCR and gene sequencing. Homozygous animals were viable and fertile. No obvious behavioral phenotype was observed. We confirmed successful knockout of PAR1 in astrocytes with no reconstitution 5 weeks post knockout. PAR1 was demonstrated in other cell types, confirming no leakage of Cre. The colocalization of PAR1 and astrocytes in the control animal shows expected expression of PAR1. In this astrocyte-specific, conditional knockout transgenic mouse line, gene deletion seems to be permanent. Further studies should confirm a longer time course since some translational trials study behavioral effects of brain injury to 90 days. The effect of this astrocyte-specific conditional PAR1 knockout on outcomes after stroke is currently being investigated.

Disclosures: S. Liska: None. **A. Brookshier:** None. **H. Chang:** None. **M. Bhuiyan:** None. **P. Lyden:** None.

Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.01/D10

Topic: C.08. Ischemia

Support: NIH Grant 1R01RAG082207A-01

Title: Clemastine Attenuates CognitiveImpairment in a Rat Model of Chronic Cerebral Hypoperfusion

Authors: *X. ZONG[1], Y. FENG[2], X. MA[3], Q.-G. ZHANG[4];
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Abstract: Clemastine Attenuates Cognitive Impairment in a Rat Model of Chronic Cerebral Hypoperfusion
Xuemei Zong, Yu Feng, Zhihai Huang, Xiaohui Ma, Quanguang Zhang*Department of Neurology, LSU Health Sciences Center, Shreveport,LA 71103,USA**Objective:** Chronic cerebral hypoperfusion has been implicated as a potentially important pathological factor in mild cognitive impairment, Alzheimer's disease (AD) and vascular dementia (VaD). Notably, myelin

loss is a prominent pathological feature of chronic cerebral hypoperfusion, while well-functioning myelin is crucial for memory and cognition. Utilizing drug repurposing to identify effective drug candidates for VaD treatment has gained attention. Recent research has highlighted the potential of clemastine, an FDA-approved allergy medication, as a promising pro-myelinating drug. Therefore, in this study, we aim to investigate whether clemastine can enhance myelination and alleviate cognitive impairment following permanent occlusion of the bilateral common carotid artery (BCCAO). **Methods:** Chronic cerebral hypoperfusion and VaD were induced by BCCAO in adult male Sprague Dawley rats. Animals were treated with either clemastine or an equivalent volume of the vehicle from day 1 to day 14 post-surgery. Following treatment, memory-related behavioral tests were conducted, and myelin pathology in the cortex and hippocampus was assessed through immunofluorescence staining and ProteinSimple® capillary-based immunoassay.**Results:** Western blot and immunohistochemical analysis revealed no significant difference in MAP2 expression and neuronal survival in hippocampal CA1 region among the three groups 3 months after BCCAO. However, clemastine increased the levels of mature oligodendrocytes and enhanced myelination in corpus callosum and hippocampus CA1 region. Furthermore, clemastine preserved spatial learning and memory performance in the Barnes maze test and contextual memory in fear conditioning training test, and improved depressive-like behaviors. Taken as a whole, the results suggest that clemastine treatment might be a potentially promising therapeutic drug to attenuate negative neurological consequences from chronic cerebral hypoperfusion and VaD.

Disclosures: X. zong: None. **Y. Feng:** None. **X. Ma:** None. **Q. Zhang:** None.

Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.02/D11

Topic: C.08. Ischemia

Support: JSPS KAKENHI Grant Number JP23K05139
JSPS KAKENHI Grant Number JP20K11523
JSPS KAKENHI Grant Number JP16K00932

Title: Neuroprotective effect of citrus compounds, 3,5,6,7,8,3',4'-heptamethoxyflavone and naringin, in the hippocampus following transient global cerebral ischemia in SHRSP

Authors: *S. OKUYAMA, T. OMASA, A. SAWAMOTO;
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Abstract: Stroke-prone spontaneously hypertensive rats (SHRSP) are a strain that was further isolated by selective breeding from spontaneously hypertensive rats (SHR), which were isolated from normotensive Wistar Kyoto rats (WKY). When a transient global cerebral ischemia surgery

is performed for SHRSP, delayed neuronal cell death in the hippocampus occurs significantly compared to WKY, making it useful as a model for neuronal damage during the acute phase of cerebral infarction. In this study, we investigated the effects of 3,5,6,7,8,3',4'-heptamethoxyflavone (HMF), a polymethoxyflavone contained in citrus peel, and naringin (NGI), a flavanone, in the brain hippocampus. WKY/Izm and SHRSP/Izm (9 weeks old, male) were used as test animals. Among the SHRSP groups, the group administered with normal feed supplemented with 0.063% HMF was designated as H1, the group administered with 0.125% HMF was as H2, and the groups administered with 0.125% or 0.25% NGI were as N1 and N2, respectively. At 12 weeks of age, rats in all groups underwent global cerebral ischemia surgery in which bilateral carotid arteries were temporarily clipped for 20 minutes and then reperfused, and dissection was performed at 13 weeks of age. The hippocampus was analyzed using immunohistochemical staining or western blotting. Regarding the expression of Iba1-positive microglia, the expression level was significantly increased in the SHRSP group compared to the WKY group, whereas this increase was significantly suppressed in the H1 and H2 groups. Regarding the expression of MAP-2 positive signals in the dendrites of neurons, the signal was significantly decreased in the SHRSP group compared to the WKY group; in contrast, this decrease was significantly suppressed in all four groups. Furthermore, regarding the number of healthy neurons in the CA1 region, it decreased significantly in the SHRSP group compared to the WKY group; on the other hand, this decrease was significantly suppressed in the H1, N1 and N2 groups. The expression level of HMGB1 was significantly increased in the SHRSP group compared to the WKY group, and this increase was significantly suppressed in the four groups. In addition, the expression level of BDNF was significantly decreased in the SHRSP group compared to the WKY group, and this decrease was significantly suppressed in the H2 group. The results of this study revealed that two different structural compounds of citrus fruit, HMF and NGI, showed a neuro-protective effect due to its anti-inflammatory effect and neurotrophic factor-producing effect.

Disclosures: S. Okuyama: None. **T. Omasa:** None. **A. Sawamoto:** None.

Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

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Program #/Poster #: PSTR398.03/D12

Topic: C.08. Ischemia

Support: DFG, FOR 2879, project A3, MA4375/6-1

Title: Microglial depletion and repopulation enhance early functional recovery after ischemic stroke in mice

Authors: *S. ISLA CAINZOS[1], J. BISKAMP[2], T. MAGNUS[1];
[1]Neurol., Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [2]Inst. for Physiol. I, Univ. of Freiburg, Freiburg, Germany

Abstract: Ischemic stroke triggers an immense inflammatory response in brain tissue. Microglia, activated by both acute and chronic phases after stroke, influence the regenerative and reparative processes in the brain. Microglia survival and proliferation are dependent upon signaling through the colony-stimulating factor 1 receptor (CSF-1R). Previous short-term CSF-1R inhibition studies supported the notion that PLX5622 (CSF-1R antagonist) induced microglia repopulation, which could reduce inflammatory cytokine expression, brain damage and resolve behavioral impairments. In this study, we aim to investigate the contribution of microglial depletion and the subsequent repopulation after ischemic stroke on post-stroke functional recovery. Wild-type mice were trained in a skilled reaching task (SPR) that evaluates fine motor function and underwent distal medial cerebral artery occlusion (dMCAO) or sham surgery. PLX5622 or vehicle was administered between day 3 and day 7 after dMCAO. Functional deficits and their recovery were monitored both behaviorally (SPR) and electrophysiologically, recording multichannel electrocorticography (ECOG) from both hemispheres in freely behaving animals. After dMCAO, the SPR performance of the sham PLX5622 (n = 6) and vehicle (n = 5) groups did not change in comparison to the baseline, while in both stroke groups PLX5622 (n = 10) and vehicle (n = 9) performance dropped significantly (p = 0.0054, two-way ANOVA analysis) compared to the sham groups on days 1 and 3 after stroke. Fine motor function in the PLX5622 stroke group recovered fully on day 7, with a significant difference (p = 0.0072, two-way ANOVA analysis) from the vehicle stroke group that did not recover until day 14. These findings provide evidence that microglia short-term depletion at the right time window leads to an earlier recovery of fine motor function. In the preliminary analysis of the ECoG potentials, we found a broadband loss of power that could be observed in the power spectral density (PSD) in the vicinity of the ischemic lesion in both stroke groups until day 3; further analysis of subsequent time points is needed. We hypothesize that the PSD aperiodic exponents, which have been shown to reflect cortical excitation-inhibition imbalance in the vicinity of stroke, will correlate to the behavioral outcome. Thus, analyzing the aperiodic exponents of ECOG signals may elucidate the link between pathophysiology and behavior. These insights provide an understanding of post-stroke pathophysiology and an appropriate spatial and temporal window for future treatment approaches.

Disclosures: S. Isla Cainzos: None. **J. Biskamp:** None. **T. Magnus:** None.

Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.04/

Topic: C.08. Ischemia

Support: Pronaces/fordecyt/2020 #140644

Title: Mesenchymal stem cell-derived extracellular vesicles preserve tight junction proteins in the blood brain barrier in vitro after ischemic stroke

Authors: *D. LOZANO LÓPEZ[1], *D. LOZANO LÓPEZ[1], M. DÍAZ-CORÁNGUEZ[2], R. CASTANEDA ARELLANO[3], L. ALARCÓN[4], D. MARTIN-TAPIA[4], J. V. SEGOVIA-VILA[4], L. HERNANDEZ-ORTEGA[5], H. NARIO-CHAIDEZ[6];
[1]Univ. de Guadalajara, Guadalajara, Mexico; [2]Pharmacobiology, Ctr. for Res. and Advanced Studies of the Natl. Polytechnic Inst. (CINVESTAV-IPN), Mexico, Mexico; [3]Univ. of Guadalajara, Tonala, Mexico; [4]Ctr. for Res. and Advanced Studies of the Natl. Polytechnic Inst., Mexico, Mexico; [5]Univ. of Guadalajara, Guadalajara, Mexico; [6]Latin American Oncology Services Syst., Guadalajara, Mexico

Abstract:
NoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNone
NoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNone
NoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNoneNone
NoneNoneNoneNone

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Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.05/D13

Topic: C.08. Ischemia

Title: Injectable GelNB Nanofibrous Hydrogels Empowering Angiogenesis, Enhancing Sensorimotor Function, Diminishing Inflammation through Growth Factors Emission In Ischemic Stroke

Authors: *D. KIM[1], S. YANG[2,3,4], Y. KIM[1], G. KIM[2], J. KIM[5];
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Abstract: Injectable GelNB Nanofibrous Hydrogels Empowering Angiogenesis, Enhancing Sensorimotor Function, Diminishing Inflammation through Growth Factors Emission In Ischemic Stroke

Donggue Kim[1, 2*], Ji Woo Lee[1*], Yangtae Kim[4*], JunHyeok Choe[1], Gaeun Kim[1], Jae Geun Kim[4#], Kwang Hoon Song[1#], Sunggu Yang[1, 2, 3#1]Department of Nanobioengineering, Incheon National University, Incheon, 22012, Republic of Korea.[2]Center for Brain-Machine Interface, Incheon National University, Incheon, 22012, Republic of Korea.[3]gBrain Inc., Incheon 21984, Republic of Korea.[4]Department of Life Sciences, Incheon National University, Incheon, 22012, Republic of Korea

Ischemic stroke, representing the majority of stroke incidents, damage the brain tissue. This condition manifests in diverse aftereffects such as motor impairment and emotional disturbances. However, the fundamental treatments to address ischemic stroke aftereffects remain lacking. Our team proposes a novel approach employing injectable gelatin-norbornene nanofibrous hydrogels (GNFs) infused with growth factors (GFs, VEGF, S1P, and PMA). We analyzed the characteristics of GNFs and administered GNFs into the motor cortex post-ischemic stroke to evaluate the therapeutic impact of GFs-loaded GNFs on ischemic stroke. GNFs mimic natural fibrous extracellular matrix architecture, release the GFs continuously, and offer the benefits of an injectable system, allowing precise injection volume by 1 μL with 30 G needle. GFs-loaded GNFs increase angiogenesis, anti-inflammation, and sensorimotor function. For further application, our biocompatible GNFs has the potential to load disease-specific drugs and inject them into brain regions, associated with diseases, to treat a range of neurological disease.Keywords: Electrospinning, Ischemic stroke, Sensorimotor function, GelNB, Nanofibrous hydrogels, VEGF, S1P, PMA, Angiogenesis

Disclosures: D. Kim: None. **S. Yang:** None. **Y. Kim:** None. **G. Kim:** None. **J. Kim:** None.

Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.06/D14

Topic: C.08. Ischemia

Support: NINDS IGNITE 1 R61 NS123195-01

Title: Efficacy of the $K_{Ca}3.1$ inhibitor senicapoc on longitudinal neurobehavioral stroke outcomes

Authors: *M. ADLER-WACHTER[1], B. SCHWEITZER[1], A. S. RUDE[2], A. MCDONOUGH[1], Y.-J. CHEN[3], H. WULFF[3], J. R. WEINSTEIN[1];
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Abstract: Background: Acute ischemic stroke is a leading cause of death and long-term disability. Both microglia (MG) and macrophages (MP) are critical effector cell types in ischemic brain injury. $K_{Ca}3.1$ is a calcium-activated potassium channel that is critical for pro-inflammatory activation of MG/MP and exacerbation of stroke pathophysiology. Previous studies have demonstrated the $K_{Ca}3.1$ inhibitor senicapoc is safe in humans. Our research group has reported that senicapoc reduces infarct volume by ~55% at 8 days post middle cerebral artery occlusion (MCAO). Here we evaluate senicapoc's ability to attenuate neurobehavioral deficits post-MCAO using a panel of dynamic, longitudinal assessments. **Methods:** Young adult male (female studies upcoming) mice underwent 60 min MCAO. Senicapoc (40mg/kg) or vehicle (miglyol 812) was administered via intraperitoneal injection twice daily for 7 days starting 12 h after MCAO. Senicapoc levels in brain and plasma were determined by UPLC/mass spectrometry. Neurobehavioral assessments were performed 1 week prior to and again 1, 2 and 4 weeks following MCAO. Motor function was tested using Noldus Catwalk for automated gait analysis and grid test for sensorimotor function and coordination. Manual, blinded analysis of grid test videos counted number of slips per paw. Spatial memory was assessed with alternating T maze by quantifying percent of trials in which the animal alternated entry of maze arms. **Results:** On Catwalk (n=32), difference in swing speed between left and right paws increased following stroke at 7 (effect size (d)=0.55) and at 14 days (d=0.80) but did not reach significance at 30 days (d=0.40). Results at 7 and 14 days indicate slower movement of limbs contralateral to MCAO, as expected. Grid test (n=20) showed significant increases by 20-30% in footfault index at 7 (d=0.98), 14 (d=1.26) and 30 days (d=1.74) indicating animals slipped more on contralateral limbs as expected. MCAO did not induce significant changes in alternating T-maze (n=20) at any post-stroke time points ($p>0.05$). Increased sample size and inclusion of females is needed to draw further conclusions about treatment efficacy. Our apriori power analysis calculations call for a minimum n of 18 animals (9M/9F) per group. **Conclusions:** We have validated dynamic longitudinal assessments of sensorimotor, but not neurocognitive, function following MCAO stroke. We are currently using these validated sensorimotor assessments to determine senicapoc's efficacy post stroke. We are also assessing other potential neurocognitive testing paradigms. These are promising, clinically relevant approaches for investigating senicapoc's efficacy.

Disclosures: M. Adler-Wachter: None. **B. Schweitzer:** None. **A.S. Rude:** None. **A. McDonough:** None. **Y. Chen:** None. **H. Wulff:** None. **J.R. Weinstein:** None.

Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

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Program #/Poster #: PSTR398.07/D15

Topic: C.08. Ischemia

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Title: Hybrid electro-optical stimulation attenuates ischemic brain damage via augmenting the brain lymphatic drainage system

Authors: *M. KIM[1,2], J. YOUN[3], H. LEE[1,2], B. CHOI[1,2], J. JEONG[3,4], H. SHIN[1,2];
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Abstract: Hybrid electro-optical stimulation attenuates ischemic brain damage via augmenting the brain lymphatic drainage system Min Jae Kim[a,b,#], Jiman Youn[c,#], Hong Ju Lee[a,b], Byung Tae Choi[a,b], Joonsoo Jeong[c,g,*], Hwa Kyoung Shin[a,b,*][a]Department of Korean Medical Science, School of Korean Medicine, Pusan National University, Yangsan, Gyeongnam 50612, Republic of Korea[b]Graduate Training Program of Korean Medical Therapeutics for Healthy-Aging, Pusan National University, Yangsan, Gyeongnam 50612, Republic of Korea[c]Department of Information Convergence Engineering, Pusan National University, Yangsan 50612, Republic of Korea[g]School of Biomedical Convergence Engineering, Pusan National University, Yangsan 50612, Republic of Korea
Ischemic brain injury not only results in significant neurological, motor and cognitive impairment but also contributes to the accumulation of toxic solutes and pro-inflammatory cytokines in the infarction region, exacerbating the ischemic brain damage. The glymphatic system, crucial for brain waste clearance and homeostasis, is impaired by ischemic injury, highlighting the importance of developing therapeutic strategies for post-stroke complications. Here, we develop a novel hybrid electro-optical stimulation device that integrates near-infrared micro-light-emitting diode (μLEDs) with transparent microneedles, enabling efficient non-invasive stimulation of the cortical area for ischemic stroke treatment. We aim to investigate whether this hybrid electro-optical stimulation enhances glymphatic system function and ameliorates ischemic brain injury in the middle cerebral artery occlusion and reperfusion (MCAO/R) mice model. Our results demonstrate that hybrid stimulation improves neurological, motor, and cognitive function, as well as reduces brain atrophy following MCAO/R. Moreover, hybrid stimulation restores impaired glymphatic system function and alleviates the accumulation of pro-inflammatory cytokines such as IL-1β and cleaved caspase-3. This enhancement of glymphatic function by hybrid stimulation is associated with modulation of the polarization of aquaporin-4 (AQP4) in the ischemic brain. Notably, inhibition of AQP4 partly reverses the improved functional outcomes of hybrid stimulation, highlighting the crucial role of glymphatic system enhancement by hybrid stimulation in restoring impaired brain function after MCAO/R. Our findings suggest that targeting glymphatic drainage by brain stimulation may provide a promising therapeutic approach for treating ischemic brain injury.

Disclosures: M. Kim: None. **J. Youn:** None. **H. Lee:** None. **B. Choi:** None. **J. Jeong:** None. **H. Shin:** None.

Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

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Topic: C.08. Ischemia

Support: American Heart Association (AHA) Postdoctoral Fellowship 1029163

Title: Fasudil Attenuates Brain Injury after Photothrombotic Stroke in Genetically Obese (ob/ob) Mice

Authors: *M. SALMAN[1], T. ISHRAT[2];
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Abstract: Background: Obesity and diabetes mellitus are a predominant risk factor for ischemic stroke with a high mortality rate worldwide. The therapeutic strategies beneficial for stroke are ineffective in obesity and diabetic stroke. Despite these, therapeutic options remain restricted. Recently, fasudil, a Rho-kinase inhibitor has emerged as a potential therapeutic approach for increasing neuroprotection. Thus, this study aimed to investigate the protective role of fasudil against photothrombosis stroke in genetically obese (ob/ob) mice.**Methods:** The adult C57BL/6J-ob/ob (B6.Cg-Lep^{ob}/J; 9-10 weeks old) mice were subjected to the photothrombosis method in which the proximal-middle cerebral artery was exposed with a laser beam by retro-orbital injection of a photosensitive dye before the laser light exposure. Fasudil (10mg/kg) was administrated intraperitoneally at 30 min, 24h, and 48h following post-stroke. After 72h, mice were euthanized, and tissues were harvested for biochemical and molecular analysis.**Results:** Fasudil treatment significantly reduced infarct volume and brain edema compared to the vehicle group. Additionally, fasudil treatment protects against vascular damage by reducing MMP-9 expression, restoring tight junction protein and immunoglobulin extravasation. Furthermore, fasudil treatment attenuated 4-hydroxynonenal and nitrotyrosine expression, and decreased TUNEL-positive cells count.**Conclusion:** Our results show that fasudil reveals neuroprotection through mitigating vascular damage and neuronal loss against pt-MCAO. Thus, fasudil might be considered a new therapeutic strategy for obese and diabetic stroke pathogenesis.**Keywords:** Ischemic stroke; Fasudil; Oxidative stress; Apoptosis

Disclosures: M. Salman: None. **T. Ishrat:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an

institution.; This work was supported by The American Heart Association (AHA) Postdoctoral Fellowship 1029163..

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PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Mäxi Foundation

Title: Neural xenografts contribute to long-term recovery in stroke via molecular graft-host crosstalk

Authors: *R. RUST[1], R. WEBER[2], A. BOSWORTH[3], M. ZHANG[4], K. KISLER[5], R. M. NITSCH[6], C. TACKENBERG[7], B. V. ZLOKOVIC[8];
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Abstract: Stroke patients are often left with permanent disabilities, as there are currently no regenerative treatment options available. Induced pluripotent stem cell (iPSC)-based therapies offer a promising strategy to enhance recovery after stroke. In this study, we show that local transplantation of iPSC-derived neural progenitor cells (NPCs) improves recovery-associated brain tissue responses and reduces long-term neurological deficits in stroke mice. Using *in vivo* bioluminescence imaging and histological analyses, we confirm the survival and differentiation of the NPCs into mature neurons, without pluripotent residuals. The NPCs promote several pro-regenerative responses, including enhanced vascular repair, improved blood-brain barrier integrity, reduced microglial activation, and increased neuro- and axonogenesis, compared to controls undergoing sham treatment. Deep learning-assisted behavioral analysis reveals significant improvements in gait and fine-motor skills in NPC-treated mice. Single-nucleus profiling shows that the graft primarily differentiates into GABAergic neurons, with other cells developing into glutamatergic neurons, astrocytes, and NPC-like phenotypes. Molecular

interaction analysis between the GABAergic graft and the host stroke brain tissue shows crosstalk via regeneration-associated Neurexin (NRXN), Neuregulin (NRG), Neural Cell Adhesion Molecule (NCAM), and SLIT signaling pathways. Our findings highlight that grafted iPSC-derived NPCs primarily form GABAergic neurons, which may play a critical role in long-term stroke recovery through specific molecular interactions with the host tissue.

Disclosures: R. Rust: None. **R. Weber:** None. **A. Bosworth:** None. **M. Zhang:** None. **K. Kisler:** None. **R.M. Nitsch:** None. **C. Tackenberg:** None. **B.V. Zlokovic:** None.

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PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

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Topic: C.09. Stroke

Support: JSPS KAKENHI Grant Number JP22K16682

Title: Cerebral blood flow changes induced by trigeminal nerve stimulation

Authors: *Y. FUKUSHI, H. MIMURA, M. KIMURA;
Shizuoka Univ., Hamamatsu, Japan

Abstract: Cerebrovascular disease has a high mortality rate and ranks as the fourth leading cause of death in both the United States and Japan, according to 2020 statistics. Cerebral infarction represents a predominant type of cerebrovascular disease, often leading to residual symptoms such as paralysis, significantly affecting patients' quality of life. Previous studies using the rat model elucidated that the cerebellar fastigial nucleus (FN) electrical stimulation reduced cerebral infarction. FN stimulation increased regional cerebral blood flow (rCBF) and systemic blood pressure (BP). As a mechanism, we have demonstrated that FN stimulation acts on the cerebrum through a cholinergic pathway, mitochondrial uncoupling protein 4 expressions in cortical neurons may inhibit cell death and have a neuroprotective effect. However, applying FN stimulation in humans is challenging due to its highly invasive properties. Therefore, we investigated electrical stimulation of the first branch of the trigeminal nerve (TNV1) as a less invasive alternative to FN stimulation, aiming to achieve similar effects. The TN is a large nerve in the face divided into three branches, and a simple and less invasive approach is expected if the forehead can be stimulated. This study clarified the relationship between stimulation parameters and cerebral blood flow. Adult male Sprague-Dawley rats weighing approximately 300g at 9 weeks old were used. TNV1 stimulation was performed under isoflurane anesthesia, with monitoring of rCBF, BP, and heart rate (HR). Initially, the skull was exposed and a 2 mm in diameter hole was drilled in the skull at specific coordinates from the bregma. A blood flow meter probe was placed to measure rCBF. In addition, BP and HR were monitored using a pressure transducer through femoral artery cannulation. For TN stimulation, surface electrodes

were placed along the line between the eye and the ear for transcutaneous stimulation on either hemisphere. First, TN stimulation was performed at a constant pulse width of 0.5 msec and current intensity of 2 mA at varying frequencies (10-100 Hz). rCBF significantly increased at frequencies of 10-30 Hz ($p<0.05$, $n=4$), but no significant difference was observed at 50-100 Hz. Subsequently, when TN stimulation was performed at 20 Hz, 0.5 msec, and 2 mA, BP and HR were increased. We thought that TN stimulation activates the rostral ventrolateral medulla regulating systemic blood flow. Since TN stimulation has similar effects on rCBF and BP compared to FN stimulation, it might also have neuroprotective effects. In conclusion, TN stimulation may be a less invasive alternative to FN stimulation.

Disclosures: Y. Fukushi: None. **H. Mimura:** None. **M. Kimura:** None.

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PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

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Topic: C.09. Stroke

Support: I01BX005127
R35 NS132184
R01 NS130763
R01 NS109459
Department of Neurological Surgery of the University of Wisconsin-Madison
IK6BX005690

Title: Post-stroke miR-21 mimic treatment modulates gut microbiome

Authors: *B. CHELLUBOINA[1], S. L. MEHTA[2], R. VEMUGANTI[3];
[1]Univ. of Wisconsin, Madison, WI; [2]Neurolog. Surgery, Univ. of Wisconsin-Madison, Madison, WI; [3]Neurolog. Surgery, Univ. of Wisconsin, Madison, WI

Abstract: As miR-21 targets many inflammatory and oxidative stress-related transcripts, it is considered as a cell protective miRNA. We recently showed that increasing the levels of miR-21 by administration of a miR-21 mimic significantly reduces brain damage and promotes long-term recovery after stroke in adult and aged mice of both sexes. Recent studies showed that gut microbiome influences inflammation in the post-stroke brain. Curiously, miR-21 was also shown to be a regulator of the gut microbiome. Hence, we tested if miR-21 mimic treatment rectifies post-stroke gut microbial dysbiosis. Adult male and female mice were intravenously administered with either control mimic or miR-21 mimic at 5 min of reperfusion following 1h transient middle cerebral artery occlusion. The miR-21 mimic cohort showed significantly reduced infarct volume (T2-MRI) and curtailed gut permeability (FITC-Dextran/histopathology)

at 1 day of reperfusion compared with the control mimic cohort (n = 6 to 8/group) in both males and females. Fecal samples collected on days 7 and 14 of reperfusion were subjected to gut microbial analysis by 16S RNA sequencing. The gut barrier of the miR-21 mimic cohort showed lower circulatory levels of FITC-Dextran and fewer peptidoglycan-positive cells compared with the control mimic cohort. Furthermore, analysis of the gut microbiome showed an altered abundance of gut bacteria genus Alistipes, Bacteroides, Clostridia, Lachnospiraceae, Lactobacillus, Muribaculaceae, Parabacteroides, Romboutsia, Ruminococcus, and Tenericutes in the miR-21 mimic cohort compared with the control mimic cohort. Thus, our studies show that post-stroke miR-21 treatment protects gut integrity and modulates the composition of the gut microbiome during the delayed phase of stroke.

Disclosures: B. Chelluboina: None. **S.L. Mehta:** None. **R. Vemuganti:** None.

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PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: C.09. Stroke

Support: AARF- Alzheimers Foundation

Title: Neuron-specific enrichment of mir20a-3p attenuates stroke-induced sensorimotor impairment in the acute phase and cognitive performance in the chronic phase

Authors: *D. SAMPATH[1,2], Z. AKBARI[3], B. GOPALAKRISHNAN[3], F. SOHRABJI[4]; [1]Neurosci. and Exptl. Therapeut., Texas A&M Univ. Syst. Hlth. Scien, College Station, TX; [2]Neuroscience and Experimental Therapeutics, Texas A&M University System Health Scien Neuroscience and Experimental Therapeutics, Bryan, TX; [3]Texas A&M Univ. Syst. Hlth. Scien Neurosci. and Exptl. Therapeut., Bryan, TX; [4]Neurosci. and Exptl. Therapeut., Texas A&M Univ. Syst. Hlth. Scien Neurosci. and Exptl. Therapeut., Bryan, TX

Abstract: We reported previously that compared to adult females, astrocytes from middle-aged females have lower functional capacity for stroke neuroprotection, including decreased trophic support and decreased capacity to detoxify the ischemic microenvironment. MiRNA profiling indicated that the mir17-92 cluster is significantly decreased in astrocytes from middle-aged females. Intravenous injections of miR-20a-3p delivered after stroke is rapidly internalized by neurons, and this treatment reduced acute sensorimotor deficits (PMID 34570349) and attenuated cognitive impairment in the chronic phase (Sampath et al., 2023). The present study tested the hypothesis that specific enrichment of mir20a-3p in neurons would replicate these beneficial effects. **Methods:** We designed a tetracycline (Tet)-induced recombinant adeno-associated virus (rAAV) construct where miR-20a-3p was located downstream of neuron specific enolase and the mCherry reporter. The construct was delivered at a concentration of

$2.5*10^{10}$ VP/ml intracerebrally in the following coordinates (A.P- +0.9mm, M.L- +3.6mm, and D.V- -6.5mm). Control vector included a scrambled oligo instead of mir20a-3p. Animals were allowed to recuperate for six weeks, followed by middle cerebral artery occlusion (MCAo). Animals received doxycycline injection at 4 hrs after stroke (50mg/Kg BWT). Sensorimotor tests were performed pre and post stroke, while tests of spatial learning was performed at 30 days, and associative learning (cued fear conditioning) was performed at pre, 30-90-days post stroke. **Results:** Stroke increased latency in the adhesive tape test in both the rAAV-scrambled and rAAV-miR20 treated stroke groups at 2- and 5-days post stroke ($p<0.05$), however the deficit was significantly higher in the control rAAV group ($p<0.05$). Remote fear memory retrieval, assessed by freezing behavior, decreased over the 30-, 60- and 90-days post stroke testing schedule. However, the rAAV-scrambled group showed deterioration of fear memory at early as 30 days post stroke ($p<0.05$) vs its pre-stroke levels, which was not observed in the Sham and rAAV-miR20 treated groups. Stroke also caused a significant impairment in spatial learning as tested with Barnes maze in the rAAV-scrambled group, as evidenced by the latency to detect the escape goal box over three days of training. **Conclusion:** Enriching mir20a-3p in neurons resulted in robust protection against stroke-induced long term cognitive deficits. While iv mir20a-3p treatment was also effective in reducing stroke-induced cognitive deficits, precision delivery of mir20a-3p to neurons indicates that this cellular target is critical for long-term recovery.

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Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

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Topic: C.09. Stroke

Support: R41HL134517
R01HL136485
P50 HL110789

Title: Lipid Receptor GPR31 (G-Protein Coupled Receptor 31) is a Novel Dual Target for Thrombosis and Ischemic Stroke

Authors: *L. COVIC;
Tufts Med. Ctr., Boston, MA

Abstract: Lipid Receptor GPR31 (G-Protein Coupled Receptor 31) is a Novel Dual Target for Thrombosis and Ischemic Stroke
Authors: Nga Nguyen, Monica Hinds, Athan Kuliopulos and Lidija Covic

Funding: This work was funded, in part, by grants from the National Institutes of Health by R41HL134517, R01HL136485, and P50 HL110789 (L. Covic and A. Kuliopulos).
DisclosuresKuliopulos and L. Covic report serving as Founders of Oasis Pharmaceuticals. The other authors report no conflicts.
Primary Theme and Topics: Ischemia-- Neuroprotection and tolerance
AbstractIschemic stroke is a leading cause of adult disability, with limited treatments available aside from fibrinolytic therapy. The development of a dual antiplatelet/neuroprotective therapy would provide a potentially safer alternative to fibrinolytic therapy. We found that GPR31 (G-protein-coupled receptor 31)—a cell surface receptor for the 12(S)-HETE bioactive lipid, is an emerging therapeutic target that may play an important role in promoting stroke. We demonstrated that targeting GPR31 with a lipidated pepducin-based inhibitor, GPR310, attenuates both ischemic stroke and thrombosis without causing a bleeding phenotype. Using CRISPR technology, we generated and characterized a GPR31 knockout (Gpr31-KO) mouse which validated GPR31 as a therapeutic target by the suppression of both stroke and arterial thrombosis without causing a bleeding phenotype. GPR310 administered 5-h post transient middle cerebral artery occlusion (MCAO) in wild-type mice, demonstrated a highly significant protective effect of stroke infarct size, similar to protection conferred by Gpr31-KO. The safety, tolerability and inhibition of platelet aggregation of GPR310 was documented in non-human primates. Based on preliminary pharmacokinetics (PK) in baboons, the favorable PK supports further therapeutic advancement of GPR310. Mouse hippocampal areas CA1 to CA3 and dentate gyrus regions, cortex, piriform cortex, and thalamus all express GPR31 with cellular co-expression with the NeuN marker. The protective role of GPR310 in neuronal toxicity was demonstrate using mouse hippocampal HT22 cells, which have high surface expression of GPR31. GPR310 inhibited glutamate-mediated pERK1/2 and calcium activation, consistent with a neuro-protective function. Current studies are investigating the mechanism of action and function of neuronal vs. platelet GPR31 in stroke sequelae as well as its function in the mechanism of neuronal cell death, to further examine the potential of GPR31 as a therapeutic target in stroke.

Disclosures: L. Covic: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; PI.

Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

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KIAT/P0020818
Grant from the Jeollanam-do Science and Technology R&D Project (Development of stem cell-derived new drug)

Title: The Therapeutic Effects of Stem Cell-Derived Extracellular Vesicles in Ischemic Stroke

Authors: *E. JANG[1], H. YU[2], J. HWANG[2], J. YOO[3], J. CHOI[2], H.-S. JEONG[2], S. JANG[2]; [2]Physiol., [1]Chonnam Natl. Univ. Med. Sch., Jeollanamdo, Korea, Republic of; [3]Physical Educ., Chonnam Natl. Univ., Gwangju, Korea, Republic of

Abstract: Ischemic stroke, one of the leading causes of morbidity and mortality, is caused by ischemia and hemorrhage resulting in impeded blood supply to the brain. According to many studies, blueberries have been shown to have a therapeutic effect in a variety disease. Therefore, in this study, we investigated whether blueberry-treated mesenchymal stem cell (MSC)-derived extracellular vesicles (B-EV) have therapeutic effects in in vitro and in vivo stroke models. We isolated the extracellular vesicles using cryo-TEM and characterized the particles and concentrations using NTA. MSC-derived extracellular vesicles (A-EV) and B-EV were round with a lipid bilayer structure and a diameter of ~150 nm. In addition, A-EV and B-EV were shown to affect angiogenesis, cell cycle, differentiation, DNA repair, inflammation, and neurogenesis following KEGG pathway and GO analyses. We investigated the protective effects of A-EV and B-EV against neuronal cell death in oxygen-glucose-deprivation (OGD) cells and a middle cerebral artery occlusion (MCAo) animal model. The results showed that the cell viability was increased with EV treatment in HT22 cells. In the animal, the size of the cerebral infarction was decreased, and the behavioral assessment was improved with EV injections. The levels of NeuN and neurofilament heavy chain (NFH)-positive cells were also increased with EV treatment, yet decreased in the MCAo group. In addition, the number of apoptotic cells was decreased with EV treatment compared with ischemic animals following TUNEL and Bax/Bcl-2 staining. These data suggested that EVs, especially B-EV, had a therapeutic effect and could reduce apoptotic cell death after ischemic injury.

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Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

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Topic: C.09. Stroke

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Title: Genetic and Pharmacologic Inhibition of Histone Deacetylase 3 Improves Functional Outcomes Post-Intracerebral Hemorrhage via Reduction of Neuroinflammation.

Authors: *N. J. WATSON[1], S. SUKUMARI RAMESH[2];
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Abstract: Secondary brain injury is a leading cause of neurological deficits after intracerebral hemorrhage (ICH), a severe stroke subtype. Stimulation of the immune system at the site of a brain hemorrhage, characterized by microglial activation, leads to neuroinflammation and secondary brain damage. Our laboratory has previously demonstrated the potential of targeting Class 1 HDACs in improving outcomes post-ICH. Furthermore, genetic knockdown of HDAC3 in a macrophage cell line significantly attenuated hemin-induced release of TNF-α and IL-6 compared to a control, while genetic knockdown of both HDAC1 and HDAC2 significantly augmented hemin-induced release of TNF-α from cells without an effect on IL-6. To further extend this observation, we have generated microglia-specific HDAC3 conditional knockout using Cre-Lox technology. Both male and female microglia-specific conditional knockouts exhibited improved neurobehavioral outcomes compared to the experimental control at both acute and long-term time points. Further, cytokine expression analysis of the ipsilateral brain regions post-ICH using RT-qPCR has demonstrated a significant reduction of pro-inflammatory cytokines with a concomitant increase in anti-inflammatory cytokines, as well as a noted increase in cholesterol efflux related cytokines. This was also associated with reduced microglial activation in both male and female subjects, as evidenced by immunohistochemistry. Additionally, post-injury administration of a selective HDAC3 inhibitor, RGFP966, in mice improved functional outcomes compared to vehicle-treated control. RGFP966 treatment also reduced pro-inflammatory cytokine expression post-ICH, implicating HDAC3 inhibition as a potential novel therapy for ICH.

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Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

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Topic: C.09. Stroke

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Neuroscience Program, College of Medicine
Department of Chemistry and Biochemistry

John G. Kulhavi Professorship in Neuroscience, and E. Malcolm Field and Gary Leo Dunbar Endowed Chair in Neuroscience at Central Michigan University and a generous gift from Joan Allinder

Title: Reduction of neuroinflammation in MCAo rat brain tissue following progesterone treatment delivered using G4 PAMAM dendrimers intraperitoneally

Authors: *L. BOLEN[1,2,3,4], A. POUDEL[5,6,4], A. UPRETY[7,2,4], S. SCHWIND[7,4,2], B. SRINAGESHWAR[5,4,6], O. SMITH[5,4], G. L. DUNBAR[5,3,4], J. ROSSIGNOL[5,3,4,6];
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Abstract: The leading cause of disability and second most prevalent cause of death is stroke, which occurs when blood flow to the brain is obstructed. Stroke results in neuroinflammation and reduced brain structure from impairment and death of cells. Currently, options for the treatment of stroke are limited. This study evaluated the therapeutic effects of polyamidoamine (PAMAM) dendrimer encapsulated progesterone when delivered intraperitoneally into an ischemic stroke rat model. The neurosteroid progesterone is known to have anti-inflammatory and neuroprotective properties. Progesterone can pass freely through the blood-brain barrier (BBB) but is water-insoluble. Therefore, progesterone was encapsulated in PAMAM dendrimers to increase bioavailability and modify transport via the BBB. Hematoxylin and eosin (H&E) staining and immunohistological techniques were used to examine changes in inflammation and infarct size in the stroke brain following treatment. H&E staining was used to quantify stroke infarct volume, while IHC assessed the expression of inflammation using GFAP and IBA-1 markers. The rats underwent either sham or middle cerebral artery occlusion (MCAo) stroke surgery, received 10 intraperitoneal injections of dendrimer encapsulated progesterone every other day for 5 weeks, and then were euthanized after 35 days. Following euthanasia, brains were extracted, frozen, and sliced into 30 µm thick sections. Slices were then stained with H&E, anti-GFAP, and anti-IBA-1 antibodies, mounted, cover slipped on slides, and imaged. Our findings observed trends in reduced stroke volume following H&E in MCAo rats treated with the PAMAM dendrimer-progesterone complex and PAMAM dendrimer treatment alone. GFAP and IBA-1 expression were reduced in the same treatment groups, indicating promising effects of progesterone in reducing inflammation. Further, these results confirm previous findings of the role dendrimer alone has in anti-inflammation following stroke in MCAo rats.

Disclosures: L. Bolen: None. **A. Poudel:** None. **A. Uprety:** None. **S. Schwind:** None. **B. Srinageshwar:** None. **O. Smith:** None. **G.L. Dunbar:** None. **J. Rossignol:** None.

Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.17/D25

Topic: C.08. Ischemia

Support: AHA 23TPA1142407

Title: Estrogen receptor-beta activation improves stroke outcomes

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Abstract: Chronic 17β-estradiol (E_2) treatment has been shown to be neuroprotective in animal brain injury models. However, the failure to reproduce its beneficial effects in the clinic raised concerns regarding its safety. Our previous study demonstrated that a single bolus of E_2 pretreatment reduces ischemic brain damage in the ovariectomized rats via activation of estrogen receptor subtype beta (ER-β). Subsequently we demonstrated that either pre- or post-treatments with ER-β agonist reduced ischemic brain damage and cognitive deficits. As a logical continuation after our findings, the goal of the current study is to investigate the underlying mechanism responsible for the ER-β agonist-mediated neuroprotection. Given the role of E_2 in brain metabolism, we aim to investigate the effects of ER-β agonist pretreatment on the global metabolic changes in the brain of reproductively senescent (RS) female rats. Retired breeder (9-10 months) Sprague-Dawley female rats were considered RS after remaining in constant diestrus phase for more than a month. The RS rats were treated with either ER-β agonist (beta 2, 3-bis(4-hydroxyphenyl) propionitrile; DPN; 1 mg/kg; s.c.) or DMSO vehicle at 48 hr intervals for 10 injections. Forty-eight hours after last injection, brains were collected for unbiased global metabolomic analysis. The data analysis showed significant alterations in the metabolites of glycolysis and other carbohydrate pathways, amino acid metabolism, nucleotide metabolism, and lipid metabolism in the brains of ER-β agonist treated RS rats as compared to vehicle treated. Many of the observed metabolic changes after ER-β agonist treatment can boost brain energy production and support the brain under stress conditions such as ischemia. For example, ER-β agonist significantly decreased Glucose 6-phosphate (G6P) in the brain of RS female rats. A decrease in G6P levels might reduce the flux through the glycolytic pathway, potentially lowering oxidative stress and promoting cellular resilience. Reduced G6P levels could trigger stress response pathways such as the AMP-activated protein kinase (AMPK) pathway or the sirtuin pathway, which are involved in cellular adaptation to metabolic stress and may confer neuroprotective effects. Activation of these pathways due to ER-β agonist treatment may enhance cellular antioxidant defenses, improve mitochondrial function, and promote cellular repair mechanisms, thus protecting the brain from ischemic damage in RS female rats.

Disclosures: I. Cabeda Diaz: None. **Z. Bassett:** None. **K.R. Dave:** None. **M.A. Perez-Pinzon:** None. **A.P. Raval:** None.

Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

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Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 1R01NS117606-01A1
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Title: Elucidating the Role of miR-335-3p and ATP1A2 in Cerebral Edema Following Subarachnoid Hemorrhage: Insights from In Vivo and In Vitro Models"

Authors: *S. WU[1,2], H. HU[2], S. TABASSUM[2], A. GUSDON[2], C.-H. LEE[3], J. LEE[2], H. CHOI[2], X. S. REN[2];
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Abstract: Cerebral edema (CE) stands out as a significant feature of early brain injury following subarachnoid hemorrhage (SAH). ATP1A2 is a pivotal gene encoding the α2 subunit of the Na^+/K^+ ATPase (NKA) pump; an essential electrogenic transmembrane enzyme vital for maintaining membrane potential and osmotic equilibrium in cells. Dysfunction of the NKA in brain cells contributes to cerebral edema and neuronal cell swelling. MiR-335-3p, a microRNA, plays a role in regulating ATP1A2 levels. Alterations in miR-335-3p expression may lead to changes in the abundance of ATP1A2 protein, thereby affecting NKA function. Understanding the interaction between miR-335-3p and ATP1A2 is crucial for unraveling the mechanisms underlying cerebral edema and identifying potential therapeutic interventions. Utilizing *in vivo* and *in vitro* SAH models, this study aims to elucidate whether miR-335-3p attenuates cerebral edema after SAH by regulating ATP1A2 and affecting the function of NKA. We constructed an in vivo mouse model of SAH by endovascular perforation and assessed cerebral hemorrhage in the mouse model via magnetic resonance images (MRI). Our *in vivo* findings demonstrate a significant elevation in miR-335-3p levels within both serum and brain tissue 24 hours following SAH induction in mice. TargetScanHuman database analysis identified ATP1A2 as a direct miR-335-3p target. *In vitro* experiments employing hemin-induced HT-22 cells provided further insight into this regulatory mechanism. HT-22 cells were exposed to diverse concentrations of hemin (50,100, 200, 500 μM) for 24 h. We assessed cell death of hemin-induced HT-22 cells by Hoechst 33342/PI double staining assay using flow cytometry and fluorescence microscopy. The results showed that hemin reduced Ht-22 cell viability. In our *in vitro* SAH model, qPCR analysis revealed an upregulation of miR-335-3p expression, while flow cytometry demonstrated a concurrent downregulation of ATP1A2 expression. Subsequent intervention with antagomir-335-3p successfully restored ATP1A2 expression levels. Fluorescence microscopy and flow cytometry using sodium indicators demonstrated increased cellular swelling in the *in vitro* SAH model, significantly mitigated by antagomir-335-3p treatment. Collectively, our results identify

miR-335-3p as a biomarker for cellular swelling in SAH, elucidating its role in modulating NKA through ATP1A2 and highlighting its therapeutic potential in mitigating SAH-induced brain cell swelling.

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Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.19/D27

Topic: C.08. Ischemia

Support: AHA 23TPA1142407

Title: Electronic cigarette vapor exposure impairs catecholamine metabolism responsible for observed post-stroke cognitive decline

Authors: V. SANCHEZ[1], *H. PRADHYUMNAN[1], G. G. PEREZ[1], A. P. RAVAL[2,3]; [1]Leonard M. Miller Sch. of Medicine, Univ. of Miami, Miami, FL; [2]Neurol., Univ. of Miami, Miami, FL; [3]Bruce W. Carter Dept. of Veterans Affairs Med. Ctr., Miami, FL

Abstract: Widespread usage of nicotine-containing electronic cigarettes (EC) has raised the importance of characterizing how vaping affects stroke outcome [1]. Our published study showed that just 16 days of EC exposure results in worsened post-stroke cognitive decline in young rats of both sexes [2]. Furthermore, our study demonstrated that EC exposure altered brain energy metabolism. Brain energy metabolism can impact neurotransmitters (NT) metabolism and NT are vital for cognitive function. Therefore, the current study investigates how EC exposure affects NT metabolism in the brains of rats before and after induction of ischemia. Adult Sprague-Dawley rats of both sexes were randomly allocated to air or EC (5% nicotine Juul pods) exposure for 16 nights. After exposure, brain cortexes were collected from a cohort of rats for unbiased global metabolomic analysis. A second cohort was exposed to transient middle cerebral artery occlusion (tMCAO; 90 min) or sham surgery and survived 21 days. After 21 days rats were perfused with saline and 4% paraformaldehyde. The brains were sectioned coronally (10μm) for immunohistochemical staining. Three serial sections per animal starting at -5.30mm from Bregma were stained with anti-tyrosine hydroxylase (TH) antibody to visualize dopaminergic cells in the ventral tegmental area (VTA). Estimated population of TH-positive cells in the VTA was obtained using a brightfield stereoscope and the optical fractionator probe function in StereoInvestigator software with a counting frame of 50x50μm and ~30 sampling sites per section. Initial quantification shows that EC reduces number of TH-positive neurons. Metabolomic analysis indicated that EC resulted in increases ($p<0.05$) in phenylalanine, tryptophan, and glutamate metabolites, and both increases ($p<0.05$) and decreases ($p<0.05$) in

histamine and tyrosine metabolites in the brains of rats. Observed changes in NT metabolites due to EC were more prominent in females than males. Altered NT metabolism and release may be in part responsible for the observed post-stroke cognitive decline. Because of the relative novelty of EC, what impact EC has on NTs remains elusive and understanding the effects of EC on NT will help elucidate how EC exposure exacerbates ischemic brain damage.
Acknowledgements: We thank Ms. Ofelia Furones-Alonso for surgical and technical support.
References:[1] Siegel, J, Patel, SH, Mankaliye, B, & Raval, AP (2022). *Transl. stroke res*[2] Pradhyumnan, H, Patel, SH, Furones-Alonso, O, Zhao, W, Bramlett, HM, & Raval, AP (2024). *Stroke*

Disclosures: V. Sanchez: None. **H. Pradhyumnan:** None. **G.G. Perez:** None. **A.P. Raval:** None.

Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.20/D28

Topic: C.08. Ischemia

Support: AHA 23TPA1142407

Title: Electronic cigarette exposure worsens innate immune response and ischemic stroke outcomes in rats of both sexes.

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Abstract: The innate immune response plays a crucial role in the pathogenesis of ischemic stroke and a key component of the innate immune response is the inflammasome. In a published report, we observed amplified inflammasome activation after ischemia in the brains of nicotine exposed female rats [1]. Apart from conventional cigarettes, nicotine is the main ingredient of currently popular electronic cigarette (EC) devices and because of its relative novelty, our understanding of what impact EC has on the brain is limited. In a recent publication, we demonstrated that EC exposure exacerbates ischemic brain damage in female rats [2]. Therefore, the current study aims to investigate the impact of EC exposure on post-ischemic inflammasome activation in the brains of male and female rats. Since microglia play a key role in both innate and adaptive immune responses following ischemia, we also aim to evaluate the impact of EC exposure and ischemic episode on microglial activation in rat brains. Adult Sprague-Dawley rats of both sexes were randomly assigned to air/EC vapor (5% nicotine Juul pods) exposure for 16 nights, followed by transient middle cerebral artery occlusion (tMCAO; 90 mins) or sham

surgery. Animals were then divided into two cohorts. In the first cohort, cortical brain tissue was collected from animals 24h after tMCAO/sham surgery and used for western blotting of inflammasome proteins including Caspase-1, Interleukin-1β (IL-1β), apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and gasdermin-D (GSDMD). Female animals exhibited elevated levels of caspase-1 and ASC proteins in the ipsilateral cortex post-stroke. In a subsequent study, rats were sacrificed 21 days post-tMCAO for immunohistochemistry analysis of microglial marker ionized calcium-binding adapter molecule-1 (Iba-1). The estimate of activated microglia was obtained across three serial coronal sections (10μm) using the optical fractionator probe function in StereoInvestigator software with a counting frame of 100x100μm and approximately 40 sampling sites per section. Results indicated increased activated microglia in the cortex of female rats exposed to EC, potentially contributing to exacerbated infarction post-stroke.
Acknowledgements: We thank Ms. Ofelia Furones-Alonso for surgical expertise.
References:[1] d'Adesky, ND, de Rivero Vaccari, JP, Bhattacharya, P, Schatz, M, Perez-Pinzon, MA, Bramlett, HM, & Raval, AP (2018). *IJMS*, 19(5), 1330.[2] Pradhyumnan, H, Patel, SH, Furones-Alonso, O, Zhao, W, Bramlett, HM, & Raval, AP (2024). *Stroke*, 55(3), 735–746.

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Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.21/D29

Topic: C.08. Ischemia

Support: TCRD110-63

Title: Glycogen synthase kinase 3 beta inhibition affords neuroprotection after asphyxial cardiac arrest

Authors: *P.-Y. CHEN[1,2], J.-H. LIN[3], P.-C. TING[5,4], Y. SU[1], T.-L. TSENG[1], K.-T. YANG[6]; [1]Cardiovasc. Res. Ctr., [2]Med. Res., [3]Div. of Exptl. Surgery, Dept. of Surgery, [4]Dept. of Surgery, Hualien Tzu Chi Hosp., Hualien, Taiwan; [5]Ph.D. Program in Pharmacol. and Toxicology, Sch. of Med., [6]Dept. of Physiol., Tzu Chi Univ., Hualien, Taiwan

Abstract: Cardiovascular disease is one of the leading causes of morbidity and mortality in a well-developed society. High-risk cardiovascular diseases include hypertension, arrhythmia, and cardiac arrest. Cardiac arrest can lead to global cerebral ischemia that can impact brain function especially in the most vulnerable area, the hippocampus. The damaged hippocampus leads to learning and memory deficits. The activated glycogen synthase kinase (GSK)-3β is reported to cause neuroinflammation after cerebral ischemia. GSK-3β inhibition attenuated ischemia-induced neuron damage and alleviated neuronal dysfunction. Our goal is to determine the

potential ability of GSK-3β inhibition against cardiac arrest-mediated cerebral ischemia. Global cerebral ischemia/reperfusion is induced by 6 minutes asphyxial cardiac arrest (ACA) and resuscitation in Sprague Dawley rat. The vehicle (DMSO, ip.) or a GSK-3β inhibitor, IM-12 (3 mg/kg, ip.), was pretreated 15 minutes prior to asphyxial cardiac arrest induction. Behavior function was evaluated in working memory utilizing Y-maze 1 day after ACA. The alternation rate in Y-maze was significantly inhibited in vehicle+ACA (47.36 ± 4.41%) as compared to sham (74.42 ± 2.51%) rats. IM-12 (63.4 ± 2.21%) pretreatment significantly revived the alternation rate as compared to vehicle+ACA rat. To further identify the possible mechanism of IM-12 pretreatment. The synaptic mechanism was determined by synaptic protein expression. The expression of synaptic Synaptosomal-Associated Protein, 25kDa (SNAP25), was enhanced in DMSO+ACA and increased in IM-12+ACA rats, suggesting synaptic function was preserved in IM-12 pretreatment. In conclusion, our data indicated that pretreatment with IM-12 15 minutes before ACA effectively alleviated neuronal dysfunction, which suggested the potential ability of GSK-3β inhibition in neuroprotection after cardiac arrest-induced global cerebral ischemic/reperfusion injury.

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Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.22/Web Only

Topic: C.08. Ischemia

Support: Heart & Stroke Foundation of Canada
Canada Foundation for Innovation (CFI)
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Title: Effects of selective brain cooling on blood flow and glucose metabolism in acute cerebral ischemia

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Abstract: Following ischemic stroke, inflammatory pathways could activate oxidative stress, release pro-inflammatory cytokines, and disrupt the blood-brain barrier. Therapeutic

hypothermia has been proposed to limit inflammation-induced (secondary) brain injuries and improve clinical outcomes. However, clinical studies have failed to demonstrate an effect, and hypothermia has yet to be translated into the clinic, partially due to the paucity of effectiveness data in large animals. We hypothesized that selective brain cooling (SBC) would maximize neuroprotection and minimize adverse effects of whole-body cooling. 54 adult pigs were divided into the ischemic (n = 16 vs. 26; untreated vs. treated) and normal (n = 12) groups. Focal ischemia was induced by intraparenchymal injection of a vasoconstrictor (ET-1), and SBC treatment (2-3°C decrease from normothermia) was administered via intranasal cooling. Standard physiological variables and CBF measured by CT perfusion were collected in all subjects. Four treated ischemic subjects also underwent perfusion-metabolism imaging using ^{18}F-fluorodeoxyglcuose (FDG). Mortality was 77% and 25% between the untreated and treated groups, respectively ($p < 0.05$). In the ischemia group, intracranial pressure (ICP) before sacrifice was significantly lower in the treated group than in the untreated group ($p < 0.01$). In untreated ischemic animals, we observed a sustained increase in CBF (hyperemia) after reperfusion; whereas in treated ischemic animals that survived, this increase was absent. Surviving-treated animals had elevated metabolic rates 4h post-injury, returning to baseline during rewarming. Conversely, those that died exhibited increased CBF but decreased metabolic rates compared with baseline. This could be due to excessive pro-inflammatory cytokines that disrupt glucose uptake and metabolism. Additionally, the fatal mismatch between CBF and metabolic demand in deceased treated animals suggests impaired autoregulation and maximal vasodilation. Secondary injuries further exacerbated tissue damage by increasing vascular permeability (i.e., raising ICP and decreasing blood pressure). In contrast, successful SBC moderated immune responses and preserved autoregulation to improve survival. Hence, hyperemia could be an effective biomarker to assess SBC efficacy. Future strategies might utilize CBF-guided SBC to tailor treatment for patients with ischemia.

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Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.23/D31

Topic: C.08. Ischemia

Title: Let-7i inhibition as a novel adjuvant intervention for the treatment of ischemic stroke

Authors: *V. R. KRISHNAMOORTHY[1,2], S. KIM[3], H. LAPORTE[4], T. NGUYEN[5], M. SINGH[6];
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Chicago, IL; [4]Physiol., Loyola Univ. Chicago Hlth. Sci., Maywood, IL; [5]Dept. of Pharmacol. and Neurosci., Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX; [6]Cell and Mol. Physiol., Loyola Univ. Chicago, Maywood, IL

Abstract: Ischemic stroke, a leading cause of death worldwide, and the leading cause of permanent disability in adults, results from disrupted blood flow to the brain. Recombinant tissue plasminogen activator (rtPA) is the only FDA-approved pharmacological treatment option for ischemic stroke but is limited by the window of therapeutic opportunity. This underscores the critical need for improved treatment options for ischemic stroke. Progesterone (P4) is a cholesterol-derive steroid hormone that is protective in experimental models of ischemic stroke, but the mechanisms by which this occurs are poorly understood. Previously, our lab demonstrated that the microRNA, let-7i, is upregulated in primary cortical astrocytes following ischemic stroke. Furthermore, this effect was associated with reduced expression of two critical mediators of P4's protective effects, namely the membrane progesterone receptor, progesterone receptor membrane component 1 (PGRMC1), and brain-derived neurotrophic factor (BDNF). Based on these findings, we hypothesized that combined treatment of P4 and a let-7i inhibitor (let-7i antagomir) will enhance P4-induced neuroprotection following stroke. While initial data support our hypothesis, the cellular mechanisms remain unclear. In this study, an in vitro model of ischemic stroke, using oxygen/glucose deprivation (OGD), was established to explore the cellular mechanisms of our novel intervention in primary cortical neurons. Neurons were isolated from post-natal day 2 mouse pups and exposed to OGD conditions (0.1% O_2) for 1 hour. Our data indicate that exposure to 1 hour of OGD results in a reduction in PGRMC1 and BDNF protein expression in primary cortical neurons. Interestingly, our data shows that gene expression of PGRMC1 and BDNF does not change in neurons immediately following exposure to our OGD conditions. These data suggest that the therapeutic effects of let-7i inhibition, at early time points following stroke, may be mediated by a cell-specific response, and implicate astrocytes as the cellular target that facilitates the protective effects of our novel intervention.

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Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Title: Sex and Circuit Specific Amygdala Dysfunction After Global Cerebral Ischemia

Authors: *J. VIGIL;
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Abstract: Modern medical advances have increased the odds of surviving an ischemic event such as cardiac arrest or stroke. With more people surviving and recovering from these ischemic insults, it is apparent that survivors experience long-term effects on brain function. However, no study has attempted to identify amygdala dysfunction after global cerebral ischemia (GCI), despite clinical evidence of emotional dysfunction. Therefore, it is important to identify the effect that GCI has on the amygdala, the emotional center of the brain. I hypothesize GCI induces dysfunction of L-type calcium channels (LTCCs) within the basolateral amygdala (BLA) thereby contributing to deficits in amygdala-dependent behavior and LTP in male mice. GCI was induced in adult mice via cardiac arrest and subsequent cardiopulmonary resuscitation (CA/CPR) for 8-minutes before resuscitation by epinephrine injection, ventilation, and mild chest compressions. Delay fear conditioning was used to assess amygdala-dependent learning and memory, synaptic plasticity was evaluated by performing LTP recordings in the BLA, LTCC function was assessed using whole-cell voltage clamp recordings, and neuronal injury was evaluated by Fluorojade staining. Behavioral testing revealed that only male mice are diminished in their ability to form associative memories. Similarly, plasticity of the cortical inputs to the BLA are impaired only in males, however, intra-amygdala recordings revealed no disruption of LTP, whole-cell LTCC mediated currents were minimally affected by GCI, and there is no cell death within the BLA of either sex. Additional 2-photon calcium imaging experiments will evaluate LTCC function at more distal synapses after GCI. These results support the role of the amygdala in cognitive-affective impairments after CA. We have revealed a sex and circuit specific deficit in amygdala function that provides new insights into the role that biological sex plays in mediating brain dysfunction following CA.

Disclosures: J. Vigil: None.

Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.25/D33

Topic: D.06. Vision

Title: Neural Networks Implicated in Cerebral Achromatopsia

Authors: *M. MARCUCCI[1], J. A. NIELSEN[2], O. BENZLEY[3], F. SCHAPER[4], M. FERGUSON[4];
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Abstract: Our objective is to identify the brain regions and networks involved in lesion induced cerebral achromatopsia. Cerebral achromatopsia is a perceptual disorder characterized by the partial or complete loss of color vision. It typically results in experiencing the world in shades of gray or sepia tones without damaging other aspects of perceptual vision. Cerebral achromatopsia can be caused by brain lesions such as strokes, encephalitis, tumors, or traumatic brain injuries. Previous research using voxel-wise lesion overlap analysis, which only relies on the lesion site, found evidence of lesion overlap in the ventro-medial occipital, temporal lobes, fusiform gyri, and right hippocampus. However, symptoms may better localize to brain networks instead of brain regions. We utilized lesion network mapping, a method that identifies the brain regions functionally connected to the lesion site rather than focusing on the lesion location itself. We performed a systematic literature review to identify relevant case studies including patients showing signs and symptoms of cerebral achromatopsia (n=58). Lesion network mapping analysis was performed on the achromatopsia cases, using a large resting state functional connectivity dataset derived from healthy controls (n=1000). Many of the cases included in the analysis where due to ischemic stroke, while others were incidents from hemorrhagic strokes, brain trauma, encephalitis, or tumors. The analysis showed functional connectivity to the parahippocampal gyrus and hippocampal zones. While our results align with previous reports finding lesions in the right hippocampus, the more general hippocampal zones were identified as novel regions in the network of cerebral achromatopsia. Further research is necessary to determine the specific roles that these networks play and how they might interact with each other to cause cerebral achromatopsia.

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Poster

PSTR398

Stroke and Ischemia: Therapeutic, Interventional, and Translational Studies

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Program #/Poster #: PSTR398.26/D34

Topic: C.08. Ischemia

Title: Effects on axonal and myelin properties in the corpus callosum of a rat model of acute cerebral ischemia following intravenous infusion of mesenchymal stem cells

Authors: *T. YOKOYAMA, H. NAGAHAMA, M. NAKAZAKI, R. UKAI, S. OKA, Y. KATAOKA-SASAKI, M. SASAKI, O. HONMOU;
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Abstract: Intravenous infusion of mesenchymal stem cells (MSCs) promotes functional improvements in rodent models of cerebral ischemia. Several mechanisms have been proposed, including induced neural plasticity as indicated by increased thickness of the corpus callosum (CC). In this study, we investigated the fine morphological changes in the CC by focusing on the characteristics of axons and myelin on the CC. We induced transient middle cerebral artery occlusion (MCAO) for 90 minutes. Two hours after MCAO induction, in vivo diffusion-weighted images (DTI) were obtained to evaluate infarct size to meet our inclusion criteria. The MCAO rats were then randomized into two groups (MSC group and vehicle group). The MCAO rats were intravenously infused with MSCs 6 hours after MCAO induction. Behavioral tests and in vivo T2-weighted images (T2WI) were performed during the study period. After 42 days, rats were transcardially perfused and their brains were harvested for histologic analysis. Functional improvement and reduction in high-intensity volume of T2WI were observed in the MSC group compared to the vehicle group, which is consistent with our previous studies. Ex vivo T2WI and light microscopic observation with toluidine blue-stained plastic section showed an increased thickness of CC in the MSC group compared to the vehicle group. In ex vivo DTI analysis, DTI tractography showed a greater number of tracks in the MSC group than in the vehicle group. In addition, the profile of DTI diffusivity showed significant changes in fractional anisotropy (FA) and radial diffusivity (RD). FA in the MSC group was higher than the vehice group, and RD in the MSC group was lower than the vehicle group. Electron microscopy in the trunk of CC also showed significant changes in axon and myelin profiles. Axon diameter and myelin thickness of myelinated axons were greater in the MSC group than in the vehicle group. These results suggest that improvement of motor function in the stroke rat model is associated not only with myelin thickness but also with axon profiles.

Disclosures: T. Yokoyama: None. **H. Nagahama:** None. **M. Nakazaki:** None. **R. Ukai:** None. **S. Oka:** None. **Y. Kataoka-Sasaki:** None. **M. Sasaki:** None. **O. Honmou:** None.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.01/D35

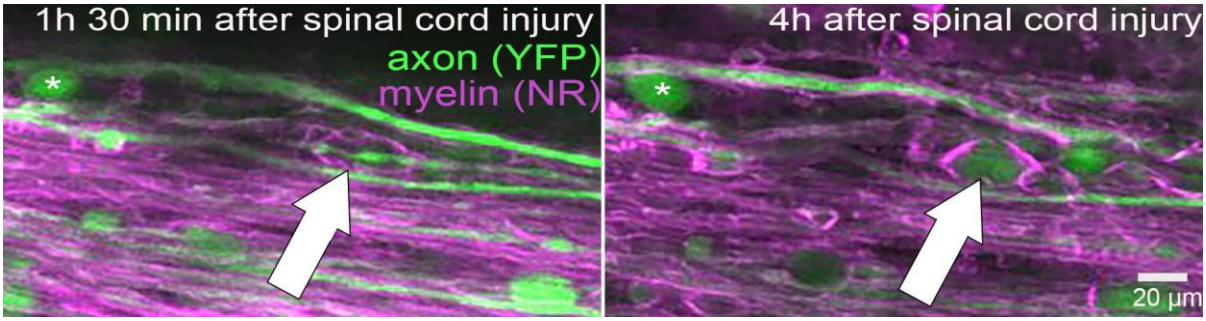
Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life Spinal Cord Research Foundation, WFL-US-14/22

Title: Na-k-cl cotransporter 1 inhibition protects the axo-myelinic interface and improves neurological outcomes after contusive spinal cord injury

Authors: *S. AMES[1,2], J. BROOKS[1,2], E. R. JONES[2,1], F. CORTEZ-THOMAS[2,1], J. MOREHOUSE[2,1], D. DESTA[2,1], D. P. STIRLING[3,2,1];
[2]Kentucky Spinal Cord Injury Res. Ctr., [3]Neurolog. Surgery, [1]Univ. of Louisville, Louisville, KY

Abstract: Ultra-structural studies of compressive and contusive spinal cord injury (SCI) have shown that the most prominent acute changes in white matter are periaxonal swelling and separation of myelin away from their axon, axonal swelling, and axonal spheroid formation. However, the underlying cellular and molecular mechanisms that cause periaxonal swelling and the functional consequences are poorly understood. Utilizing *in vivo* longitudinal imaging of *Thy1*YFP^{+} axons and myelin labeled with Nile red, we have shown that periaxonal swelling significantly (ANOVA on Ranks, $p < 0.001$; post hoc Dunn's method, $p < 0.05$; n =2-11/ timepoint) increases acutely (24 hours) following a contusive SCI (T13, 30 kdyn, Infinite Horizons Impactor) and precedes axonal spheroid formation. In addition, using longitudinal imaging to visualize the fate of the same myelinated fibers acutely after SCI, we have determined that ~73% of myelinated fibers present with periaxonal swelling at one hour post SCI and ~51% of those fibers transition to axonal spheroids by four hours post SCI (Binomial proportion test, $p < 0.005$, n = 5). As cation-chloride cotransporters are localized to regions of the internode and regulate cell volume, we hypothesized that inhibiting Na-K-Cl cotransporter 1 (NKCC1) or activating K-Cl cotransporter 2 (KCC2) would prevent periaxonal swelling after SCI. We found that inhibition of NKCC1 using bumetanide (30 mg/kg, 1h and 4h post-SCI) significantly (Mann Whitney U test, $p < 0.05$) reduced acute periaxonal swelling and increased (One-way ANOVA, $p < 0.05$; Bonferroni post hoc t-test, $p < 0.05$) axonal survival at 24h after T9, 50 kdyn contusive SCI versus vehicle controls (n=6-7/group). Furthermore, NKCC1 inhibition significantly improved finer aspects of locomotor recovery (Binomial proportion test, $p < 0.001$) and increased white matter sparing (One-way ANOVA, $p < 0.05$; Bonferroni post-hoc t-test, $p < 0.05$) at 6 weeks after SCI. Collectively, these data reveal a novel role for NKCC1 in periaxonal swelling and secondary axonal loss after SCI.



Disclosures: S. Ames: None. **J. Brooks:** None. **E.R. Jones:** None. **F. Cortez-Thomas:** None. **J. Morehouse:** None. **D. Desta:** None. **D.P. Stirling:** None.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.02/D36

Topic: C.11. Spinal Cord Injury and Plasticity

Support: R01 5R01NS129987-02

Title: Investigating the effects of SOCE inhibition on functional and histological recovery in a murine contusive spinal cord injury model

Authors: *E. R. JONES[1], J. BROOKS[1], J. MOREHOUSE[1], D. DESTA[1], D. P. STIRLING[1,2,3]; [1]Kentucky Spinal Cord Injury Res. Ctr., Univ. of Louisville, Louisville, KY; [2]Department of Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY; [3]Department of Neurological Surgery, University of Louisville, Louisville, KY

Abstract: Store-operated calcium entry (SOCE) plays a crucial role in cellular processes, including cellular calcium homeostasis and signaling. We and others have shown that dysregulation of SOCE has been implicated in neurological disorders and CNS trauma; however, the underlying mechanisms remain poorly understood. To further our knowledge of the key SOCE effectors stromal interaction molecules (STIM) and Orai channels on functional and histological outcomes after spinal cord injury (SCI), we investigated the effects of three pharmacological agents known to interfere with this signaling pathway: DPB162-AE (STIM inhibitor), YM-58483 (Orai channel inhibitor), and 2-APB (IP3R inhibitor). Following acclimation and baseline testing, female 6-8-week-old C57Bl/6 mice were randomized into seven treatment groups and given a 50 kilodyne contusion at T9/10. DPB162-AE (3 µM), 2-APB (100µM), 2-APB + DPB162-AE (combined treatment), and QD vehicle control were all given treatments intrathecally (IT) 1 hour post SCI and then QD for 7 days post SCI. Additionally, DPB162-AE, YM-58483 (500 nM), and BID vehicle control were each given treatments IT 1 hour post SCI and then BID for 5 days post SCI. Each treatment paradigm was compared to respective vehicle controls. Behavioral assessments, including open-field (Basso Mouse Scale (BMS) and BMS subscore) and horizontal ladder, were conducted by blind assessors. Mice were euthanized 6 weeks post SCI, and the cords were prepared for histological evaluation. 2-APB + DPB162-AE QD treated mice exhibited improved functional outcomes in comparison to controls, attaining significantly (Repeated measures ANOVA, $p<.001$; Bonferroni, $p<.05$, n=10-11/group) higher BMS scores at weeks 4 and 6. DPB162-AE QD and 2+APB + DPB162-AE QD treated mice also had greater proportions of mice with high BMS subscores at weeks 3-6 (Binomial proportions, $p=.035$, n=10-11/group). Histological assessments of white matter sparing at the injury epicenter revealed no significant differences between treatment groups versus their respective controls. Surprisingly, 2-APB + DPB162-AE QD treated mice had a significantly smaller percentage of white matter spared 2 mm rostral (Repeated measures ANOVA, $p=.004$, Bonferroni, $p=.007$, n=9-10/group) of the epicenter in comparison to controls. These findings suggest usage of DPB162-AE QD and 2-APB + DPB162-AE QD as a therapeutic treatment for improving functional recovery following SCI and introduces a novel target for SOCE inhibition following SCI.

Disclosures: E.R. Jones: None. **J. Brooks:** None. **J. Morehouse:** None. **D. Desta:** None. **D.P. Stirling:** None.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.03/D37

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant R01-NS-105987
NIH Grant F31-DK-136279
Eberly Endowment Fund from PSUCOM

Title: Voltage-gated calcium channel dysfunction after spinal cord injury

Authors: H. GOUDSWARD[1], V. RUIZ-VELASCO[2], S. L. STELLA, Jr.[3], *G. M. HOLMES[3]; [1]Penn State Col. of Med. Neurosci. Grad. Program, Hershey, PA; [2]Anesthesiol., Penn State Col. of Med., Hershey, PA; [3]Penn State Univ. Col. of Med., Hershey, PA

Abstract: Previous studies have demonstrated gastric vagal afferents are less sensitive to mechanical and chemical stimuli after traumatic spinal cord injury (SCI). This altered sensitivity may be due to changes in the electrophysiological properties of voltage-gated Ca^{2+} (Ca_V) channels in gastric-projecting nodose ganglia (NG) neurons. Ca_V channels play a critical role in the transduction of sensory information, as they have a primary role in neurotransmitter release at central synapses. Therefore, reduced function of Ca_V channels after SCI would significantly impair vagal afferent signaling. Using whole-cell patch clamp electrophysiology and immunohistochemistry, we assessed the biophysical properties and expression profile of Ca_V channels in gastric NG neurons from male Wistar rats (age ≥8 weeks) 3-days or 3-weeks following T3-SCI. We found that while there were no changes in the biophysical properties of Ca_V channels in gastric NG neurons 3-days following T3-SCI ($p=0.3957$), there was a significant ($p=0.0006$) reduction in the peak current density in those isolated after 3-week SCI (n=19, peak current density=16.41±2.41 pA/pF) as compared to surgical controls (n=12, peak current density=39.92±5.63 pA/pF). We also used subtype-specific blockers to evaluate which Ca_V channel subtype carried the majority of Ca^{2+} currents in these cells, and found that N-type Ca^{2+} channels were the predominant subtype in acute SCI (%Ca^{2+} current inhibition by ω-conotoxin= 60.0±6.6%, n=13), chronic SCI (63.1±9.7%, n=10), and surgical control groups (63.4±5.0%, n=20). These findings suggest reduced Ca_V channel function develops following chronic SCI in gastric NG neurons, contributing to reduced vagal output at central synapses.

Disclosures: H. Goudsward: None. **V. Ruiz-Velasco:** None. **S.L. Stella:** None. **G.M. Holmes:** None.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.04/D38

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Molecular Docking Study of Dapsone on the NMDA Receptor to Establish a Potential Anti-hyperalgesic and Antioxidant Mechanism

Authors: *H. ROMERO SÁNCHEZ, Jr[1], A. MATA-BERMUDEZ[2], C. RIOS[3], A. DIAZ-RUIZ[4];
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Abstract: Research Objective andRationale:The objective of this study is tounderstand, through molecular docking techniques, the interaction betweendapsone and the NMDA receptor to elucidate its potential anti-hyperalgesicmechanism. The activation of the NMDA receptor is crucial in the etiology ofpain, and previous studies by our group have shown that dapsone is a partialantagonist of this receptor. Understanding this interaction could guide thedesign of new therapies for chronic pain conditions, providing new insights inpharmaceutical chemistry and biomedical research.**Methods:**Three-dimensional structures ofdapsone and the NMDA receptor were prepared in formats compatible with AutoDockVina. Docking parameters were set, including the search box size and searchparameters. Scientific rigor was ensured through appropriate controls andreplication of experiments.**Results:**Molecular docking analysisrevealed the formation of stable complexes between dapsone and the NMDAreceptor with specific binding affinities at the binding sites. This indicatesthe potential ability of dapsone to modulate NMDA receptor activity.**Conclusions:**These findings highlight theimportance of understanding at the molecular level how dapsone interacts withthe NMDA receptor, which can have significant implications in the design oftargeted therapeutic compounds. This study lays the foundation for furtherinvestigations to validate and explore these mechanisms more deeply, aiming todevelop effective therapies. **Scientific Rigor**:Blinding techniques were used,and experiments were replicated to ensure reproducibility. Sample sizes andcontrols were adequate to ensure the validity of the findings.**Biological Variables**:Computational models were used tostudy the interaction between dapsone and the NMDA receptor, without the directinvolvement of biological subjects at this stage of the study.**Funding Sources and Conflictsof Interest:**This study was funded by HéctorAlonso Romero Sánchez. No conflicts of interest are declared**Licensing and Compliance:**Thiswork complies with SfN policies and principles, including guidelines for theuse of animals and humans in research.

Disclosures: H. Romero Sánchez: None.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.05/D39

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH R01 NS128086
Lone Star Paralysis Foundation Gift
Neuraptive Texas

Title: Comparing and contrasting natural versus artificial repair of plasmalemmal lesions in giant axons

Authors: *M. L. MENCEL[1], R. SOOD[2], G. D. BITTNER[3];
[1]Cell and Mol. Biol., The Univ. of Texas at Austin, Austin, TX; [2]Univ. of Texas at Austin, Austin, TX; [3]Inst. for Neurosci., Univ. of Texas at Austin, Austin, TX

Abstract: Lesioned or transected membrane-bound cytoplasmic processes (such as nerve axons) must rapidly seal to prevent degeneration/apoptosis. We report that neurons (and likely all eukaryotic cells) seal traumatic plasmalemmal lesions by the progressive accumulation of injury-induced vesicles that form a functional barrier at the lesion site. This process is initiated by calcium influx across the lesion site that induces vesicle formation. Upon transection, the cytoskeleton is disrupted and reorganizes to direct transport towards the injury site. Vesicle migration may be facilitated by transport along cytoskeletal tracks or the bulk flow of axoplasm out of the injury site. Inhibitors of cytoskeletal polymerization or molecular motor proteins increase the time to form a vesicle seal. Both vesicle formation and transport require the use of molecular motor proteins. Natural (vesicle-mediated) repair seals transected axons such as crayfish giant axons (GAs) by formation of a vesicular plug within 15-20 minutes. Repair of transected GAs can also be artificially-induced through the use of amphoteric substances such as polyethylene glycol (PEG). When PEG is applied to the cut end of a cytoplasmic process, closure (sealing) of the transection occurs by the very rapid (milliseconds to seconds) collapse and fusion of axolemmal leaflets. PEG-sealing of transected GAs occurs in both calcium-containing and calcium-free solutions, indicating that PEG bypasses all known biochemical pathways involved in natural repair. Inhibition of cytoskeletal dynamics and molecular motor proteins appears to have no effect on PEG-sealing of GAs. The presence of vesicles at the cut end does not interfere with PEG-sealing of transected GAs. The rapid sealing of plasmalemmal lesions and/or complete transections by PEG-sealing could aid in the repair of ischemic cells. Better understanding of PEG-sealing should also help develop protocols to enhance behavioral recovery following PEG-fusion repair of peripheral nerve injuries.

Disclosures: M.L. Mencel: None. **R. Sood:** None. **G.D. Bittner:** None.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.06/D40

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life, Spinal Cord Research Foundation (Grant Number: WFL-UY-13/23, Project # 290)
Morton Cure Paralysis Fund
Agencia Nacional de Investigación e Innovación (grant FCE_3_2022_1_172524)

Title: Role of purinergic and connexin signaling in the awakening of a stem cell niche in the spinal cord

Authors: *M. G. FABBIANI CARLOS[1], M. V. FALCO PASTORINO[1], C. MACIEL[1], S. VALDIVIA[2], N. VITUREIRA[3], R. E. RUSSO[1];
[1]Neurofisiologia Celular y Mol., IIBCE, Montevideo, Uruguay; [2]Inst. of Physiol. and biophysics "Bernardo Houssay "(Univ. of Buenos Aires, UBA - Natl. Council for scientific and Tech. Res., CONICET), Buenos Aires, Argentina; [3]Physiol., Facultad de Medicina, Univ. de la República, Montevideo, Uruguay

Abstract: The ependyma of the spinal cord is a latent stem cell niche that is reactivated by injury to contribute new cells to the glial scar. However, the mechanisms by which ependymal cells are reactivated by spinal cord injury (SCI) remain poorly understood. Purinergic signaling may have a role as extracellular ATP rises after SCI and EC have functional P2X7 receptors (P2X7r). In addition, we have shown that after SCI, gap junction blockade prevents injury-induced proliferation. We speculate that purinergic and connexin (Cx) signaling may be important to the reactivation of the ependymal stem cell niche.To explore the role of P2X7r we injected the selective analog BzATP nearby the central canal. We tested ependymal cell proliferation by EdU uptake and Cx26 expression by immunohistochemistry after SCI or in vivo injection of BzATP. Glial fibrillary acidic protein (GFAP) expression was monitored by using a GFAP-EGFP transgenic mouse. To address the impact of Cx26 we used a Cre-lox system in adult mice to selectively delete Cx26 from EC. We found that similar to injury, injection of BzATP induced the proliferation of ependymal cells and shifted ependymal cells to a GFAP phenotype. BzATP did not induce these changes in ependymal cells of P2X7r knock out mice. In vivo blockade of P2X7r with the potent and selective antagonist AZ10606120 reduced significantly the injury-induced proliferation of ependymal cells. Like injury, P2X7r activation led to the expression of Cx26. Remarkably, genetic deletion of Cx26 in EC prevented the effect of P2X7r activation on ependymal cell proliferation. Our results show that purinergic and Cx signaling are key pathways for the reactivation of the ependyma by injury. Cx26 seems to be downstream to P2X7r and appears as a relevant target to modulate the reaction of EC to injury to achieve better self-repair.

Disclosures: M.G. Fabbiani Carlos: None. **M.V. Falco Pastorino:** None. **C. Maciel:** None. **S. Valdivia:** None. **N. Vitureira:** None. **R.E. Russo:** None.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.07/D41

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH ROI NS047567
CIHR PJT 165823

Title: Gaba receptors at nodes of ranvier facilitate sensory perception and movement

Authors: *K. HARI[1], A. M. LUCAS-OSMA[1], H. ZHANG[2], N. DE SILVA[1], K. K. FENRICH[2], D. J. BENNETT[3];
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Abstract: While accurate proprioceptive feedback is critical for precise movement, less accurate feedback is needed for more routine movements, implying that feedback sensitivity may be modulated on the fly, and the brain must be informed of these sensitivity changes to avoid perceptual mismatch and errors. Even though presynaptic GABAergic innervation of sensory axons has traditionally been assumed to simply modulate sensory transmission to motoneurons (reflexes), this view has recently been challenged by the discovery that GABAA receptors on the nodes of Ranvier in sensory axons in the dorsal columns directly increase sodium spike propagation, implying that GABA may directly modulate sensory perception, as well as reflexes. To begin understanding the role of axonal GABA in sensory feedback sensitivity and perception, we knocked out the function of specialized GABAergic neurons that innervate sensory axons (GAD2 KO mice), the alpha5 GABA receptors that plays an outsized role in axon modulation (GABRA5 KO mice), or the V3 neurons that innervate GAD2 neurons (Sim1//VGLUT2 KO mice). These mice exhibited no gross motor deficits, including walking overground fairly normally. However, when challenged to walk on a horizontal ladder, they appeared to have little precise perception of limb position, missing rungs ~50% of the time. By directly recording sensory transmission in proprioceptive afferents in vitro, we demonstrate that this deficit is due to increase spike transmission failure through nodes of Ranvier, which is associated with a loss of GABAergic depolarization of axon nodes (PAD). This leaves animals in a permanent low sensitivity state. Furthermore, chronic spinal cord injury led to an opposite situation with permanently increased sensory transmission and PAD, and upregulated nodal GABAA receptors, making a heightened sensitivity state. We discuss how this may be a compensation to not only help restore reflexes but also augment sensory perception mediated by residual ascending sensory axons spared by the injury. Together these findings suggest that GABAergic systems play a critical role in proprioception and execution of precise movements, the understanding of which may help designing improved treatments for spinal cord injury.

Disclosures: K. Hari: None. **A.M. Lucas-Osma:** None. **H. Zhang:** None. **N. De Silva:** None. **K.K. Fenrich:** None. **D.J. Bennett:** None.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.08/D42

Topic: C.11. Spinal Cord Injury and Plasticity

Support: SC1GM144032
NIH Grant P20-GM103642
PRSTT 2022-00125

Title: The effect of Tamoxifen on metabolic patterns and contractile properties of skeletal muscle after spinal cord injury

Authors: *V. GONZALEZ[1], S. OCASIO[1], N. E. CHORNA[1], I. SALGADO[2], M. E. SANTIAGO-GASCOT[1], J. M. SANTIAGO SANTANA[3], A. TORRADO-TAPIAS[1], J. COLON MERCADO[1], W. FRONTERA[4], J. D. MIRANDA[1];
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Abstract: Treating spinal cord injuries (SCI) come with a series of challenges since it is a condition for which no cure is available. Severe symptoms can be observed in SCI patients, including somatosensory deficits, chronic pain, muscle mass reduction and loss of movement. Tamoxifen (TAM), a selective estrogen receptor modulator demonstrated beneficial effects in the spinal cord. However, the effect of TAM in skeletal muscle is unknown and this should be established because nerve regeneration and cell survival after SCI is hopeless if muscle deterioration (muscle atrophy) is beyond repair. We hypothesize that administration of TAM after SCI will maintain the metabolite profile similar to the sham group in skeletal muscles and increase the expression of myosin heavy chain (MHC) in these muscles. This study sought to elucidate important changes in skeletal muscle physiology and its contractile properties after SCI and TAM treatment. Adult female rats (Sprague Dawley) received a moderate contusion and control group had only laminectomy, animals from both groups received either TAM or placebo pellets and hindlimbs muscles extracted at 28 days post injury, to which metabolomic analysis, western blot and single fiber contractile properties measurements were performed. Orthogonal Partial Least Square Discriminant Analysis (OPLS-DA, n= 6) showed changes after SCI related to purine metabolism and other metabolites; exhibited that the treatment with TAM had favorable metabolic changes in skeletal muscle ($p < 0.001$). Western Blot analysis revealed downregulated levels of MHC in skeletal muscle after SCI ($n=3$, $p<0.0045$) and the treatment with TAM prevent the decrease of MHC expression ($p\leq 0.01$) on skeletal muscle. Our results suggest that the treatment with TAM after SCI can revert unfavorable metabolic changes,

preserve the structural mechanical integrity of the contractile apparatus, its properties and its normal function.

Disclosures: V. Gonzalez: None. **S. Ocasio:** None. **N.E. Chorna:** None. **I. Salgado:** None. **M.E. Santiago-Gascot:** None. **J.M. Santiago Santana:** None. **A. Torrado-Tapias:** None. **J. Colon Mercado:** None. **W. Frontera:** None. **J.D. Miranda:** None.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.09/D43

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life Grant WFL-UY-13/23, Project # 290

Title: The role of connexins in selfrepair induced by endogenous spinal progenitors

Authors: *M. V. FALCO PASTORINO[1], M. G. FABBIANI CARLOS[2], R. E. RUSSO[1]; [1]IIBCE, Montevideo, Uruguay; [2]Dept. de Neurofisiología Celular y Mol., IIBCE, MONTEVIDEO, Uruguay

Abstract: The ependyma of the adult spinal cord is a latent stem cell niche that contributes to the glial scar after spinal cord injury. The mechanisms by which injury reactivates the ependymal stem cell niche remain unknown. Ependymal cells are coupled via connexin (Cx) 43 and Cx26 in the active niche of neonatal mice, but uncouple with downregulation of Cx26 in the adult. Injury induces re-coupling and the upregulation of Cx26, suggesting a role for Cx signaling in the reactivation of the ependymal stem cell niche (1,2). We hypothesized that Cx26 is a main regulator of the response of ependymal cells to tissue damage. To analyze the specific role of Cxs in ependymal cells we used transgenic mice to selectively delete Cx26 or Cx43 by crossing mice with floxed Cx26 or Cx43 genes with a FoxJ1CreER-tdTomato transgenic line. We found that in $Cx26^{fl/fl}$ mice, recombination with tamoxifen strongly affected the CC response to injury 5 and 15 days post injury. The deletion of Cx26 in ependymal cells modified their contribution to the glial scar. Surprisingly, the deletion of Cx43 also reduced proliferation. To test whether Cx43 effect on proliferation was related to Cx26, we quantified the expression of Cx26 in Cx43 fl/fl mice and found a significant reduction, suggesting that the effect of Cx43 deletion on ependymal cell proliferation was due to the lack of Cx26 expression. Our findings suggest that Cx26 is a key molecular component of the signaling pathways that lead to the reactivation of ependymal cells and thus represents a potential target to improve the contribution of the ependymal stem cell niche to self-repair. We speculate that Cx43 hemichannels may be involved in the mechanisms activated by injury that mediate Cx26 expression.1. Fabbiani et al. (2020) Connexin signaling is involved in the reactivation of a latent stem cell niche after spinal cord injury. J Neurosci

40:2246-2258. 2. Falco et al. (2023) P2X7 receptor activation awakes a dormant stem cell niche in the adult spinal cord. Front Cell Neurosci 17:1288676. doi:10.3389/fncel.2023.1288676

Disclosures: M.V. Falco Pastorino: None. **M.G. Fabbiani Carlos:** None. **R.E. Russo:** None.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.10/D44

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Neilson Foundation 410893

Title: Effects of thoracic contusion spinal cord injury on serotonin innervation and receptor expression in the spinal ejaculation generator in the lumbar spinal cord of male rats.

Authors: *P. A. GREEN[1], J. COREY[2], L. M. COOLEN[1];
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Abstract: Spinal cord injury (SCI) in men is commonly associated with sexual dysfunction, including anejaculation, and chronic mid-thoracic contusion injury in male rats also impairs ejaculatory reflexes. Ejaculation is controlled by a spinal ejaculation generator (SEG) consisting of a population of lumbar spinothalamic (LSt) neurons. LSt cells control ejaculation through the release of neuropeptides and their connections at the level of the lumbosacral spinal cord. This spinal control center is under descending inhibitory influence of supraspinal inputs which include possible inputs from the caudal raphe nuclei and nucleus paragigantocellularis. Much remains unknown about supraspinal inputs to the SEG. There is evidence that serotonin plays a role, however the neural pathways and receptors that are involved are unknown. The current study tested the effects of mid-thoracic contusion injury in male rats on serotonin innervation of LSt cells using immunofluorescence for serotonin (5HT) and galanin and at 2 or 4 weeks after SCI or Sham (n=4-6). Analysis thus far revealed that LSt cells receive serotonin inputs primarily on dendrites and not on soma. Moreover, SCI reduced portion of LSt cells with serotonin inputs, but without significant reduction of 5HT-labeled axon contacts on LSt cells. Analysis of earlier times after injury are ongoing. Next, we examined the expression of serotonin 1a and 2a receptors within LSt cells at 4 weeks after contusion SCI or Sham (n=4-6). RNAscope was used to visualize *Galanin* (LSt cell marker), *Htr1a (*5-HT 1A receptor) and *Htr2a* (5-HT 2A receptor). Quantitative analysis revealed that 5-HT1A receptor was highly expressed in LSt neurons and significantly lower in SCI (70% of LSt cells) than in Sham (93% of LSt cells; p = 0.02). Likewise, 5-HT2A receptor was expressed n LSt cells in Sham (53% of LSt cells) and lower in SCI (26% of LSt cells; p=0.07). These results support the hypothesis that supraspinal serotonin inputs innervate the SEG while contusion SCI causes a mild disruption of such inputs while

reducing serotonin receptor expression in LSt cells. Future experiments are exploring treatments of serotonin 1A and 2A agonists on restoring ejaculatory function after SCI.

Disclosures: P.A. Green: Other; Kent State University, Brain Health Research Institute. **J. Corey:** None. **L.M. Coolen:** Other; Kent State University, Brain Health Research Institute.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.11/D45

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life

Title: Role of alternative heme binding proteins after SCI

Authors: *S. KAFURA[1], A. BACHMANN[2], J. PAGE[3], A. KRONER-MILSCH[3];
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Abstract: Traumatic spinal cord injury (SCI) is a severely debilitating condition, and affected individuals experience worsened quality of life as well as a life-long increased need for health care services. Intraparenchymal hemorrhage has long been associated with worsened locomotor outcomes for patients compared to those with non-hemorrhagic lesions and is a known contributor to secondary tissue damage. We and others have reported that the hemoglobin (Hb) released from damaged red blood cells (RBCs) and its breakdown products worsen SCI-associated tissue damage by exacerbating inflammation and oxidative tissue injury. High affinity proteins can capture Hb and heme and mitigate their deleterious effects. However, while present in the CNS, their concentration is insufficient to rescue the hemorrhage-induced secondary injury. While alternative heme-binding proteins like the lipocalin family member alpha-1 microglobulin (A1M) and the serine proteinase inhibitor alpha-1 antitrypsin (A1AT) have weaker Hb and heme affinity, they have additional tissue homeostatic function, including inhibition of reactive oxygen species (ROS). Our recent data demonstrate A1AT and A1M are significantly upregulated after SCI and suppress heme mediated cell death and ROS production in vitro. They are differentially expressed in neurons and glia. Preliminary data on viral overexpression of A1M and A1AT in the spinal cord tissue suggests a tissue-protective effect and improved functional recovery after cervical SCI, suggesting that A1M and A1AT have the potential to mitigate secondary damage and improve function after SCI.

Disclosures: S. Kafura: None. **A. Bachmann:** None. **J. Page:** None. **A. Kroner-Milsch:** None.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.12/D46

Topic: C.11. Spinal Cord Injury and Plasticity

Support: 2018/05006-0
2019/02714-7
2022/06609-6

Title: Tlr2 and tlr4 influence on motoneuron survival and glial responses after ventral root crush in mice

Authors: *L. CARTAROZZI[1,2], B. LIMA[3], A. MIDORI ROSSI TOMIYAMA[3], F. RABELO SANTOS[4], A. L. OLIVEIRA[3];
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Abstract: Lesions at the interface between the peripheral nervous system (PNS) and the central nervous system (CNS) are followed by pronounced motoneuron death and a robust activation of innate immunity. It has been increasingly described that after nerve lesion, there is an upregulation of classically known immune molecules, such as the major histocompatibility complex of class I (MHC-I) and toll-like receptors (TLRs). These molecules exert pleiotropic functions in the CNS and PNS, strongly related to a signaling system established retrogradely in the spinal cord, between axotomized motoneurons and surrounding glial cells. In this context, this study aimed to evaluate the lack of TLR2 and TLR4 expression on motoneuron survival and glial responses in the spinal cord after ventral root crush (VRC). For that, 8-week-old female C57BL/6J, TLR2 knockout, and TLR4 knockout mice were subjected to VRC. Three- or seven-days post injury (dpi), spinal cords were dissected and processed for either RT-qPCR and Western blotting (3 dpi) or cryoprotected, frozen, and sectioned for motoneuron counting and immunohistochemistry (7 dpi). Animal use and handling protocols were approved by the Institutional Committee for Ethics in Animal Use (CEUA/IB/UNICAMP, protocol number 4458-1). Analysis of motoneuron survival revealed an increased susceptibility to degeneration in TLR4 KO mice ($p = 0.0012$ and $p = 0.016$) compared to C57BL/6J and TLR2 KO groups, respectively. In addition, the microglial response was more intense in the ipsilateral sides of the spinal cord in the TLR2 KO group compared to the wild type ($p = 0.03$). No differences in reactive astrogliosis were found between the experimental groups at 7 dpi. Cellular changes were preceded by an intense upregulation of gene transcripts for IL-1β, IL-10, and arginase 1 in the TLR4 KO group, coupled with an increase in protein levels for NF-κβ. The increased susceptibility to degeneration observed in TLR4 KO mice underscores the significance of TLR4 signaling in neuroprotection. Furthermore, the heightened microglial response in TLR2 KO mice suggests a nuanced interplay between TLR2 and microglial activation post-injury. These findings

highlighted the pivotal role of TLRs in modulating motoneuron survival and glial responses following VRC.

Disclosures: L. Cartarozzi: None. **B. Lima:** None. **A. Midori Rossi Tomiyama:** None. **F. Rabelo Santos:** None. **A.L. Oliveira:** None.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.13/D47

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Upstate Foundation

Title: The Effect of Neurotrophin Treatment on Macrophage Populations After Spinal Cord Injury

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Abstract: Spinal cord injury (SCI) results in a devastating loss of motor and sensory function, with limited treatment options that will fully restore nerve function. Immediately after an SCI, there is tissue destruction, swelling and inflammation at the lesion. The tissue damage will promote a robust inflammatory response, appearing hours after injury, but persisting for months. Chronic inflammation can contribute to a local environment that is inhibitory to axonal regeneration post-injury, including the creation of a glial scar. The nature of the inflammatory response is complex and not fully understood. Infiltrating activated macrophages for example, have two phenotypes: the M1 macrophage exacerbates the tissue damage, while M2 macrophages enhance phagocytosis and tissue repair. The balance between these two subtypes may determine the capacity for axonal regeneration. Many treatments for SCI are focused on promoting neuronal survival (neuroprotection) and encouraging axonal regrowth (neuroregeneration). Several neurotrophins, including GDNF, NT3 and BDNF have been identified as potential therapeutic agents for SCI. These neurotrophins can directly promote axonal sprouting and maintain neurons. Recent evidence has shown that neuregulin can modulate the immune response after a spinal cord injury. Specifically, it promotes the appearance of macrophage subtypes (M2) that facilitates the repair process, creating a more permissive environment for axonal growth. In this study, the effects of various neurotrophins on the macrophage profile were examined in a spinal contusion model. The results suggest that certain neurotrophins can alter the relative balance of macrophage subtypes in the lesion over time post-injury.

Disclosures: A. Kestay: None. **D. Osterhout:** None.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.14/D48

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant 5R25GM061151-22

Title: Comparative analysis of molecular and cellular events in radial nerve cord and intestinal regeneration in the sea cucumber Holothuria glaberrima

Authors: *J. TORRES RODRIGUEZ, J. E. GARCIA-ARRARAS;
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Abstract: Understanding the genetic regulation of CNS regeneration, particularly the involvement ofsignaling pathways, remains a crucial area of investigation. Notch signaling, a highly conservedpathway in multicellular organisms, plays a pivotal role in various cellular processes, includingproliferation, dedifferentiation, and fate specification. However, its specific function in post-traumatic regeneration remains poorly characterized. In this study, we employ a small-moleculeinhibitor of the Notch pathway, DAPT, to investigate its role in CNS and intestinal regeneration ina novel model system, the sea cucumber *Holothuria glaberrima*. We induce injury to the radialnerve cord (RNC), induce evisceration of the intestines, and study the subsequent regeneration.Our observations reveal that DAPT inhibition of the Notch pathways causes a significant delay incell dedifferentiation in both tissues. Additionally, DAPT treatment results in decreased cellproliferation in both tissues at 8 days post-injury, which is typically the peak period of proliferation.The observed delays in cell dedifferentiation, reduced proliferation, and altered tissue morphologysuggest that Notch signaling is involved in regulating these aspects of regeneration. The fact thatDAPT treatment affects similarly, both CNS and intestinal regeneration, suggests that Notchsignaling plays a significant role in the amazing regenerative abilities of echinoderms and pointsto a process that could provide insights into the regeneration processes in other organisms.Further studies are needed to fully elucidate the mechanisms by which Notch signaling influencesregeneration in sea cucumbers and to explore its potential as a target for therapeutic interventionsin regenerative medicine.

Disclosures: J. Torres Rodriguez: None. **J.E. Garcia-Arraras:** None.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.15/D49

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH NINDS Grant 1R01NS114007-01A1
NIH NINDS Grant R01NS117749- 01
Shriners Viral Core Grant #84051-PHI-21

Title: Evaluation of intrathecal delivery of MaCPNS as a simple method to locally transduce dorsal root ganglia in rats

Authors: *E. V. ROOT[1], G. KOMA[2], T. J. CAMPION, III[3], J. RAJAVONG[1], A.-L. STEIN[1], G. M. SMITH[4], A. J. SPENCE[2];
[2]Bioengineering, [3]Shriners Hosp. Pediatric Res. Ctr., [4]Dept of Neurosci., [1]Temple Univ., Philadelphia, PA

Abstract: Targeted genetic transduction of specific neurons is a foundational tool in neuroscience. For example, specific targeting of afferent neurons with cell bodies in the dorsal root ganglia (DRG) supports a number of important research questions. One example is in spinal cord injury, where encouraging progress has been made in treating human patients using electrical epidural stimulation that targets these afferents. It remains unclear, however, which afferents are critical to the recovery, and what the upstream pathways are that undergo helpful plasticity. Rodent models with genetically transduced DRG neurons are critical to unraveling these mysteries. At present, however, these are typically transduced using direct injection into individual DRGs after surgical exposure, a procedure that can take several hours for a single animal. An exciting new method to target the peripheral nervous system (PNS), including these DRG neurons is the engineered genetic vector MaCPNS. Studies showing the distribution of MaCPNS transduction in rats are only just emerging. Previous work has shown that intravenous administration of MaCPNS appears to transduce DRGs across all vertebral levels in rodents. An important refinement of this system would be to restrict expression to within certain levels of the spinal cord (eg., lumbar but not cervical roots). Systematic delivery may also increase the risk of toxicity and, after activation of neurons with DREADDS, has the potential to influence thoracic or cervical afferents which may affect important physiological mechanisms, such as breathing. Therefore, a noninvasive method which can target a localized region of the spinal cord would be ideal for many studies. Here we sought to determine the degree of localization of MaCPNS driven expression after a lumbar intrathecal injection. We hypothesized that intrathecal injection of 10 uL of MacPNS1-hM3Dq-mCherry into the subdural space below lumbar vertebrae L2-L3 would give strong transduction of lumbar DRGs but not thoracic or cervical. Early histological examination of four rats shows labeling in DRGs that may be restricted to several neighboring spinal cord segments. Some spinal cord labeling occurred but may be due to depth of injection. Preliminary treadmill kinematic data suggest that activation of transduced afferents caused walking gait at low speeds to be more variable, including hopping or skipping steps. Ongoing work is systematically mapping the transduction across the neuraxis, and expanding the kinematics study.

Disclosures: E.V. Root: None. **G. Koma:** None. **T.J. Campion:** None. **J. Rajavong:** None. **A. Stein:** None. **G.M. Smith:** None. **A.J. Spence:** None.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.16/D50

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Wings for Life
NIH/NCATS Grant TL1TR001440
Mission connect and TIRR foundation
UT system STAR Award

Title: Rescuing excitatory neurons from prolonged neuronal swelling improves locomotor recovery after spinal cord injury in mice

Authors: *Q. LI[1], A. SANDOVAL[1], J. SU[3], M. HENWOOD[2], B. CHEN[4];
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Abstract: In addition to acute damage, spinal cord injury (SCI) triggers secondary injury responses, with sustained neuronal loss and dysfunction. However, the underlying mechanisms for these delayed neuronal pathologies are not entirely understood. A notable consequence of SCI is the resultant swelling of neurons, but the contribution of cell swelling to neuronal loss and functional deficits after SCI has not been systematically characterized. In this study, we devised a three-dimensional imaging pipeline to evaluate spinal neurons - examine their types, quantities, volumes, and spatial distribution - in a double-lateral hemisection SCI model. We discovered that both excitatory and inhibitory neurons swell and die, yet with distinct temporal patterns. Inhibitory neurons demonstrated marked swelling and death two days after SCI, with these observations resolving by day 14. In contrast, there is a persistent swelling and continuous loss of excitatory neurons for at least 35 days after SCI. Further investigation of the mechanisms underlying prolonged neuronal swelling revealed sustained expression of the Na^+-K^+-Cl^- Cotransporter 1 (NKCC1) in excitatory neurons, with contrasting downregulation in inhibitory neurons. Treatment with a clinically approved NKCC1 inhibitor, bumetanide, effectively mitigated swelling in excitatory neurons, and reduced their loss in the secondary phase after SCI. Optimizing the administration of bumetanide after SCI significantly improved locomotor recovery, with benefits persisting for at least four weeks after treatment cessation. This study advances our understanding of SCI-related pathology, while also introducing the use of

bumetanide as a novel treatment to mitigate sustained neuronal swelling and enhance recovery after SCI.

Disclosures: Q. Li: None. **A. Sandoval:** None. **J. Su:** None. **M. Henwood:** None. **B. Chen:** None.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

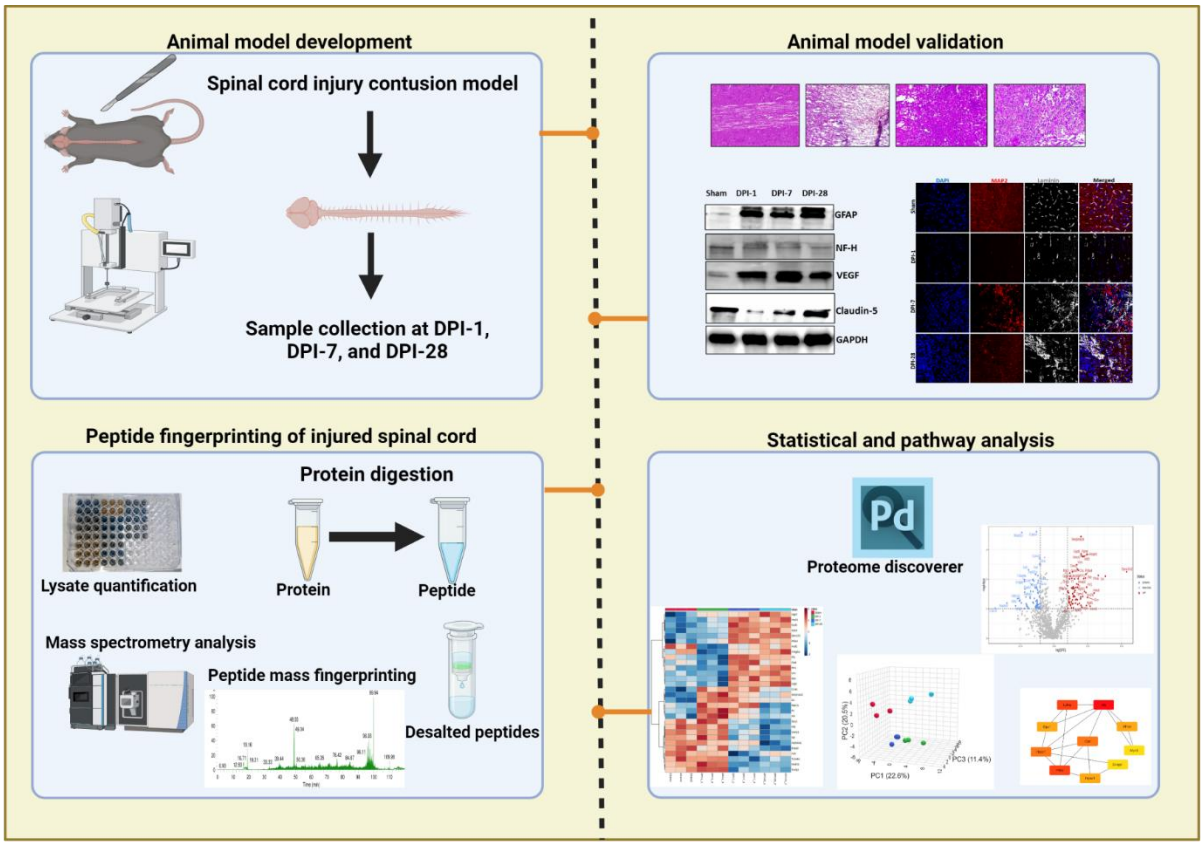
Program #/Poster #: PSTR399.17/D51

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Lactate dehydrogenase a is a key mediator of angiogenesis after spinal cord injury

Authors: *M. CHOPRA, H. KUMAR;
Pharmacol. and Toxicology, Natl. Inst. of Pharmaceut. Educ. and Res., Ahmedabad, India

Abstract: SCI (spinal cord injury) is a very devastating condition, that can cause damage to the neuronal tissues resulting in sensory and motor neuron dysfunctions. Following primary mechanical injury, secondary injury leads to progressive damage in the epicenter, such as apoptosis, inflammation, demyelination, and vascular disruption resulting in a glial scar. Primary injury not only destroys the neuronal cells but also damages the endothelial cells (ECs) in blood vessels. During the progression of vascular pathology after SCI, proteomics alteration occurs in the endothelial cells. Identifying the regulatory proteomic signatures results in enhancing the understanding of vascular pathology after SCI and deciphering the novel therapeutic target. Hence, the current study focused on the identification of the proteomic signature of endothelial cells in SCI pathology. The results of the current study suggested that the experimental model of SCI was developed successfully which is evidenced by H&E staining, western blot, and IHC. The expression of neuronal marker NF-H found to be decreased suggests a significant neurodegeneration and increased GFAP expression suggests astrocytic activation. For vascular pathology progression, increased VEGF and vwf expression suggested an increase the angiogenesis. Overall findings from the data suggest experimental SCI model has been developed successfully with vascular pathology progression. Further, to identify the proteomic signature of endothelial cells, we have performed a bottom-up proteomic analysis by which we have identified the differential expression of proteins (DEP). The pathway analysis of upregulated DEP suggests an enrichment of the VEGFA-VEGFR2 signaling pathway at DPI-7 and DPI-28. Moreover, we have identified Lactate dehydrogenase as a key mediator in regulating angiogenesis through VEGFA-VEGFR2 signaling.



Disclosures: M. Chopra: A. Employment/Salary (full or part-time):; Ministry of Chemical and Fertilizer, Govt. of India. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Central Instrumentation Facility, NIPER Ahmedabad. **H. Kumar:** A. Employment/Salary (full or part-time):; Ministry of Chemical and Fertilizer, Govt. of India.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.18/D52

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH RO1 (NS131806-01) (ADG)
Mission Connect, a program of the TIRR foundation
Wings For Life

Title: MerTK promotes inflammatory resolution to facilitate neuroprotection and repair after spinal cord injury (SCI)

Authors: *A. R. SCHEINFELD[1,2], J. ALDRICH[1,2], S. LEE[1,2], K. SOTO DUSENBERY[1,2], A. D. GAUDET[1,2];
[1]Univ. of Texas at Austin, Austin, TX; [2]Neurology, Dell Medical School University of Texas at Austin, Austin, TX

Abstract: Spinal cord injury (SCI) causes persistent neuroinflammation that leads to secondary damage, worsening neurologic recovery and chronic neuropathic pain. Inflammatory resolution is an underexplored mechanism to support long-term recovery and is aided by *phagocytosis* via Phagocytic receptor MerTK. Although MerTK has been explored for inflammatory diseases such as MS and some forms of cancer, the role of MerTK in resolution of CNS trauma remains underexplored. Here, we hypothesize that deletion of MerTK in mice will exacerbate secondary damage and locomotor deficits after T9 contusion SCI, and further anticipate that activation of MerTK by addition of its ligand will dampen inflammatory gene expression in cultured macrophages. Compared to wildtype mice, MerTK knockout mice show impaired locomotor recovery after SCI. In accordance, MerTK knockout spinal cord epicenters had a larger lesion volume and cross-sectional area. qRT-PCR analysis shows that MerTK knockout spinal cord epicenters have increased expression of potentially damaging pro-inflammatory mediators. Further, the addition of Gas6 to macrophages stimulated with lipopolysaccharide dampens induction of inflammatory genes. Our results suggest that MerTK is required for typical healing and locomotor recovery after SCI and point to MerTK as a viable target for modulating immune cell reactivity. Ongoing studies involve MerTK modulation as a novel neuroimmune therapy to enhance recovery after SCI.

Disclosures: A.R. Scheinfeld: None. **J. Aldrich:** None. **S. Lee:** None. **K. Soto Dusenbery:** None. **A.D. Gaudet:** None.

Poster

PSTR399

Spinal Cord Injury: Cellular and Molecular Mechanisms II

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Program #/Poster #: PSTR399.19/D53

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NSFC Grant 82071393

Title: Ce-accumulated microglia sustain a proinflammatory microenvironment during the subacute phase after traumatic spinal cord injury in mice

Authors: Q. QIAO[1], *N. LI[2];
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Abstract: Impaired cholesterol metabolism is increasingly recognized as a pivotal factor contributing to the proinflammatory microenvironment following traumatic spinal cord injury (TSCI). However, the specific cell types most affected and their contributions to subsequent neuroinflammation remain poorly understood. To elucidate this, we combined single-cell sequencing with lipid metabolomics in mouse TSCI models. Our findings reveal a distinct population of microglia with upregulated cholesterol ester (CE)-related gene expression 14 days post-injury. These microglia accumulated CE-rich lipid droplets to a greater extent than their counterparts, as confirmed by flow cytometry ($CD45^{+}$/$CD11b^{+}$/$BODIPY^{493/503}$), Oil Red O staining, and cholesterol quantification assays. We refer to this unique microglial population as CE-accumulated microglia (ceMG) in subsequent analyses. Further investigations into ceMG characteristics demonstrated that myelin debris, rather than lipopolysaccharide, more effectively activated these cells. CeMG exhibited elevated levels of reactive oxygen species (DCFH-DA) and markers of endoplasmic reticulum stress (XBP1s, ATF4), underscoring their proinflammatory phenotype. Importantly, inhibition of CE synthesis using avasimibe (20 mg/kg) in mice enhanced myelin integrity and reduced cell death. In conclusion, we identify a novel microglial subtype following TSCI, marked by significant CE accumulation and proinflammatory activity. This discovery highlights a new avenue for exploring microglia-mediated neuroinflammation post-TSCI and warrants further detailed investigation.

Disclosures: Q. Qiao: None. **N. Li:** None.

Poster

PSTR400

Brain Mechanisms and Processing of Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.01/D54

Topic: D.01. Somatosensation – Pain and Itch

Support: P30GM145497
U01EY034709

Title: Whole brain mapping of activity-dependent genetically labeled neurons after corneal stimulation

Authors: *S. S. REYNOLDS[1,2], P. CARADONNA[1], K. NIYONKURU[1], W. RENTHAL[3], I. D. MENG[4,2,5];
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Abstract: Dry eye disease is marked by ocular discomfort and pain, negatively impacting the quality of life. Previous studies have identified two distinct regions within the trigeminal nucleus

activated by corneal stimulation, yet a detailed mapping of the brain regions activated by corneal pain is lacking. The aim of this study was to perform whole brain mapping of brain regions activated by corneal stimulation and examine changes induced by dry eye. Activity-dependent genetic labeling was performed using targeted recombination in activated populations (TRAP) mice. Male and female TRAP2 mice (Fos 2A-iCreER , Jackson Laboratory) were crossed with the CAG-tdTomato reporter line (Ai14, Jackson Laboratory) to visualize neurons activated by corneal stimulation following 4-OH-Tamoxifen (75 mg/kg, i.p.). In some animals, lacrimal gland excision (LGE) was performed 2-weeks prior to 4-OH-Tamoxifen treatment to create aqueous tear deficiency. After 3 days of habituation to the holding chamber and injection procedure, 4-OH-Tamoxifen was administered 15 min prior to either corneal hypertonic saline (5M NaCl, 5 applications, once every 3 min), no stimulation (mock application of saline), or no treatment (remained in holding chamber after i.p. injection). Two weeks later mice were perfused and brains with attached spinal cord through C2 extracted. Utilizing a whole brain mapping service provided by LifeCanvas Technologies, volumetric light sheet images were acquired from cleared tissue. Images were aligned to the Allen Brain Atlas and a searchable database of cell counts was created. Image files were provided to allow for additional analysis using Imaris software. An increase in “TRAPed” neurons was observed in regions of the trigeminal nucleus previously shown to increase c-Fos expression after corneal stimulation. Furthermore, whole brain mapping revealed several additional brain regions with an increase in “TRAPed” neurons after corneal stimulation when compared to unstimulated controls. These results indicate that whole brain analysis of genetically labeled neurons can provide novel insight into brain networks activated by noxious corneal stimulation and their modifications in dry eye.

Disclosures: S.S. Reynolds: None. **P. Caradonna:** None. **K. Niyonkuru:** None. **W. Renthal:** None. **I.D. Meng:** None.

Poster

PSTR400

Brain Mechanisms and Processing of Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.02/D55

Topic: D.01. Somatosensation – Pain and Itch

Title: Altered cortical connectivity in chronic low back pain

Authors: *G. KENEFATI[1], M. ROCKHOLT[1], K. EISERT[1], R. WU[1], D. OK[1], M. MCCARTIN[1], Q. ZHANG[1], G. SUN[1], E. VOIGT[2], L. DOAN[1], Z. CHEN[3], J. WANG[1]; [1]Dept. of Anesthesiol., Perioperative Care and Pain Med., NYU Grossman Sch. of Med., New York, NY; [2]Dept. of Otolaryngology-Head and Neck Surgery, NYU Grossman Sch. of Med., New York, NY; [3]Dept. of Neurosci. and Physiol., NYU Grossman Sch. of Med., New York, NY

Abstract: Generalized hypersensitivity, or widespread pain, is commonly found in individuals with chronic low back pain. In this study, we investigated resting and pain-evoked EEG recordings in chronic pain patients who exhibit widespread pain vs. patients with localized pain vs. healthy controls. EEG data were source-localized for six regions of interest (dorsolateral prefrontal cortex (dlPFC), insular cortex (IC), dorsal and rostral anterior cingulate cortices (dACC, rACC), medial orbitofrontal cortex (mOFC), and primary somatosensory cortex (S1). Amplitude- and phase-based functional connectivity were assessed for the resting and evoked data, respectively. We found that chronic pain patients demonstrate increased theta-band connectivity between dlPFC and ACC compared to healthy controls. Graph theory analysis further revealed enhanced theta-band centrality of left-hemisphere IC in nociceptive processing, indicating increased aversive processing. These results indicate that nociceptive processing in chronic pain is altered by a unique set of cortical mechanisms.

Disclosures: G. Kenefati: None. **M. Rockholt:** None. **K. Eisert:** None. **R. Wu:** None. **D. Ok:** None. **M. McCartin:** None. **Q. Zhang:** None. **G. Sun:** None. **E. voigt:** None. **L. Doan:** None. **Z. Chen:** None. **J. Wang:** None.

Poster

PSTR400

Brain Mechanisms and Processing of Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.03/D56

Topic: D.01. Somatosensation – Pain and Itch

Support: ANR-21-CE16- 0018-01

Title: Brain network alterations associated with corneal neuropathic pain in mice

Authors: *J. HUANG[1], F. MORIER[2], S. PEZET[3], G. VETERE[2], L. BOURGEAIS[4,1], A. REAUX LE GOAZIGO[1];
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Abstract: Ocular surface pain has a negative impact on the quality of life and the management of this debilitating condition is still a therapeutic challenge. To date, the central mechanisms underlying ocular pain remain inadequately explored. This work aims to elucidate the central regions activated in response to ocular pain. To achieve this, we constructed a "c-Fos connectome" using c-Fos neuronal staining in a mouse model of ocular pain. Adult male mice (n=5) received topical instillation of 0.2% benzalkonium chloride (BAC), twice a day for 7 days. Control (naive) mice (n=4) did not receive any instillation. Corneal pain was evaluated by spontaneous eye closing ratio and mechanical von Frey test. Corneal integrity and nerve damage were evaluated using slit-lamp examination and nerve staining, respectively. At Day 7, mice

were perfused with 4% PFA and brains were sectioned in the sagittal plane except for the brainstem (coronal sections). Sections were processed for c-Fos staining and were imaged using Nanozoomer, a digital slide scanner. Brain sections were then aligned with the Allen Brain Atlas using ABBA plug-in in Image J software. c-Fos+ cells were detected and quantified in 820 regions using QuPath software. Finally, the c-Fos connectome was built using a Python script developed in our team and Cytoscape software. Our investigations revealed that mice subjected to 0.2% BAC instillation exhibited corneal nerve damage accompanied by spontaneous ocular pain and mechanical allodynia compared to naive mice. Analysis of c-Fos expression in the trigeminal brainstem indicated a significantly increase in c-Fos positive cells in BAC-treated mice compared to naive mice, specifically within two regions of the spinal trigeminal nucleus, Vc/C1 and V1/Vc, which are known to receive the central terminals of the corneal nociceptors. Moreover, higher c-Fos expression was observed in various brain regions associated with pain and visual pathways. In addition, our data demonstrated that the pain network in BAC condition exhibited greater connected compared to the control network, which comprised more isolated sub-graphs. In conclusion, our study introduces a novel and effective analytical pipeline for examining neuronal activation (c-Fos) throughout the entire mouse brain, facilitating the construction of a comprehensive c-Fos connectome. These findings constitute the initial anatomical and functional characterization of neural pathways implicated in ocular pain. Subsequent analysis aims to pinpoint hub regions within the pain network, which could serve as potential targets for disrupting pain signaling pathways.

Disclosures: J. Huang: None. **F. Morier:** None. **S. Pezet:** None. **G. Vetere:** None. **L. Bourgeais:** None. **A. Reaux le goazigo:** None.

Poster

PSTR400

Brain Mechanisms and Processing of Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.04/D57

Topic: D.01. Somatosensation – Pain and Itch

Support: MOST 111-2320-B-001-007-MY3

Title: Sensory and affective aspects of pain differentially regulated by separate Anterior Paraventricular Thalamus projections

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Abstract: The experience of pain is complex, involving both sensory and affective components, yet the underlying neural mechanisms remain unclear. Here we employed a combination of axonal tracing, behavioral tests, electrophysiology, in vivo calcium imaging, optogenetic, and chemogenetic approaches in murine pain model. We demonstrate that formalin-induced pain

behaviors coincide with increased responses in glutamatergic neurons within the anterior paraventricular nucleus of the thalamus (PVA). Using microendoscopic imaging, we identified non-overlapping sub-populations of PVA^{VgluT2} neurons engaged in sensory and affective pain processing, their activity varying across different pain states. Activating PVA glutamatergic neurons was sufficient to induce mechanical hypersensitivity and aversion behaviors, whereas suppression ameliorate formalin-induced pain. Furthermore, we reveal the segregation of PVA^{VgluT2} projections to the bed nucleus of the stria terminalis (BNST) and nucleus accumbens (NAc), each influencing specific aspects of pain-like behavior. This finding provides an important insight into the mechanism of distinct components of pain, highlighting the pivotal role of PVA in mediating different aspects of pain-like behavior with distinct circuits.

Disclosures: S. Mindaye: None. **W. Chen:** None. **Y. Chen:** None. **C. Chen:** None.

Poster

PSTR400

Brain Mechanisms and Processing of Pain

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Program #/Poster #: PSTR400.05/D58

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant R01DA045664
NIH Grant R01MH116904
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Terman Fellowship
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Stanford Neuroscience Institute's Neurochoice Initiative

Title: A closed-loop circuits for chronic mechanical pain

Authors: Q. WANG[1], L. JOO HAN[1], H. SHIM[1], L. YUAN[1], ***W. QI**[2], Y. YUAN[1], S. FAN[1], X. CHEN[1];
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Abstract: Spinal cord-projecting neurons in the rostral ventromedial medulla (RVM^{SC} neurons) play active roles in pain facilitation. However, the underlying circuitry and molecular mechanisms remain largely unknown, and the therapeutic potential of targeting RVM^{SC} neurons to treat chronic pain has not been explored. Here we show that acute activation of OPRM1+ RVM^{SC} neurons does not facilitate pain in normal mice, but activity of these neurons is required for both initiation and maintenance of chronic mechanical hypersensitivity in mouse models of inflammatory and neuropathic pain.Additionally, we uncover a novel pathway wherein the somatosensory cortex receives input from the spinal cord via the thalamic region, subsequently modulating pain thresholds via the lateral superior colliculus and RVM. Together, our results reveal a spinal cord-cortex-medulla-

spinal cord pathway that drives persistent pain, offering insights for future therapeutic interventions targeting this pathway.

Disclosures: Q. Wang: None. **L. Joo Han:** None. **H. Shim:** None. **L. Yuan:** None. **W. Qi:** None. **Y. Yuan:** None. **S. Fan:** None. **X. Chen:** None.

Poster

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Brain Mechanisms and Processing of Pain

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Topic: D.01. Somatosensation – Pain and Itch

Support: National Medical Research Council, Singapore
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MOE Tier 1 grant

Title: Septal p75 neurotrophin receptors mediate experimental neuropathic pain

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Abstract: An unmet need in the modulation of chronic pain has triggered a search for newer experimental therapeutics. In this regard, very recently our laboratory showed that septo-dorsal hippocampal cholinergic projection mediate nociception in the rat chronic constriction injury (CCI) model of neuropathic pain. The septal cholinergic neurons are enriched in neurotrophin receptors (NTRs), including the p75NTR. Given the preceding, we hypothesized that septal p75NTRs mediate experimental neuropathic pain and, thus, are a potential target for experimental therapeutics. Experiments were conducted on male rat. An AAV viral vector containing p75NTR short hairpin RNA (shRNA) was microinjected into the MS to induce p75NTR knockdown (KD) via RNA interference. Electrophysiological experiments showed that intraseptal microinjection of nerve growth factor (NGF; 100 ng/µl, 0.5 µl), an agonist at NTRs, evokes a suppression of dorsal hippocampus CA1 population spike (PS) and a concomitant theta activation in control, urethane anaesthetized rat (n = 8). These responses evoked by intraseptal NGF are consistent with the effect of stimulation of the septal cholinergic neurons. Conversely, KD of septal p75NTR attenuated NGF-induced suppression and theta activation (n = 10), but not the similar responses evoked by intraseptal nicotine (6 µg/µl, 0.5 µl), an agonist at cholinergic

nicotinic receptors. This suggests that p75NTR plays a selective role in facilitating the effect of NGF in enhancing the putative cholinergic tone in the septo-hippocampus. Behavioral experiments conducted on the CCI model showed that septal p75NTR KD attenuated the CCI-induced peripheral hypersensitivity to mechanical and thermal stimuli as compared to control animal (n = 34). However, physiological nociceptive reflexes were not affected by KD (n = 37). This mimics the effect of destruction of septal cholinergic neurons. The preceding underlines the potential of p75NTRs as a therapeutic target for the management of neuropathic pain in part via modulation of the septal cholinergic network.

Disclosures: R. Yong: None. **M. Ariffin:** None. **S. Lo:** None. **C. Low:** None. **S. Khanna:** None.

Poster

PSTR400

Brain Mechanisms and Processing of Pain

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.07/D60

Topic: D.01. Somatosensation – Pain and Itch

Support: NIDA R01-DA053752
NIDA F31-DA054792

Title: Pain's divergence: unraveling sex-specific differences in kappa opioid receptor endpoints

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Abstract: Chronic neuropathic pain is a multidimensional condition that is highly co-morbid with Opioid Use Disorder and manifests differently between males and females. The Dynorphin/Kappa Opioid Receptor (KOR) system is an emerging target for treating anhedonia associated with chronic pain and stress-induced relapse of drug seeking. Recent work from our lab suggests males are more susceptible than females to the functional upregulation of the dynorphin/KOR system induced by chronic pain, specifically in the Nucleus Accumbens and Ventral Tegmental Area. In continuation of this work, the present set of studies sought to determine whether KOR expression and function were similarly increased in the Basolateral Amygdala (BLA), a key region in processing stress, reward, and learning. All studies used a peripheral nerve injury neuropathic pain model in C57BL/6J adult male and female mice. We first found increased KOR expression in male pain mice 2 weeks post-injury using fluorescence *in situ* hybridization. Activation of BLA KOR measured via GTPgS autoradiography was also higher in male pain mice but not female pain mice 2 weeks post-injury. Surprisingly, using a conditioned place preference (CPP) assay, we found that the KOR agonist U50,488H (5 mg/kg, i.p.) induced reinstatement of oxycodone CPP in chronic pain female but not male mice.

Reinstatement of oxycodone CPP was not evident in the sham mice of either sex. Overall, these data support previous findings that chronic pain-mediated changes in the kappa opioid system are sexually dimorphic and provide important implications for stress-induced drug-seeking in chronic pain patients with a history of opioid use. These findings also reinforce that multiple endpoints (behavioral, molecular, etc.) between sexes are necessary when studying animal disease models.

Disclosures: J.E. Mondello: None. **C.M. Cahill:** None.

Poster

PSTR400

Brain Mechanisms and Processing of Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.08/E1

Topic: D.01. Somatosensation – Pain and Itch

Title: Glun2d subunit containing nmda receptors in the nucleus accumbens mediate cisplatin-induced neuropathic pain

Authors: *S. GAKARE[1], D. GAWANDE[2], G. SHELKAR[3], S. DRAVID[4];
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Abstract: Neuropathic pain presents a significant challenge, with its underlying mechanisms still not fully understood. Here, we investigate the role of GluN2D-containing NMDA receptors in the development of neuropathic pain induced by cisplatin, a widely used chemotherapeutic agent. Through genetic and pharmacological strategies, we found that GluN2D-containing NMDA receptors play a targeted role in regulating cisplatin-induced neuropathic pain, while sparing inflammatory or acute pain responses. Specifically, both GluN2D knockout (KO) mice and pharmacological blockade of GluN2D-containing NMDA receptors mitigate cisplatin-induced increase in mechanical nocifensive response. Moreover, animals with conditional deletion of GluN2D receptors from parvalbumin (PV) interneurons similarly display resistance to neuropathic pain. Importantly, ablating GluN2D-containing NMDA receptors in the nucleus accumbens leads to decreased sensitivity to cisplatin-induced neuropathic pain. Furthermore, we demonstrate that cisplatin treatment increases excitatory neurotransmission in wildtype mice, whereas this effect is dampened in PV-GluN2D KO mice, suggesting a mechanism through which GluN2D-containing receptors modulate neuroplastic changes linked with neuropathic pain. These findings unveil a novel pathway implicating GluN2D-containing NMDA receptors within the nucleus accumbens, specifically governing cisplatin-induced neuropathic pain, thus offering potential therapeutic avenues for managing chemotherapy-induced neuropathy.

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Poster

PSTR400

Brain Mechanisms and Processing of Pain

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Topic: D.01. Somatosensation – Pain and Itch

Support: Brain and Behavior Research Foundation (NARSAD Young Investigator grant 27197 to M.C.C.)
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Whitehall Foundation grant 2017-12-54 (to M.C.C.)
Rita Allen Scholar Award in Pain (to M.C.C.)

Title: Temporally distinct adaptations in accumbal-projecting mesolimbic dopamine neurons following peripheral nerve injury

Authors: *J. M. THOMPSON, Y.-H. CHANG, M. C. CREED;
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Abstract: Patients with chronic pain (CP) frequently present with comorbid mood disorders that negatively impact their quality of life and complicate treatment, however the mechanisms underlying this association are not well understood. Affective symptoms of CP are generally thought to be related to a hypodopaminergic state with decreased activity of dopamine neurons in the ventral tegmental area (VTA). The VTA is a heterogeneous region with multiple projection targets, each of which have unique roles in reward processing and reward-guided behaviors. Activity in VTA dopamine neurons projecting to the nucleus accumbens core (NAc) is critical for reward-guided decision making and positive reward prediction error, however it is unknown if function of these neurons is altered in the acute or chronic pain states. Moreover, it is unknown how adaptations in this pathway may drive CP-related mood symptoms including amotivation and anhedonia. Using ex vivo patch clamp recordings, we found the ability of NAc-projecting VTA dopamine neurons is reduced at a chronic but not acute timepoint following SNI. This parallelled behavioral results where we found that mice exhibited reduced effort spent in pursuit of rewards and impaired reward sensitivity on a reward-guided decision making task at chronic but not acute time points following SNI. These results suggest that impaired VTA-NAc activity correlates to pain-related anhedonia and supports development of pharmacological or neuromodulatory strategies targeting this hypoactivity for treatment of CP-related affective disorders.

Disclosures: J.M. Thompson: None. **Y. Chang:** None. **M.C. Creed:** None.

Poster

PSTR400

Brain Mechanisms and Processing of Pain

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.10/E3

Topic: D.01. Somatosensation – Pain and Itch

Support: R21NS132590-01

Title: D-serine disrupts GluD1-Cbln1 interaction and blocks the nociceptive effects of Cbln1 in the central amygdala

Authors: *S. SABNIS, K. S. NARASIMHAN, S. DRAVID;
Texas A&M, College Station, TX

Abstract: D-serine disrupts GluD1-Cbln1 interaction and blocks the nociceptive effects of Cbln1 in the central amygdala Sabnis Siddhesh[1], Narasimhan Kishore[1], Dravid Shashank[11]Texas A&M University, Department of Psychiatry and Behavioral Science **ABSTRACT**D-serine in addition to being an NMDA receptor co-agonist, is a ligand for GluDs and shows weak binding with the ligand-binding domain of GluDs. D-serine binding, however, may promote synaptic plasticity rather than resulting in the usual ion channel currents through GluD1 or GluD2. D-serine binding to GluD2 promotes long-term depression at the parallel fiber-Purkinje cell synapse in the developing brain. Moreover, D-serine binding seems to be crucial for GluD1's for forming inhibitory synapses. Nevertheless, there is no evidence on how D-serine binding to GluD1 affects the amino-terminal domain interaction of GluD1 with Cbln1 and how this influences behavior. In the in-vitro cell-binding experiment, we discovered that D-serine inhibits the Cbln1 and GluD1 interaction with the IC50 of 0.31±0.169 mM, similar to the previously reported value. The inhibitory effect of D-serine was concentration dependent. Furthermore, we also tested the effects of D-serine in-vivo. Cbln1 serves as a nociceptive signal in the central amygdala and exerts analgesic effect in the inflammatory pain condition. Thus, we tested whether D-serine blocks these effects of Cbln1. D-serine at the dose of 30μg inhibited the nociceptive effects of Cbln1. In addition, it was also able to counteract the analgesic effect of Cbln1 in the inflammatory pain. We also confirmed that the D-serine's effect is GluD1 dependent and not NMDAR dependent by blocking the NMDARs using MK-801. Interestingly, we also found that D-serine reduces Cbln1 in the ex vivo experiments. In the ongoing studies, we are looking into the mechanism through which D-serine reduces the Cbln1.

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Poster

PSTR400

Brain Mechanisms and Processing of Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.11/E4

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH R25 NS090978
AAN Clinical Research Training Scholarship in Parkinson's Disease
NIH R01 DK116178

Title: Dopamine reduces excitability of pain-responsive neurons in the central nucleus of the amygdala

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Abstract: The central nucleus of the amygdala (CeA) is a heterogenous region of primarily GABAergic neurons that contributes to numerous behaviors, including fear learning, feeding, reward, and pain. Dopaminergic input to the CeA has been shown to regulate many of these behaviors, but how dopamine (DA) exerts these effects at the cellular level has not been well characterized. We used the Transient Recombination of Active Populations (TRAP) mouse line to label pain-responsive CeA neurons, and then targeted these cells for patch-clamp recordings in acute slices (both male and female mice, age 8-16 weeks). DA reduced the intrinsic excitability of CeA neurons, as well as the amplitude of excitatory inputs from the basolateral amygdala (BLA). The D1 agonist SKF-38393 and D2 agonist quinpirole also had inhibitory effects, suggesting the effects of DA are mediated by a combination of both receptor types. These results provide insight into how DA regulates CeA activity and support a role for dopaminergic regulation of pain processing within the amygdala.

Disclosures: R.J. Heuermann: None. **R.W. Gereau:** None.

Poster

PSTR400

Brain Mechanisms and Processing of Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.12/E5

Topic: D.01. Somatosensation – Pain and Itch

Title: An Amygdala Circuit for Injury-Induced Hypersensitivity and negative valence of pain in Mice

Authors: *T. D. WILSON[1], M. THOUAYE[1], B. NEUGEBAUER[1], S. SINGH[2], S. GHODRATI[1], Y. CARRASQUILLO[3];
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Abstract: The central nucleus of the amygdala (CeA) is a critical center for pain processing and modulation of pathological pain. Our lab has shown that the CeA can bidirectionally modulate pain-like behaviors in a cell-type-specific manner. Nociceptive inputs into the CeA come from the lateral parabrachial nucleus (PBN) whereas polymodal information, including those related to affective and cognitive states, reaches the CeA via inputs from the basolateral amygdala (BLA). Previous studies have shown that synaptic transmission is strengthened in both pathways in various rodent models of persistent pain. We have further demonstrated that PBN to CeA pathway contributes to injury-induced hypersensitivity. However, the contribution of BLA to CeA circuit to injury-induced hypersensitivity remains unknown. To address this question, we are using an intersectional chemogenetics approach in combination with behavioral assays. The sciatic nerve cuff model of neuropathic pain is used with von Frey, Hargreaves, Acetone and Randall-Selitto to measure hypersensitivity to tactile, heat, cold and pressure stimuli at different time-points after nerve injury. In addition, negative valence of pain was measured during these tests by scoring the affective-motivational behavior of the animals in response to application of noxious stimuli. The results from our experiments show that inhibition of the BLA to CeA pathway one week after nerve injury reverses tactile hypersensitivity and reduces affective-motivational responses to noxious tactile stimuli, but has no effect on cold, heat or pressure hypersensitivity, and returned to baseline 24hrs after chemogenetic inhibition. We also show that inhibition of the BLA to CeA pathway four weeks after nerve injury reverses tactile and pinch hypersensitivity, associated with a reduction of affective-motivational responses to noxious tactile stimuli. Interestingly, this reversal lasted 48hrs following chemogenetic inhibition. These results show the BLA to CeA pathway is necessary for modality-specific hypersensitivity after injury and for the affective dimension of pain. Ongoing experiments are examining longer time-points to determine the duration of the contribution of BLA to CeA circuit to injury-induced hypersensitivity. Additional experiments are exploring whether chemogenetic activation of this pathway is sufficient to induce long-term hypersensitivity in the absence of injury and whether injury alters the activity of CeA-projecting BLA neurons both in-vivo and in an ex-vivo acute brain slice preparation.

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Poster

PSTR400

Brain Mechanisms and Processing of Pain

Location: MCP Hall A

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Program #/Poster #: PSTR400.13/E6

Topic: D.01. Somatosensation – Pain and Itch

Support: R01 DK115478
R15 NS128624

Title: Quantifying pro vs. anti-nociceptive PKC-δ positive neurons in the central nucleus of the amygdala foruse in our web-based virtual model of pain

Authors: *A. NOFAL[1], C. D. PERUMAL[2], I. ANANDAN[1], K. KRAEUTER[2], R. NEILAN[3], B. J. KOLBER[4];
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Abstract: The central nucleus of the amygdala (CeA) plays an important role in processing neuropathic pain-like behavior. Within the CeA, there are different cell-types with markers such as protein kinase C-δ (PKC-δ), somatostatin (SST), and calcitonin gene-related peptide receptor (CGRPR; also known as Calcrl). Our lab recently published a 3-D agent-based computational model of several pain-related neuronal populations to simulate their interactions, behaviors, and emergent pain-related output from the CeA. This work aims to advance our modeling framework by (1) estimating key model parameters from data collected in wet-lab studies of pro-nociceptive and anti-nociceptive PKC-δ neurons and (2) increasing the visibility and accessibility of our model by developing a web-based platform for model simulation. Key parameters in our 3-D model include the quantity and location of neurons in the CeA and their role pain regulation. To estimate parameters specific to PKC-δ neurons, we used dual antibody immunohistochemistry (IHC) to distinguish between putative pro-nociceptive and anti-nociceptive PKC-δ expressing neurons in the CeA by taking advantage of isoflurane-dependent cFos expression in anti-nociceptive PKC-δ neurons. Imaris cell counting software was used to quantify these neuron subpopulations and their overlap. The wet lab experiments showed the distribution of pro-nociceptive versus anti-nociceptive PKC-δ expressing neurons across the capsular (CeC), medial (CeM), and lateral (CeL) sub-divisions within the CeA. Imaris cell counting showed a significant split between pro:anti PKC-δ cells suggesting the potential for functional differences of these two populations. The quantified cell-type specific markers will be used in the future to functionally distinguish between the two subpopulations of PKC-δ neurons. Additional analysis experiments can be performed to further distinguish between pro- and anti-nociceptive PKC-δ neurons as well other bi-directionally modulated CeA populations. To increase the accessibility of our computational model of CeA neurons, we developed an interactive web-version of the model. Our website provides 3-D visualization of the CeA and neural activity generated via a Java server running NetLogo3D internally. The website allows for users to simulate pain-related neural activity within the CeA and view emergent pain output. Ongoing work to the website aims to include new parameters estimated from wet-lab experiments such as those mentioned above and to expand functionality of the site to allow other researchers to upload their physiology and histology data for use in the model.

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Poster

PSTR400

Brain Mechanisms and Processing of Pain

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Program #/Poster #: PSTR400.14/E7

Topic: D.01. Somatosensation – Pain and Itch

Support: R01NS112632
F31NS125974
R01DA037621
R01NS045954
R01NS062306

Title: Somatostatin Neurons and Sphingosine-1-Phosphate Receptor 1 in the Amygdala Reduce Inflammatory Pain

Authors: S. R. LAMERAND[1], P. L. SHEETS[2], ***B. K. TAYLOR**[1];
[1]Anesthesiol., Univ. of Pittsburgh, Pittsburgh, PA; [2]Dept. of Pharmacol. and Toxicology, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: The central nucleus of the amygdala (CeA) modulates both the sensory/discriminative and affective/emotional components of pain processing. Recent studies suggest that the somatostatin-expressing subpopulation (SOM+) of CeA neurons are pain inhibitory since: 1) Nerve injury decreases their excitability; and 2) Chemogenetic inhibition induces behavioral signs of the discriminative component of pain, e.g. mechanical hypersensitivity (Wilson et al., 2019). We first evaluated the contribution of SOM+ neurons to the affective/emotional component of pain. To do this, SOM-Cre mice were injected with inhibitory (AAV8-hM4Di) or excitatory (AAV8-hM3Dq) DREADD into the CeA, two weeks later they were fitted with intracranial cannulae into the right CeA (AP -1.25mm, ML 2.8mm, DV -3.65mm), and then 1 week later were injected with CNO (0.1µg/.2µL) and tested for conditioned place aversion (CPA) or preference (CPP). We found that CNO did not induce conditioned place preference in DREADDq mice two days after CFA but did induce CPA in uninjured DREADDi mice. The CeA expresses Sphingosine-1-Phosphate receptors (S1PRs) and so we next tested the hypothesis that pharmacological agonism at CeA S1PR1 receptors would inhibit mechanical hypersensitivity. Male and female C57BL/6J mice were fitted with intracranial cannulae into the right CeA and then one-week later 10µL of complete Freund's adjuvant (CFA) was injected into the left plantar hindpaw skin. CFA induced mechanical hypersensitivity (vFrey) that could be reduced ($p<0.05$) with intra-CeA microinjection (0.2µL) of the S1PR1 agonists fingolimod (0.1µg) or SEW2871 (1µg), but not vehicle. The S1PR1 agonists SEW2871 (1µg) also inhibited mechanical hypersensitivity associated with inhibition of CeA SOM+ neurons. We conclude that: 1) Inhibition of CeA SOM+ neurons causes affective/emotional pain; 2) S1PR1 agonists into CeA inhibit inflammatory pain; and 3) S1PR1 agonists into CeA prevent hypersensitivity

associated with SOM+ neuron inhibition. These studies point to S1PR1 in CeA, and perhaps S1PR1 coupled to stimulatory G-proteins on SOM+ neurons, as promising pharmacotherapeutic targets for the relief of persistent pain.

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Poster

PSTR400

Brain Mechanisms and Processing of Pain

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Program #/Poster #: PSTR400.15/E8

Topic: D.01. Somatosensation – Pain and Itch

Support: HI22C0467
R2022020030

Title: 40-hz acoustic stimulation reduces pain sensitivity and tumor necrosis factor-alpha in the ventrolateral periaqueductal gray in a mouse model of neuropathic pain

Authors: *C. CHEONG, A. HO, S. YU, T. KIM;
Gwangju Inst. of Sci. and Technol., Gwangju-si, Korea, Republic of

Abstract: Background: Sensory stimuli at 40 Hz have been shown to induce gamma oscillations in the brain and reduce microglia-induced inflammation in mouse models of Alzheimer's disease. In neuropathic pain, inflammatory cytokines released by microglia, such as tumor necrosis factor-alpha (TNF-α), are crucial. They excite GABAergic neurons, which inhibit dopaminergic neurons, within ventrolateral periaqueductal gray (vlPAG), a key area for pain modulation. Peripheral nerve injury has been demonstrated to trigger microglial activation in the vlPAG, thus enhancing pain sensitivity. However, the therapeutic potential of 40-Hz sensory stimuli in treating neuropathic pain has not been unexplored. Therefore, we hypothesized that 40-Hz acoustic stimulation could alleviate neuropathic pain by suppressing microglial activation in the vlPAG and examined this therapeutic possibility in an animal model of neuropathic pain. **Methods:** The mouse model of neuropathic pain was established using sciatic nerve crush (SNC) surgery. Pain sensitivity was assessed through the Hargreaves and the von Frey test for thermal and mechanical nociception, respectively. The 40-Hz acoustic stimulation protocol delivered click sounds composed of 5-millisecond pulses with 25-millisecond interpulse intervals at 90 dB for two hours. Local field potentials were recorded using microelectrodes implanted via stereotactic surgery. Levels of TNF-α in the vlPAG were quantified using punch biopsy, ELISA, and BCA assay. **Results:** First, 40-Hz acoustic stimulation reduced bilateral pain sensitivity when administered from day 1 to day 14 or from day 15 to day 28 after SNC surgery. In addition, 40-Hz acoustic stimulation increased the gamma power in the vlPAG and the somatosensory cortex. Furthermore, SNC surgery induced an elevation of TNF-α in the vlPAG contralateral to the injury site after one week. 40-Hz acoustic stimulation reduced the TNF-α

levels in the vlPAG. **Conclusion:** These findings suggest that 40-Hz acoustic stimulation can reduce pain sensitivity and TNF-α in the vlPAG in neuropathic pain. Further investigation is needed to explore the underlying mechanisms of 40-Hz sensory stimulation's impact on pain modulation and inflammation, potentially leading to new therapeutic strategies for neuropathic pain.

Disclosures: C. cheong: None. **A. Ho:** None. **S. Yu:** None. **T. Kim:** None.

Poster

PSTR400

Brain Mechanisms and Processing of Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.16/E9

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: Pain Foundation Ltd.
Australian Government Research Training Postgraduate scholarship

Title: Uncovering the role of GlyT2+ periaqueductal gray neurons in pain and pain-related behaviours

Authors: *C. FENECH[1,2], N. ASSAREH[3,1], K. R. AUBREY[1,2];
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Abstract: The debilitating condition of chronic pain is a significant health issue that affects 1 in 5 people around the world and is associated with many comorbidities such as depression, anxiety, and sleep disorders. A general understanding of pain pathways in the central nervous system is known, however, knowledge about the contribution of different neuronal subtypes is still emerging. A population of glycinergic neurons have been reported in the midbrain ventrolateral periaqueductal grey (vlPAG), a key area involved with descending pain modulation. We have previously demonstrated in mice that vlPAG neurons that express GlyT2, a marker of glycinergic neurons, bidirectionally modulate acute nociception such that their chemogenetic activation increases nociceptive sensitivity whereas inhibition reduces it. However, it is unknown how these neurons contribute to coordinating pain-associated behaviours and if their role is altered in a persistent pain state. Hence, we extended our findings by chemogenetically modulating vlPAG-GlyT2 neurons in anxiety-like behavioural tests and in a model of persistent inflammatory pain. Chemogenetic activation of GlyT2+ PAG neurons increases the time in the light zone in the light-dark box and time spent in open arms in the elevated plus maze test, suggesting vlPAG-GlyT2 neuronal activation is anxiolytic. Interestingly, in mice following CFA-induced persistent inflammation, chemogenetic modulation of GlyT2+ PAG neurons does not alter the deficits in nociception and maintains the ability to modulate

anxiety-like behaviours. These results demonstrate that activation of GlyT2+ PAG neurons is both pro-nociceptive and anxiolytic, suggesting these neurons contribute to not only nociceptive signalling but also the wider affective pain experience.

Disclosures: C. Fenech: None. **N. Assareh:** None. **K.R. Aubrey:** None.

Poster

PSTR400

Brain Mechanisms and Processing of Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.17/E10

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant NS120486

Title: Exploring Corticosterone-Driven Adaptations in the Endocannabinoid System Following Chronic Inflammatory Pain in the vlPAG

Authors: *B. COUTENS[1], C. BOUCHET[2], K. B. MCPHERSON[1], L. PATTI[1], B. BOSTON[1], D. C. JEWETT[3], S. INGRAM[1];
[1]Univ. of Colorado, Anschutz Med. Campus, Aurora, CO; [2]Colorado State Univ., Fort Collins, CO; [3]Psychology, Univ. of Wisconsin, Eau Claire, Eau Claire, WI

Abstract: Chronic pain represents an enormous personal and economic burden affecting over 30% of the world's population, and its clinical management remains a pressing challenge. Endogenous opioids and endocannabinoids activate neurons in the ventrolateral periaqueductal gray (vlPAG) to modulate pain. Our previous studies found that chronic inflammation induced by injections of Complete Freund's adjuvant (CFA) into a hind paw result in hyperalgesia, cannabinoid 1 receptor (CB1R) desensitization, and elevation of endocannabinoids in the vlPAG. The current studies investigate the hypothesis that CFA-induced elevation of corticosterone mediates the observed CB1R desensitization via increased endocannabinoid synthesis. Whole-cell patch-clamp recordings from vlPAG neurons in *ex vivo* slices of male and female Sprague Dawley rats were used to measure evoked GABAergic inhibitory postsynaptic currents (eIPSCs) in vlPAG neurons. Corticosterone (1 µM) activates membrane glucocorticoid receptors (mbGRs), resulting in inhibition of eIPSCs that was reversed by both rimonabant (3 µM) and RU486 (3 µM), CB1R and GR antagonists, respectively. These results indicate that corticosterone augments endocannabinoid tone and inhibits presynaptic GABA release via CB1R activation. Interestingly, CB1R desensitization develops faster in females than in males (1 day vs 7 days), indicating a sex-specific modulation of the endocannabinoid system. However, this sex difference is not reflected in the emergence of CFA-induced hyperalgesia. The alterations in endocannabinoid tone, observed in slices from CFA-treated rats and corticosterone-exposed naive rats by using a depolarization-induced suppression of inhibition (DSI) protocol, are both dependent on protein kinase A (PKA) activation and reversed by inhibiting diacylglycerol lipase

(DAGL), the enzyme that synthesizes 2-arachidonyl glycerol (2-AG). The DSI protocol induced a prolonged inhibition in eIPSC amplitudes, indicating an increase in 2-AG tone in CFA-treated and CORT-exposed naive rats. A PKA inhibitor directly in the recording electrode or a DAGL inhibitor (DO34) reversed this effect, resulting in transient inhibition of eIPSC similar to DSI recordings from naive rats. In summary, our findings indicate that corticosterone regulates the endocannabinoid system in the vlPAG in a sex-dependent manner and could mediate the alterations induced by CFA. We are currently investigating the ability of glucocorticoid receptor antagonists to block CFA-induced hyperalgesia.

Disclosures: B. Coutens: None. **C. Bouchet:** None. **K.B. McPherson:** None. **L. Patti:** None. **B. Boston:** None. **D.C. Jewett:** None. **S. Ingram:** None.

Poster

PSTR400

Brain Mechanisms and Processing of Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.18/E11

Topic: D.01. Somatosensation – Pain and Itch

Support: European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreement No. 956414.
UKRI Medical Research Council (MRC; project number: MR/019484/1).

Title: The relationship between fear extinction and affective state susceptibility in neuropathic pain: the role of cerebellar-PAG interactions.

Authors: *V. SPATHARIOTI[1], R. DRAKE[1], R. APPS[1], B. M. LUMB[1], C. L. LAWRENSON[2]; [1]Univ. of Bristol, Bristol, United Kingdom; [2]Univ. of Exeter, Exeter, United Kingdom

Abstract: Studies in rats have demonstrated a relationship between fear extinction phenotypes and susceptibility to pain-related behaviours in a neuropathic pain state [1]. Fast extinction learners (FE+) show reduced pain-related behaviours compared to slow extinction learners (FE-). Both the cerebellum and the periaqueductal grey (PAG) are involved in pain processing and the medial cerebellar nuclei-ventrolateral periaqueductal grey pathway (MCN-vlPAG) is involved in fear extinction (as measured by conditioned freezing [2]). The present study tested the hypothesis that the link between fear extinction learning and susceptibility to neuropathic pain is dependent, at least in part, on activity in the MCN-vlPAG pathway. Two groups of Sprague-Dawley male rats (total n=26) underwent auditory cued fear conditioning followed by extinction testing. Subsequently, in all animals, the MCN-vlPAG pathway was targeted with cre-dependent inhibitory DREADDs. Animals in group 1 (n=11) underwent tibial nerve transection as a model of neuropathic pain and group 2 (n=15) were controls (naïve in relation to pain). In both groups, the effect of chemogenetic inhibition of the MCN-vlPAG pathway using CNO was tested on hindlimb evoked nociceptive responses (Von Frey and Hargreaves) and affective pain state

(conditioned place preference test, CPP). Both groups 1 and 2 were separated into FE+ (n=16, 62 %) and FE- (n=10, 38 %) based on rate of extinction. Group 1 animals developed mechanical allodynia as tested using Von Frey. When compared with FE+ animals, FE- animals had a significantly decreased paw withdrawal threshold (p=0.009, anova, Bonferroni's mct). This difference was abolished following inhibition of the MCN-vlPAG pathway (p>0.999, anova, Bonferroni's mct). In group 1 animals, inhibition of the MCN-vlPAG pathway also significantly increased time in the CNO paired chamber of the CPP (p=0.003, paired t test), but not in the vehicle treated chamber when compared to baseline. This effect was found for both FE+ (p=0.028, paired t test) and FE- (p=0.083, paired t test) phenotypes. No effect was found in group 2, naïve animals, when comparing the CNO and vehicle chamber. These data support the hypothesis that the inhibition of the MCN-vlPAG pathway contributes to the relationship between fear extinction and neuropathic pain susceptibility. 1. Ji et al. Fear extinction learning ability predicts neuropathic pain behaviors and amygdala activity in male rats. Mol Pain. 2018 14:1744806918804441. 2. Lawrenson et al. Cerebellar modulation of memory encoding in the periaqueductal grey and fear behaviour. Elife. 2022 15;11:e76278.

Disclosures: V. Spatharioti: A. Employment/Salary (full or part-time):; School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, United Kingdom. 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.; European Union’s Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreement No. 956414, UKRI Medical Research Council (MRC; project number: MR/019484/1). **R. Drake:** A. Employment/Salary (full or part-time):; School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, United Kingdom. **R. Apps:** A. Employment/Salary (full or part-time):; School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, United Kingdom. **B.M. Lumb:** A. Employment/Salary (full or part-time):; School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, United Kingdom. **C.L. Lawrenson:** A. Employment/Salary (full or part-time):; Medical School, University of Exeter, Exeter, United Kingdom.

Poster

PSTR400

Brain Mechanisms and Processing of Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.19/E12

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH R01

Title: Identifying brainstem structures required for visceromotor response and aversive learning associated with colorectal distension pain

Authors: *S. A. LEE[1], J. E. LOEZA ALCOCER[2], M. S. GOLD[2], R. P. SEAL[3];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA;
[3]Neurobio., Univ. of Pittsburgh Ctr. For Neurosci., Pittsburgh, PA

Abstract: Visceral pain is one of the most prevalent types of pain that negatively impacts patients' quality of life. It is common in preclinical studies to use visceromotor response (VMR) caused by colorectal distension as a measurement for colorectal pain. Behavioral paradigms that measure colorectal distension associated aversive learnings (Step-down assay) also have been used to study affective aspects of colorectal pain. However, the neural substrates underlying those behaviors are not well understood. In this study, we aim to investigate the role of three brainstem structures - the parabrachial nuclei (PBN), dorsal column nuclei (DCN), and nucleus tractus solitarius (NTS) - in colorectal distension induced VMR and aversive learning. All three structures are known to receive colon afferent information, but their functional importance has yet to be established. Utilizing knock-in mice for activity-dependent genetic labeling (TRAP2), we identified neurons that were responsive to noxious (70mmHg) colorectal distension. In the DCN, more neurons were active in the condition that received noxious colorectal distension compared to the condition of colorectal stimulation. Within the PBN, there was an increase in the number of active neurons in the external lateral PBN at the noxious colorectal distension condition. In the NTS, we observed a large number of neurons active in the no colon stimulation condition, but there was still a trend of increase in the number of neurons active in the noxious colorectal distension condition. To test the functional role of these brainstem structures, we plan to inhibit the activity of excitatory brainstem neurons using the designer receptor exclusively activated by designer drug (DREADD) and measure changes in colorectal distension induced VMR as well as step-down behavior to colorectal distension. We found that inhibition of VGLUT2 positive neurons in the lateral PBN prevented aversive learning to noxious CRD but did not alter colorectal distension induced VMR, suggesting that the lateral PBN is necessary for processing aversion-motivational aspects of mechanical colon pain but dispensable for reflexive responses. Results about the functional relevance of NTS and DCN will also be presented in the poster.

Disclosures: S.A. Lee: None. **J.E. Loeza Alcocer:** None. **M.S. Gold:** None. **R.P. Seal:** None.

Poster

PSTR400

Brain Mechanisms and Processing of Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.20/E13

Topic: D.01. Somatosensation – Pain and Itch

Support: F32NS128392
K00NS124190

Title: A parabrachial subpopulation responsible for modulating inflammatory pain in mice

Authors: *H. N. ALLEN, T. S. NELSON, R. KHANNA;
Pharmacol. & Therapeut., Univ. of Florida, Gainesville, FL

Abstract: Pain is a multifaceted experience that starts with activation of peripheral nociceptors that transmit nociceptive signals to the spinal cord before relaying them to the brain, where the perception of pain is ultimately produced. The parabrachial nucleus (PBN) is a hindbrain region involved in a variety of homeostatic functions and one of the first supraspinal regions to receive noxious input via the spino-parabrachial pathway. Parabrachial neurons have increased excitability in the context of pain, and glutamatergic neurons, which make up approximately 90% of the PBN, are both necessary and sufficient for neuropathic pain. Recent transcriptomic studies reveal a heterogeneity of glutamatergic neuronal subpopulations marked by various neuropeptides and receptors. We have performed *in situ* characterization of PBN neurons activated by nociceptive stimuli and identified neurons expressing Bombesin Receptor Subtype 3 (*Brs3*) as a subpopulation of interest for pain processing. Although no role for parabrachial *Brs3* in pain modulation has been reported, *Brs3* is highly expressed in parabrachial neurons that also express other GPCRs involved in pain modulation, including opioid receptors, tachykinin 1 receptors, and neuropeptide Y Y1 receptors. Here, we identify a role for parabrachial neurons expressing *Brs3* in inflammatory pain-like behaviors in rodents. Parabrachial *Brs3* neurons are activated in response to nociceptive stimuli, measured via *in situ Fos* immunoreactivity and *in vivo* calcium imaging. Further, activation of *Brs3* PBN neurons with an excitatory designer receptor exclusively activated by a designer drug (DREADD) produces spontaneous and evoked pain-like behaviors in otherwise naïve rodents. Finally, inhibition of *Brs3* neurons in the PBN reverses allodynia induced by the Complete Freund's Adjuvant (CFA) model of inflammation. Ultimately, these findings suggest that parabrachial *Brs3* neurons are involved in pain modulation and may provide a more precise target for understanding the supraspinal circuitry involved in inflammatory pain.

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Poster

PSTR400

Brain Mechanisms and Processing of Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.21/E14

Topic: D.01. Somatosensation – Pain and Itch

Support: Duke University Anesthesiology Research Funds
NIH R01 grant 1NS13181201A1
DoD grants W81XWH2110885 and W81XWH2110756

Title: Lps-induced transcriptional changes in the brain stem and role of auricular stimulation and resolvin d2 in lps-induced sickness behaviors and pain

Authors: *W. HE[1], S. BANG[2], Y. WANG[3], R.-R. JI[4];
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Abstract: LPS-induced transcriptional changes in the brain stem and role of auricular stimulation and resolvin D2 in LPS-induced sickness behaviors and pain Wei He[1], Sangsu Bang[1], Yu-Qing Wang, Ru-Rong Ji[1,2,3] **[1]**Center for Translational Pain Medicine, Department of Anesthesiology, **[2]**Departments of Neurobiology[2] and Cell Biology[3], Duke University Medical Center, Durham, NC, United States of America Auricular electrostimulation (aES) is a type of ambulatory electrical stimulation applied to the ear, known for its efficacy in pain management. In our previous study we have observed that auricular electrostimulation plays a protective role against LPS-induced neuroinflammation sickness behaviors, and pain. Furthermore, we found aES increased the release of resolvin D2 (RvD2), which can recapitulate the benefits of aES. We further investigated transcriptional changes in the dorsal vagal complex (DVC) after aES and RvD2 administrationusing RNA sequencing. The results showed that compared with naïve group, 1612 genes were upregulated, and 1312 genes were downregulated after LPS injection. Strikingly, these transcriptional changes are largely prevented by aES and RvD2 treatments. Our results indicate that auricular stimulation may protect against LPS-induced sickness behaviors via RvD2-mediated regulation of neuroinflammation. **Conflict of interest** The authors declare that they have no conflict of interest. **Link of group information** Ji Lab | Duke Neurobiology

Disclosures: W. He: None. **S. Bang:** None. **Y. Wang:** None. **R. Ji:** None.

Poster

PSTR400

Brain Mechanisms and Processing of Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.22/E15

Topic: D.01. Somatosensation – Pain and Itch

Support: GRF Grant 14117122
HMRF Grant 09203466
Shenzhen Technology Innovation Committee Grant
SGDX20210823103534005

Title: A Probiotic Bacterium Produces Butyrate for Alleviating Neuropathic Pain--Role of the Vagus Nerve-NTS-CeA Circuit

Authors: *X. LIU;
The Chinese Univ. of Hong Kong, HONG KONG, Hong Kong

Abstract: Approximately 20% of patients with shingles develop postherpetic neuralgia (PHN). We investigated the role of gut microbiota in shingle- and PHN-related pain. Significant alterations of the gut microbiota were observed in patients with shingles and PHN. Microbial markers predicted PHN development among patients with shingles. Functionally, fecal microbiota transplantation from patients with PHN to mice heightened pain sensitivity. Administration of a probiotic bacterium, PR, a depleted bacterium in patients with shingles and PHN, alleviated peripheral nerve injury-induced pain in mice by enhancing vagal neurotransmission to nucleus tractus solitarius (NTS) to suppress the central amygdala (CeA). PR was found to produce butyrate to activate the vagal neurons through GPR41. Vagal knockout of *Gpr41* abolished the effects of RI on the NTS-CeA circuit and pain behaviors. In conclusion, our study established a gut microbiota-based model for PHN risk assessment and identified PR as a pain-alleviating probiotic through modulating the gut-brain axis.

Disclosures: X. Liu: None.

Poster

PSTR400

Brain Mechanisms and Processing of Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.23/E16

Topic: D.01. Somatosensation – Pain and Itch

Support: HORIZON-MSCA-2021-PF-01_ RAB-PPS 101068450

Title: Brodmann area 7 mediates the value dependent modulation of the blink reflex

Authors: *F. ROCCHI[1,2], R. J. BUFACCHI[3], G. GABRIELI[1], S. M. ROMANELLA[4], S. ROSSI[5], G. D. IANNETTI[1,6];
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Abstract: The blink reflex (BR) is a prototypical defensive behaviour, mediated by a medullary brainstem circuit (Kofler et al 2024). Despite being a reflex response, the BR size reflects the nervous system's implicit estimation of the potential to harm of the eliciting stimulus. When elicited by somatosensory electrical stimulation of the human median nerve (hand-blink reflex, HBR), the HBR is dramatically modulated by a number of experimental factors related to the

value of blinking. For example, the HBR doubles in magnitude when the stimulated hand is close to the face rather than far away (Sambo et al 2012; Bufacchi et al 2016). Although there are different pieces of evidence suggesting that such modulation of the HBR medullary circuit relies on top-down cortical efference, it is unclear which cortical areas are involved. Here, in a group of healthy human participants, we assessed the proximity-dependent HBR enhancement following manipulation of Brodmann area 7 (BA7). We chose BA7 because it contains the human homologue of the ventral intraparietal area (VIP) in monkeys, which likely encodes the value of actions related to interacting with objects near the body. When stimulated, VIP elicits equifinalistic defensive actions (Cooke and Graziano 2004). We used high-definition transcranial direct current stimulation (tDCS) to either enhance or inhibit BA7 activity. We found that enhancing BA7 activity significantly increased both the overall HBR magnitude as well as the proximity-dependent HBR enhancement compared to a sham condition. These results indicate that BA7 modulates the excitability of the brainstem circuitry subserving the HBR, and hence is part of a cortical network involved in estimating the value of defensive blinking.

Disclosures: F. Rocchi: None. **R.J. Bufacchi:** None. **G. Gabrieli:** None. **S.M. Romanella:** None. **S. Rossi:** None. **G.D. Iannetti:** None.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.01/E17

Topic: D.01. Somatosensation – Pain and Itch

Title: The role of keratinocyte-derived exosomes on dorsal root ganglia excitability and neurite outgrowth in painful diabetic neuropathy

Authors: *J. COY-DIBLEY;
Northwestern Univ., Chicago, IL

Abstract: Painful diabetic neuropathy (PDN) is a debilitating and intractable complication of diabetes with patients suffering from a painful, burning sensation in their extremities. Current available treatments have limited effect in masking the pain without remediating the underlying mechanisms of the disease. The cellular hallmarks of PDN are cutaneous nerve-fiber degeneration and the hyperexcitability of the dorsal root ganglia (DRG) neurons. Epidermal keratinocytes are closely juxtaposed to cutaneous nerve terminals in the skin, enabling bidirectional communication between keratinocytes and cutaneous nerves. Exosomes are secreted extracellular vesicles that can produce substantial transcriptional and translational changes. The role of keratinocyte-derived exosomes in mediating DRG neuron hyperexcitability and axonal degeneration in PDN is unknown. Using primary adult mouse keratinocyte cultures, we characterized keratinocyte-derived exosomes (KDEs) for the first time in the established

high-fat diet (HFD) induced mouse model of PDN and their role on DRG excitability and neurite growth both in vitro and in vivo. Using size exclusion chromatography, we isolated highly enriched and purified KDEs and performed an extensive, unbiased, and robust molecular characterization with proteomics and RNAsequencing in mice and found significantly altered cargos between HFD and regular diet control KDEs. Additionally, using an in vivo, conditional EV reporter mouse, we have demonstrated that keratinocyte-originating nanovesicles are retrogradely trafficked into the DRG neuron cell body, suggesting a potentially new pathway for DRG transcriptome and proteome regulation originating from the skin. Using these unbiased molecular characterization methods to study keratinocyte-derived exosomes is an exciting novel investigation into neuron-keratinocyte communication in normal and diabetic skin. Our results could be translated into new topical interventions, which could fulfil the unmet need for new therapies for both small-fiber degeneration and neuropathic pain in diabetes.

Disclosures: J. Coy-Dibley: None.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.02/E18

Topic: D.01. Somatosensation – Pain and Itch

Support: CTSC Grant Number: UL1TR001449
KL2 Grant Number: KL2TR001448

Title: Characterization of Dermal Interstitial Metabolic Markers of Diabetic Neuropathy in Rat using Thermal Microneedle Arrays coupled with Liquid Chromatography—Tandem Mass Spectrometry

Authors: *S. GADAM[1], R. M. TAYLOR[2], E. S. RIVERA[3], E. M. MCBRIDE[3], E. D. MILLIGAN[4], K. WESTLUND HIGH[5], I. ADAMS[6], J. T. BACA[2];
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Abstract: INTRODUCTION: Diabetes and prediabetes are considered global epidemics, and the burden of diabetic complications including diabetic neuropathy are substantial. The absence of effective therapies for diabetic peripheral neuropathy (DPN), particularly in Type II diabetes, is in part, due to a lack of understanding the mechanisms that drive peripheral neuropathy. One potential mechanism of DPN may be the accumulation of neurotoxic metabolites in the dermal interstitium that contribute to local metabolic dysregulation of peripheral nociceptors leading to

metabolically driven hyperalgesia. The peripheral metabolic mechanisms of DPN remain poorly understood. Dermal interstitial fluid (ISF) has recently gained traction as an alternative to blood for rapid and minimally painful analysis of biomarkers, as supported by our previous work establishing that ISF, serum, and plasma have similar biochemical profiles. Therefore, we posit that peripheral dyslipidemia and glycemic anomalies at the axon-Schwann cell interface of dermal interstitium lead to metabolic dysregulation of peripheral sensory neurons contributing to neuropathy.
METHODS: 10-week-old Sprague Dawley rats (n = 6) with matched age and sex were fed a high fat diet (HFD, *D12492*) and at week 19 given streptozotocin (STZ, 35 mg/kg) to establish diabetes. A separate group of rats were fed HFD alone to establish prediabetes. Weekly hind paw thermal hyperalgesia assessment using the Hargreaves test beginning at 9 weeks to study completion, at week 27, was performed. At week 9, week 14, and week 20, we collected peripheral ISF at the axon-Schwann cell interface of dermal interstitium by our novel minimally invasive thermal microneedle arrays (TMIA), using conventional microneedle arrays coupled with heat therapy (41 ^{o}C). At the study completion, quantification of ISF free-fatty acids and their metabolic intermediates acylcarnitines, sphingolipids like ceramides, and advanced glycated end products and their precursor methylglyoxal was performed using TMIA and liquid chromatography-tandem mass spectrometry (LC-MS/MS).
RESULTS: Diabetic and prediabetic rats gained significant body weight and yielded elevated blood glucose and blood lactate levels, confirming previous published work. Rats also have a significant reduction in the response threshold to heat stimulus on the Hargreaves apparatus, demonstrating thermal hyperalgesia.
CONCLUSION: Collectively, findings highlight the role of peripheral tissue-specific lipidomic alterations in the pathogenesis of DPN. The study also presents the potential for novel TMIA assisted ISF extraction and analysis technique for the study of ISF.

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Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.03/E19

Topic: D.01. Somatosensation – Pain and Itch

Support: SNSF

Title: Inulin improves neuropathic pain, modifies PNS immune cells and gene expression in diet-induced obese mice

Authors: *C. K. GAVINI, V. MANSUY AUBERT;
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Abstract: Peripheral neuropathy (PN) arising from metabolic disorders such as obesity leads to loss of sensory perception, pain, and reduced quality of life. Currently, there are no disease modifying drugs available, as the neurobiology underlying this neuropathic pain is still unclear. Our previous work suggests western diet (WD) disrupts gut microbiome and that fecal transplantation (FMT) from lean to diet-induced obese (DIO) mice decreases obesity-induced hypersensitivity. This is accompanied by changes in peripheral nerve system (PNS) gene expression and inflammation. Our results also suggest that short chain fatty acids (SCFAs) - metabolites secreted by gut microbiome may be involved by acting directly on PNS immune cells and gene expression. As FMT studies have shown considerable heterogeneity, finding the right method of delivery is necessary to establish SCFAs as an effective strategy, and to study the mechanisms driving the interaction between the gut microbiota and host physiology. Inulin is a fermentable polysaccharide that has been shown to reduce weight gain. In our study, we investigated the underlying mechanisms behind inulin-mediated changes in energy balance, glucose homeostasis, and PN by comprehensive analysis of gut bacterial composition, plasma metabolome, gene expression using RNAseq, immune responses, and evoked pain-associated behaviors. Using 16S sequencing of DNA isolated from the cecal contents we show that Lachnospiraceae, and Lactobacillus - producers of SCFAs, were among the top genera decreased in WD-fed mice and their absence corroborated with decreased levels of SCFAs in both portal and systemic circulation. Inulin supplementation reshaped the gut microbiome in WD-fed mice and increased circulating levels of SCFAs, mainly that of propionate. Inulin supplementation improved insulin resistance, glucose sensitivity, and alleviated WD-induced weight gain. Inulin supplementation improved markers of systemic inflammation, and the pathophysiology of the PN by decreasing evoked pain-associated behaviors and loss of nerve fibers in the skin. Analysis of gene expression pathways using RNAseq of dorsal root ganglia from the WD-fed and inulin supplemented WD-fed mice show modulation of pathways involved in cholesterol homeostasis, unfolded protein response, fatty acid metabolism, calcium signaling, and inflammatory response that are consistent with inulin-mediated improvement in pain indices. These data demonstrate a distinctive physiological impact of inulin, in elevating levels of SCFAs, accompanied with different effects on the plasma metabolome, gut bacterial populations, and markers of systemic inflammation.

Disclosures: C.K. Gavini: A. Employment/Salary (full or part-time):; University of Lausanne.
V. Mansuy Aubert: A. Employment/Salary (full or part-time):; University of Lausanne.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.04/E20

Topic: D.01. Somatosensation – Pain and Itch

Title: Methylglyoxal promotes pain through the ISR kinase GCN2

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Abstract: Translational control plays an essential role in models of persistent pain. Nociceptors are a type of sensory neuron tasked with the detection and propagation of pain signals. Many conditions cause persistent changes in nociceptor excitability and neuropathy. Diabetes is the most common cause of peripheral neuropathy. Methylglyoxal (MGO) has been linked to diabetic neuropathy through direct activation of nociceptors. However, the mechanisms that underlie pain caused by MGO are incompletely understood. Prior work established that MGO engages a specific translational control mechanism known as the integrated stress response (ISR) in sensory neurons (Barragán-Iglesias P et al Pain 2019). The ISR is a mechanism for repressing global translation in response to cell stress - blockade of the ISR prevents nociceptive sensitization by MGO. Here, we show that MGO induces the ISR through general non-derepressible control 2 (GCN2) kinase in primary sensory neurons. GCN2 activation is the result of impaired elongation and the accumulation of ribosome collisions. Importantly, inhibition of GCN2 prevents pain in mice treated with MGO. This work implicates GCN2 as potential therapeutic target for a diabetic peripheral neuropathy.

Disclosures: A. Meyer: None. **Z. Campbell:** None.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.05/E21

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant 1R01DK134893-01
TTUHSC Start-up Fund

Title: Changes in integrated stress response in hyperglycemic sensory neurons

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Abstract: Diabetic neuropathy is a serious complication caused by prolonged exposure to hyperglycemia in diabetic patients which in turn causes peripheral nerve damage. Uncontrolled

glucose levels may lead to oxidative stress and inflammation, thus causing alterations in pain and temperature sensation in the damaged nerve areas. Cellular responses to stress are activated when homeostasis is altered and such responses include the Integrated Stress Response (ISR) and the activation of the Endoplasmic Reticulum (ER)-Mediated Unfolded Protein Response (UPR) elements. ISR-mediated phosphorylation of eukaryotic translation initiation factor (eIF)2-alpha and alteration in protein synthesis may demonstrate as an important physiological process which could be crucial for the development of painful neuropathy in diabetics. Blocking the activated ISR by inhibiting ISR-inducing kinase Protein kinase R (PKR), or by altering eIF2, may protect the sensory neuron dysfunction under hyperglycemic condition. In this study, we examined how hyperglycemia-mediated ER stress and UPR activation induce pathophysiological changes in neuronal cells of the dorsal root ganglion (DRG). To evaluate the damaging effect of high glucose in DRG neurons, the DRGs of spontaneous type 2 diabetic animals (leptin receptor-deficient) as well as F11 DRG cell culture were investigated simultaneously. F11 DRG cells were exposed to high glucose conditions at various time points. Our studies revealed that there was an activation of both the ISR and UPR elements, including the alterations in BiP, pJNK, IRE1α, ATF6, CHOP, XBP1, Calnexin and peIF2α, and these changes in expressions were ameliorated by treatment with Integrated Stress Response Inhibitor (ISRIB) in both in vivo and in vitro studies as confirmed by Western Blot Analysis and Immunohistochemical studies. This study also revealed that inhibition of ISR using small molecule inhibitor ISRIB at early time point could alleviate diabetic neuropathic pain including thermal and mechanical pain response. These findings suggest that ISR elements and ER stress could lead to nerve damage in diabetic patients, which could be alleviated by treatment with ISRIB, suggesting that ISRIB could be an effective therapeutic drug for diabetic neuropathy.

Disclosures: E. Chen: None. **V.S. Thakur:** None. **M. Chattopadhyay:** None.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.06/E22

Topic: D.01. Somatosensation – Pain and Itch

Support: Department of Anesthesiology and Perioperative Medicine at Loyola University Medical Center, Maywood, IL
Surgical Service at Edward Hines Jr. VA Hospital, Hines, IL
National Heart, Lung, and Blood Institute T35 grant (HL120835)

Title: Long-term sensorimotor changes after a sciatic nerve block with bupivacaine and liposomal bupivacaine in a high-fat diet/low-dose streptozotocin rodent model of diabetes

Authors: *K. M. LOTESTO[1,6], M. VOLYANYUK[2,6], J. E. EXLINE[2,6], E. A. SAGER[3], E. FOECKING[4,6,5,1], S. C. BYRAM[8,7];
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Abstract: It is unclear whether patients with diabetes are more susceptible to nerve toxicity of local anesthetics or whether nerve blocks can accelerate the progression of diabetic peripheral neuropathy. Bupivacaine is one of the most widely used local anesthetics for regional anesthesia, despite many pre-clinical studies demonstrating neurotoxicity. Herein, we report the long-term functional consequences of sciatic nerve block with bupivacaine and liposomal bupivacaine (Exparel®) in an animal model of diabetes. Male Sprague Dawley rats were subject to standard chow/vehicle or high-fat diet/low-dose streptozotocin to induce a diabetic phenotype. Animals were then subdivided into groups that received repeat sciatic nerve blocks of saline, bupivacaine, or liposomal bupivacaine. Mechanical allodynia and thermal hyperalgesia were assessed prior to and 12 weeks following nerve blocks utilizing the von Frey and Hargreaves tests, respectively. Exploratory and locomotor activity were assessed with open field testing, and nerve conduction velocity testing was conducted prior to the termination of the study at 28 weeks. Animals in the diabetic group developed sustained hyperglycemia >200mg/dL and signs of peripheral neuropathy six weeks after treatment with streptozotocin, which persisted until the end of the study. Twelve weeks after a repeat sciatic nerve block with saline, bupivacaine, or liposomal bupivacaine, we found significant interaction effects of the disease group (control versus diabetic) and local anesthetic treatment. Bupivacaine worsened all outcome measures in control animals. Furthermore, bupivacaine worsened tactile allodynia and slowed nerve conduction velocity in diabetic animals compared to their disease-matched saline and liposomal bupivacaine groups. In contrast, liposomal bupivacaine did not cause any worsening in functional outcomes for control or diabetic animals. Our data indicate that bupivacaine, and not liposomal bupivacaine, causes long-term changes in tactile allodynia, thermal hyperalgesia, locomotor activity, and nerve conduction velocity in a high-fat diet/low-dose streptozotocin rodent model of diabetes. These results highlight the necessity to investigate safe peripheral nerve block strategies to preserve long-term functional independence in patients with or at risk for diabetic peripheral neuropathy.

Disclosures: K.M. Lotesto: None. **M. Volyanyuk:** None. **J.E. Exline:** None. **E.A. Sager:** None. **E. Foecking:** None. **S.C. Byram:** None.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.07/E23

Topic: D.01. Somatosensation – Pain and Itch

Support: ANR-PHARMHCN
UCA-iSite
Pack Ambition Recherche Région AURA-PeptHCN
INSERM
Institut Analgesia

Title: Regulation of HCN channels activity by targeting its interaction with the TRIP8b auxiliary subunit in peripheral sensory neurons reduces chemotherapy-induced peripheral neuropathy symptoms

Authors: *E. WERSINGER[1], K. DELANOE[1], M. MOREZ[1], E. TORRE[2], Y. AISSOUNI[1], L. PRIVAL[1], O. ROY[3], C. TAILLEFUMIER[3], M. MANGONI[2], Y. HAN[4], D. M. CHETKOVICH[4], E. BOURINET[5], J. BUSSEROLLES[1];
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Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is a common adverse side effect experienced in patients undergoing treatment with various anticancer chemotherapeutics. CIPN symptoms have no efficient treatment, can seriously affect patients' quality of life and lead to treatment adaptation with the risk of limiting the overall survival outcome. The HCN ion channels family plays an important role in the development and maintenance of neuropathic pain and may offer excellent drug development opportunities. Non-selective HCN blockers have been shown to alleviate pain symptoms in CIPN models but the high expression of HCN channels in the heart increases the risk of cardiac side effects and limits their clinical interest. Interestingly, the function of HCN is tightly regulated by the tetratricopeptide repeat-containing Rab8b interacting protein (TRIP8b), which is not expressed in the heart. Targeting TRIP8b-HCN interaction may be a new strategy to modulate HCN activity and provide an efficient and well-tolerated CIPN treatment. Our strategy was tested in a mouse model of acute CIPN triggered by a single injection of oxaliplatin. The RNA fluorescent multiplex assay was used to characterize the population of peripheral neurons expressing HCN and TRIP8b transcripts. Immunocytochemistry and WB experiments were performed to check the colocalization of the proteins and their expression levels. To modulate the HCN/TRIP8b interaction, we designed peptoids based on the co-cristal X-Ray structure of the TRIP8b tetratricopeptide repeat region with the HCN2 C-terminus sequence. A hit peptoid compound was tested *in vitro* with patch clamp recordings of I_h currents and *in vivo* on cold hypersensitivity in CIPN wild-type and TRIP8b KO mice. In a preliminary attempt to evaluate potential cardiac side effects, we recorded the I_f current in cardiac pacemaker cells in the presence of our hit peptoid. We show high colocalization of HCN1, HCN2, and TRIP8b mRNA and proteins in DRG and TG neurons with increased expression of transcripts in particular subsets of neurons in OIPN mice. I_h current density was significantly increased in small/medium-sized neurons in OIPN but not in TRIP8b KO mice and was reduced after the application of our compound. We did not detect any significant change of I_h current in cardiac cells. *In vivo*, our compound exerted a robust dose-dependent reduction of cold hypersensitivity in wild-type OIPN mice, which was absent in TRIP8b KO mice. Similar *in vivo* effects have been observed in other CIPN models. Overall, our

results confirm that modulating TRIP8b-HCN interaction decreases CIPN symptoms without inducing any effect on HCN function in cardiac cells .

Disclosures: E. Wersinger: None. **K. Delanoe:** None. **M. Morez:** None. **E. Torre:** None. **Y. aissouni:** None. **L. Prival:** None. **O. Roy:** None. **C. Taillefumier:** None. **M. Mangoni:** None. **Y. Han:** None. **D.M. Chetkovich:** None. **E. Bourinet:** None. **J. Busserolles:** None.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.08/E24

Topic: D.01. Somatosensation – Pain and Itch

Support: P30GM122733

Title: Investigating Cannabichromene's Efficacy for Chemotherapy-Induced Neuropathic Pain

Authors: *M. A. DE LEON[1], W. GUL[2], M. ELSOHLY[3], N. M. ASHPOLE[1];
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Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is a debilitating adverse symptom experienced in over 60% of patients receiving chemotherapy treatment. Unfortunately, current therapeutic strategies require long-term treatment with limited efficacy, often requiring opioid-based medications. To mitigate adverse effects of current therapeutics, studies suggest cannabis-based medicines to alleviate neuroinflammation and related pain. Multiple studies have explored delta^9-tetrahydrocannabinol and synthetic cannabinoids, however, an ideal cannabinoid candidate for drug development would be devoid of psychoactive effects. Our current study evaluates the effectiveness of cannabichromene (CBC), shown to have anti-inflammatory properties and devoid of psychoactive effects, on alleviating cisplatin and paclitaxel-induced neuropathic pain. To assess the efficacy of CBC against CIPN, mice were subjected to either a cisplatin (2.3 mg/kg; 6 total injections) or paclitaxel (4 mg/kg; 4 total injections) protocol. Following the administration protocols, single doses of CBC were administered 30 minutes prior to CIPN assessment. Mechanical sensitivity was assessed using an electronic Von Frey (eVF) to measure changes in response to mechanical stimulation of the hind paw at various stages (e.g., pre-chemotherapy, post-chemotherapy, and with treatment onboard). To determine the duration of relief of CBC against CIPN pain, mechanical sensitivity was assessed at 30 min, 4, 24, and 72 hrs. Acute administration of CBC in both male and female mice ablated the allodynia associated with cisplatin-induced neuropathy in a dose-dependent manner. Mice that received greater than 10 mg/kg CBC or CBC derivative showed withdrawal responses at the level of non-cisplatin treated control mice. When examining the duration of relief, we see that CBC maintains its protective properties in male mice for up to 24 hours. However, CBC was not effective in

attenuating paclitaxel-induced neuropathy at any dose tested, suggesting CIPN-specific efficacy. These data indicate that CBC can delay the onset of cisplatin-induced neuropathic pain, suggesting its potential as a novel therapeutic for alleviating CIPN. Further studies will explore CBC's efficacy against other chemotherapies, such as oxaliplatin and vincristine, and examine its cellular and molecular effects on intraepidermal nerve fibers and spinal cords. Additionally, the research will elucidate the effects of repeated dosing, sex-specific differences, and the optimal therapeutic window for CBC.

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Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.09/E25

Topic: D.01. Somatosensation – Pain and Itch

Title: Bioenergetic and mitochondrial alterations in sensory neuron subtypes underlie distinct forms of chemotherapy-induced peripheral neuropathy

Authors: *F. DEEN[1], M. ALQATARI[2], L. E. BROWNE[3], M. R. DUCHEN[2], M. KOLTZENBURG[4];
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Abstract: Cancer patients receiving potentially life-saving cytotoxic therapies often develop chemotherapy-induced peripheral neuropathy (CIPN), a prevalent, progressive, and usually irreversible syndrome characterised by chronic pain and diminished quality of life in individuals. At present, the aetiology of CIPN remains poorly understood; consequently, no effective therapy has yet been identified to prevent or reverse this condition. In this study, we investigate mitochondrial dysfunction and concurrent altered neuronal excitability as a central theme underlying this pathology. For the first time, by using a combination of functional assays, high-resolution microscopy, and microelectrode array electrophysiological techniques, we show unique differences in metabolic and electrophysiological characteristics between putative cholera toxin B staining non-nociceptive (CTB+) and nociceptive (CTB−) mouse sensory neurons in two phenotypically distinct *in vitro* models of oxaliplatin (platinum compound) and bortezomib (proteasome inhibitor) induced neuropathy. Oxaliplatin-treated CTB+ neurons specifically exhibited increased excitability at initial stages (1-3 hours), followed by a significant decline in excitability at 24 hours. Interestingly, both mitochondrial and glycolytic respiration were significantly reduced during periods of decreased excitability, accompanied by reduced glucose uptake and cytosolic acidification. Notably, CTB+ neurons also exhibited a marked

dysregulation of mitochondrial calcium homeostasis, rendering these organelles prone to calcium overload. In contrast, both CTB+ and CTB− neurons were silenced following bortezomib treatment for 24 hours. Interestingly, bioenergetic reprogramming was observed in CTB+ neurons, whereby glycolysis was upregulated to compensate for impaired mitochondrial respiration. However, in CTB− neurons, mitochondrial respiratory inhibition and a significant reduction in glycolytic capacity were observed. Analysis of morphometric data unveiled cytosolic vacuolation and mitochondrial rearrangement exclusively in small-diameter (nociceptive) neurons, signifying a profound stress response. In conclusion, bioenergetic and mitochondrial dysfunction-induced metabolic insufficiency in sensory neuron subtypes are key acute events leading to persistent energy deficits and altered excitability in distinct models of CIPN.

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Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.10/E26

Topic: D.01. Somatosensation – Pain and Itch

Support: HHMI

Title: Visualizing lung cancer-neuron interaction using a novel fast tissue clearance strategy

Authors: *S. DOWNES TONEY[1], A. ZAVITSANOU[1], I. ABDUS-SABOOR[2];
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Abstract: The lungs are one of the most vital organs in the body. Damage to the lungs can heavily impact quality of life and even lead to death. As constant subjects to environmental insult, the lungs require an elaborate sensory network to monitor changes in their internal state. In the context of cancer, the precise role of sensory neurons remains unclear. In addition to transmitting a wide range of vital sensory information, lung afferents likely play an unrevealed role in anti-tumor immunity and tumor development. We induce the development of tumors in lung adenocarcinoma in Kras G12D/+ ; p53 -/- mice to enable our study of cancer-neuron interactions. To study in situ relationships between neuronal innervation and the tumor microenvironment, we utilize novel tissue clearance-based tools to compare the innervation of healthy and tumor-bearing lungs. Specifically, we use “AKS”, a new, hyper-fast tissue clearance method, in tandem with light-sheet microscopy to clear the lungs and visualize tumor development and involved neuronal mechanisms. AKS enables rapid (< 24 hours) clearance of whole lungs, expediting lung visualization and enabling faster developments in research. We show this method’s compatibility with neuronal antibody labeling, viral-based labeling, and

transgenic fluorescent proteins as well as tumor visualization in the lungs. In addition to the lungs, we also clear and image whole-mount dorsal root ganglia (DRG) and vagal ganglia (VG), the peripheral cell clusters in which cell bodies of lung-innervating sensory neurons are found. Through high-resolution visualization of lung-innervating sensory neurons, we will be able to probe neuron-neuron and neuron-cancer interactions, elucidating the nature of lung cancer-induced neuronal changes. This research will not only elucidate cancer-specific mechanisms but also broader, unknown mechanisms underlying visceral sensations and sensory neuron-mediated immunity.

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Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.11/E27

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH R01DE027223

Title: Contribution of Truncated TrkB in Oral Cancer Pain

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Abstract: We previously demonstrated that brain-derived neurotrophic factor (BDNF) released from oral squamous cell carcinoma (OSCC) cells orchestrate oral cancer-induced pain transmission, that is reversed by locally inhibiting its receptor, TrkB, at the site of oral tumor growth. The truncated isoform (TrkBTK-), lacking the tyrosine kinase domain, was shown to be the predominant isoform expressed in oral cancers. Therefore, our current study explores the contribution of peripheral truncated TrkB isoform in mediating oral cancer pain. We conducted single-cell RTPCR to test the expression of both TrkB isoforms in subgroups of tongue-innervating neurons, human tongue cancer specimens from patients, and human OSCC cell lines. Additionally, we assessed whether knocking down of truncated TrkB using siRNA within the tongue tumor in mice reversed tumor-induced pain behaviors.Our data showed that TrkBTK- (35%) was expressed in a wider proportion of lingual sensory neurons compared to full length TrkB (TrkBTK+, 10%). TrkBTK- was found to be expressed in nociceptor and non-nociceptor population and was the predominant expressed isoform in human tongue tumor samples with levels of expression correlated in pain levels of patients. These data were corroborated in our mouse model where tumors induced by truncated TrkB-expressing OSCC cell line showed greater pain behaviors. Additionally, intratumoral knockdown of TrkBTK-, significantly reversed tumor-induced feeding behavior, as well as mechanical sensitivity in the face. Our data

for the first time suggests a significant contribution of the truncated TrkB isoform in oral cancer pain at the peripheral site of tumor growth.

Disclosures: J. Merlo: None. **T. Ibrahim:** None. **S. Ruparel:** None.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

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Program #/Poster #: PSTR401.12/E28

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH grant 1R21NS121946

Title: Parkinson's disease protein DJ-1 controls peripheral neuronal activity and painful neuropathies

Authors: *S. LEE[1], R. TONELLO[2], K. LEE[3], J. ROH[4], A. PRUDENTE[5], C. PARK[6], T. BERTA[5];
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Abstract: Parkinson's disease (PD) is a brain disorder that is widely recognized for causing motor impairments. However, it often manifests with prodromal pain and peripheral sensory neuropathies, which are not well understood and frequently undertreated. The antioxidant deglycase protein DJ-1 (encoded by the Park7 gene) is associated with the onset of both genetic and sporadic PD. *Dj-1* knockout (*Dj-1*$^{-/-}$) mice have been used as a PD animal model presenting nigrostriatal dopaminergic deficits and progressive motor impairments. However, it is unclear whether these mice also exhibit prodromal pain and sensory neuropathy. It is also unknown whether therapeutic strategies targeting DJ-1 could be used to treat PD-linked and other painful peripheral neuropathies. Here, we found prodromal cold hypersensitivity and a decrease in cutaneous nerve fibers in *Dj-1*$^{-/-}$ mice, indicating the presence of peripheral painful sensory neuropathy. We also demonstrated that DJ-1 is linked to TRPA1, a cold-sensing channel, expression and activity in sensory neurons, which controls both cold sensitivity and peripheral neuropathy. Furthermore, DJ-1 plays a major role among multiple painful neuropathies. In particular, its activation can alleviate pain, oxidative stress, and decrease of cutaneous nerve fibers in a mouse model of chemotherapy-induced peripheral neuropathy (CIPN). Moreover, it can mitigate CIPN-induced neuronal hyperexcitability in mouse and human cultured sensory neurons. Together, these findings suggest that targeting DJ-1 offers a new disease-modifying therapeutic approach for PD and other painful neuropathies.

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Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.13/E29

Topic: D.01. Somatosensation – Pain and Itch

Title: Exploring the therapeutic potential of novel Nrf2 activators in neuropathic pain management

Authors: *S.-Y. NA, B. KO, J. KIM, Y. YOON, H. LEE, K. RADHAKRISHANAN, J. LEE, K. PARK, L. PARK;
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Abstract: Neuropathic pain poses a significant clinical challenge due to its complex etiology and resistance to conventional treatments. Dysregulated oxidative stress pathways are implicated in its development and persistence. Nuclear factor erythroid 2-related factor 2 (Nrf2) emerges as a promising therapeutic target, orchestrating cellular antioxidant responses. Here, we explore the potential of novel Nrf2 activators in managing neuropathic pain through primary astrocyte studies and two peripheral neuropathic pain models, the spinal nerve ligation (SNL-Chung) model and the reserpine-induced fibromyalgia model. We report that novel Nrf2 activators enhance cell viability in reactive oxygen species (ROS)-induced astrocyte death. This result demonstrates the well-known cytoprotective potential of Nrf2 activation in alleviating oxidative stress and mitochondrial dysfunction, ultimately resulting in cellular protection. Additionally, these activators upregulate Nrf2 target genes including antioxidant enzymes and detoxifying enzymes, thereby promoting cellular resilience against oxidative stress in primary astrocytes. In vivo studies using the SNL (Chung) and fibromyalgia models, which exhibit neuropathic pain, show that novel Nrf2 activators alleviate mechanical allodynia in both models as measured by the von Frey test. These results indicate that Nrf2 activators have analgesic effects in neuropathic pain. Our study highlights the therapeutic potential of novel Nrf2 activators in neuropathic pain management. Additionally, Nrf2 activators demonstrate neuroprotective effects, which could further contribute to pain relief in neuropathic conditions. Given the diverse causes of neuropathic pain, including postherpetic neuralgia, trigeminal neuralgia, diabetic neuropathy, chemotherapy-induced neuropathy, and spinal cord injury, Nrf2-based therapies are applicable to a variety of neuropathic pain conditions. While further research is needed to fully understand the efficacy and safety of Nrf2-based therapies for neuropathic pain, our findings suggest that they hold considerable promise as a novel approach to pain management.

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Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

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Program #/Poster #: PSTR401.14/E30

Topic: D.01. Somatosensation – Pain and Itch

Support: CIHR Project Grant

Title: Fate-mapping CCR2+ cells to determine monocyte-derived macrophage dynamics and morphology in the DRG in neuropathic pain

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Abstract: Typically rated as “the worst pain imaginable” by patients, neuropathic pain (NeP) results from damage to the somatosensory nervous system. NeP presents in many chronic pain conditions including but not limited to peripheral nerve injury (PNI) neuropathies such as from phantom limb pain, trigeminal neuralgia, and post-chemotherapy pain in cancer patients. Common yet debilitating, peripheral neuropathies are very difficult to treat due to a poor understanding of the complex underlying mechanisms. A fundamental initiator of NeP is the innate immune response to injury: the macrophages of the neuroimmune system initiate the NeP’s well-characterized phenotype of prolonged hypersensitivity. Recent studies have found the impacts of monocyte-derived macrophages (MDMs) at the dorsal root ganglion (DRG) under PNI. However, the MDMs' roles throughout the shift from acute establishment to chronic maintenance of NeP remain unclear. To address a cell-specific targeting approach, our study employs a fate-mapping strategy with transgenic mouse lines to better define the dynamics and morphology of the MDMs in the spared nerve injury (SNI) model of PNI-NeP. Using $CCR2^{CreER}$ mice crossed with Ai9 mice (mice presenting the constitutive promoter line $ROSA26^{tdTomato}$), we study the monocyte-derived macrophages throughout the acute and chronic timepoints in the DRG. At the acute phase of PNI-NeP, we observe MDMs increase in population density at 7 days post injury (DPI). We also find that a subset of these MDMs take on a “spooning” morphology onto primary sensory neurons, specifically towards injured myelinated Aβ afferents. At the chronic phase of PNI-NeP, we observe that MDMs decrease in density by 120DPI, with some taking on an abnormal, blebbing morphology. Ultimately, our findings provide evidence that MDMs dynamically change throughout the timecourse of the SNI model of PNI-NeP. By studying how monocyte-derived macrophages change throughout NeP, we can understand the pathophysiology of NeP chronicity, thus providing context for future targets for immune-based neuropathic pain therapies.

Disclosures: M.S. Ho: None. **N. Rawani:** None. **C. Wangshou:** None. **J.R. Plemel:** None. **B.J. Kerr:** None.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.15/E31

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH/NCATS KL2TR00316
NIH UL1TR003015

Title: Quantitative immunohistochemistry and electrophysiology to measure nicotinic acetylcholine receptors in dorsal root ganglia neuronal subtypes during mouse pain states

Authors: D. H. WEI, S. SIDDIQUI, ***R. L. PARKER**;
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Abstract: New pain treatments that improve efficacy and reduce the risk of opioid addiction are necessary due to the high frequency of people experiencing chronic pain and addictive potential of current treatments. This study will evaluate the role of α6-containing nicotinic acetylcholine receptors (nAChRs) in mice that develop chronic inflammatory or neuropathic injury. This nAChR subunit has previously been reported to be down-regulated during the development of allodynia.
Quantification of GFP-tagged α6-containing nAChRs was performed in mice that were either sham treated, underwent injection by complete Freund's adjuvant, or spared nerve injury. The development of mechanical allodynia was quantified using Von Frey testing and the up-down method to determine the 50% withdrawal threshold. The dorsal root ganglia were collected, fixed, sliced, and immunolabeled with a NeuN antibody to detect neurons, an IB4-isolectin, and either CGRP or NF200 antibodies. The person performing image analysis was blinded to treatment. Quantification was performed on confocal slices using FIJI imaging software. NeuN-positive cells were selected at random to evaluate cell size, average GFP pixel intensity, integrated density, and raw integrated density. GFP was quantified in 357 cells, of which 146 were CGRP positive/IB4 negative and 68 were NF200 positive/IB4 negative positive cells.
Whole cell patch clamp electrophysiology was used to evaluate neurons in both neuronal culture and acute DRG preps using the same mouse line and induction of neuropathic or inflammatory pain as above. GFP positive cells (expressing α6 nAChR subunits) were selectively patched and their membrane properties, as well as response to nicotine were measured. Specifically, resting membrane potential (Rm) was determined, then action potentials (APs) evoked by step current injection in current clamp mode. For APs, rheobase, AP half-width, AP upstroke, and decay time (τ) were measured to classify the DRG cell subtype. Furthermore, the response to nicotine

application measured by determining the peak response to both 10 μM and 100 μM nicotine and then measured again in the presence of conotoxin PIA, which specifically blocks the α6 subunit. Over 20 cells have been recorded to date and data analysis is ongoing.
Therefore, the role of α6-containing nAChRs during the development of mechanical allodynia has been evaluated using cell subtype specificity. This will determine whether specific DRG cell classes preferentially alter nAChR expression during pain states.

Disclosures: D.H. Wei: None. **S. Siddiqui:** None. **R.L. Parker:** None.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

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Program #/Poster #: PSTR401.16/E32

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant R01 NS126252-01

Title: Enhanced Efferocytosis Alleviates Neuropathic Pain And Promotes Clearance Of Apoptotic Cells In The Injured Nerve

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Abstract: Chronic inflammation is an important contributor of the development and maintenance of neuropathic pain. Upon nerve injury, damaged cells secrete pro-inflammatory signaling molecules that recruit circulating immune cells to the site of injury. The persistent inflammation can activate nociceptive hypersensitivity through immune mediators and result in chronic pain. The resolution of inflammation and maintenance of tissue homeostasis depends upon the clearance of apoptotic cells by infiltrating phagocytes through a process known as efferocytosis. Macrophages are the key cells responsible for the efferocytotic clearance of damaged cells through different specialized receptors like tyrosine kinase MER (MerTK). However, MerTK can be cleaved in an inflammatory environment, dysregulating efferocytosis. In this study, we used knockin mice which are resistant to proteolytic cleavage of MerTK ($Mertk^{CR}$ mice) to test whether a cleavage resistant MerTK can enhance the efferocytosis of damaged cells by macrophages and thus help in the alleviation of neuropathic pain. Following chronic constriction injury (CCI) of the sciatic nerve, both male and female $Mertk^{CR}$ mice had higher von Frey thresholds, compared to wild-type littermate controls. We confirmed that MerTK expression on macrophages was downregulated in wild-type mice compared to $Mertk^{CR}$ mice. At the nerve injury site, we observed that apoptotic markers (caspase 3 and cleaved caspase 3), as well as necroptotic markers (pRIPK3), were decreased in $Mertk^{CR}$ mice, compared to wild-type controls. Reciprocally, cleaved, and total caspase 3, and pRIPK3 levels were

increased when Mertk was knocked out in macrophages ($Lysm2^{Cre/-}Mertk^{fl/fl}$ mice). We observed increase in pro-inflammatory macrophage (M1) phenotypes at the site of nerve injury in WT mice and this increase was significantly less in $Mertk^{CR}$ mice. Efferocytosis was assessed as a percentage of $TUNEL^{+}$ live macrophages through flow cytometry. We observed an increase in $TUNEL^{+}$ live macrophages in $Mertk^{CR}$, compared to wild-type mice, suggesting that the presence of cleavage-resistant macrophages enhances the efferocytotic clearance of apoptotic cells at the site of nerve injury in $Mertk^{CR}$ mice. These results indicate that MerTK is downregulated at the peripheral nerve injury site, and its protection from cleavage accelerates the clearance of apoptotic cells to ultimately alleviate neuropathic pain. Acknowledgment: This work is supported by National Institutes of Health Grant R01 NS126252-01.

Disclosures: V.K. Pandey: None. **Y. Zuberi:** None. **C.J. Heijnen:** None. **P.M. Grace:** None.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: D.01. Somatosensation – Pain and Itch

Support: R01NS125413-01

Title: Par2 antagonism with nanoparticles block nociception in osteoarthritis model

Authors: *M. DE AMORIM FERREIRA[1], S. POUDEL[2], D. D. JENSEN[3];
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Abstract: A major symptom of patients with osteoarthritis (OA) is pain that is triggered by peripheral as well as central changes within the sensory pathways. The current treatments for OA pain such as NSAIDS or opiates are neither sufficiently effective nor devoid of detrimental side effects. The family of proteinase-activated receptors (PARs) are G protein-coupled receptors (GPCRs) activated by proteases. PAR_2, one of PAR family receptors, is expressed on a variety of inflammatory cells and sensory neurons where PAR_2 has been linked to chronic joint inflammation and pain. PAR_2 is also expressed on various joint structures like the synovium, cartilage, chondrocytes, and bone and is activated by the protease cathepsin s, which is found in the synovial fluid. Herein, we investigate the role of PAR_2 signaling in a mouse model osteoarthritis and the use of nanoparticles to deliver PAR_2 antagonists to treat arthritic pain and inflammation.Male C57BL/6 mice (20-30 g) were used, and the experiments followed the ARRIVE guidelines. The project was approved by the Institutional Animal Care and Use Committee (IACUC-PROTO202000064). After isoflurane anesthesia, the mice received a knee joint injection with iodoacetic acid (1 mg/kg) to induce osteoarthritis model. To block PAR_2, animals were treated with PAMAM nanoparticles containing AZ3451, a PAR_2 antagonist (5 µM)

or empty nanoparticles intra-articular in the knee joint, 7 days after OA induction. Before and after OA induction and treatments (1 to 28 days), paw mechanical thresholds (PWT), as well as weight bearing deficit and paw thickness were evaluated. We also detected spontaneous nociception and some adverse effects using a behavioral spectrometer.Intra-articular injection induced mechanical sensitivity in the ipsilateral hind paw with allodynia peaking at day 7 (96% ±5%), as well as weight bearing deficits (34% ±10% deficit) and paw thickness (0.45 mm ± 0.03 mm). Nociceptive behaviors started from the first day until 14 days after intra-articular injection, and on day 21 mice reverted to baseline values. Seven days after knee join injection, we treated mice with PAMAM-AZ nanoparticles to investigate the role of PAR_2. The treatment with PAMAM-AZ nanoparticles was attenuated mechanical allodynia starting 2 hours after nanoparticles treatment.Our findings indicate that PAR_2 mediates nociception in osteoarthritis model and can be a potential target for the development of drugs to the treatment of osteoarthritis.

Disclosures: M. de Amorim Ferreira: None. **S. Poudel:** None. **D.D. Jensen:** None.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

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Topic: D.01. Somatosensation – Pain and Itch

Support: Duke University Anesthesiology Research Funds
NIH R01 Grant 1NS13181201A1
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DoD Grant W81XWH2110756

Title: Syntaphilin upregulation in sensory neurons regulates mitochondrial axonal transport and neuropathic pain

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Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) and diabetic neuropathic pain (DNP) are disease conditions characterized by nerve degeneration and neuropathic pain, significantly impacting patient quality of life. Accumulating evidence suggests that CIPN and DPN may result from mitochondrial dysfunction within primary sensory neurons of the dorsal root ganglia (DRG). However, it remains unclear how the axonal transport of mitochondria from the neuronal soma to the peripheral nerve terminal is regulated by chemotherapy and diabetes. In this study, we discovered that both mitochondrial function and trafficking were impaired following chemotherapy. Notable, syntaphilin (SNPH), a static anchor protein that is known to

halt mitochondrial transport in axons, is significantly upregulated in DRG after chemotherapy. Moreover, SNPH is specifically expressed in DRG sensory neurons. Functionally, knocking down of SNPH expression in DRG neurons by siRNA and AAV-virus was effective in preventing neuropathic pain symptoms and epidermal nerve degeneration induced by CIPN and DNP. Our findings indicate that impaired axonal trafficking of mitochondria from sensory neurons plays a critical role in the development of CIPN and DNP. Targeting SNPH to enhance mitochondrial trafficking could offer novel strategies for nerve regeneration and the relief of neuropathic pain following these disease conditions.

Disclosures: J. Xu: None. **W. He:** None. **R. Ji:** None.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.19/E35

Topic: D.01. Somatosensation – Pain and Itch

Support: R01NS111553
RFNS113881
R01NS117484

Title: Rna-binding protein syncrip contributes to neuropathic pain through stabilizing ccr2 expression in primary sensory neurons

Authors: *X. FENG, H. HU, Y. TAO;
Rutgers New Jersey Med. Sch., Newark, NJ

Abstract: Background: Nerve injury-induced changes in gene expression in the dorsal root ganglion (DRG) contribute to the genesis of neuropathic pain. SYNCRIP, an RNA binding protein, is critical for the stabilization of gene expression. Whether SYNCRIP participates in nerve injury-induced alternations in DRG gene expression and nociceptive hypersensitivity is unknown. **Methods:** The expression and distribution of *Syncrip* mRNA and its coding SYNCRIP protein in mouse DRG after chronic constriction injury (CCI) of unilateral sciatic nerve were assessed. Effect of microinjection of *Syncrip* siRNA into the ipsilateral L3/4 DRGs on the CCI-induced expression of chemokine (C-C motif) receptor 2 (CCR2) and nociceptive hypersensitivity were examined. Additionally, effects of microinjection of adeno-associated virus 5 expressing full length *Syncrip* mRNA (AAV5-Syncrip) on basal DRG CCR2 expression and nociceptive thresholds were observed. **Results:** SYNCRIP is expressed exclusively in DRG neurons, where it co-exists with CCR2. Levels of *Syncrip* mRNA and SYNCRIP protein in injured DRG increased time-dependently on days 3-14 after CCI. Blocking this increase through microinjection of *Syncrip* siRNA into the injured DRG attenuated CCI-induced upregulation of DRG CCR2 and the development and maintenance of nociceptive hypersensitivities. Mimicking

this increase through DRG microinjection of AAV5-Syncrip elevated CCR2 expression in microinjected DRGs, enhanced the responses to mechanical, heat and cold stimuli, and induced ongoing pain in naive mice. Mechanistically, SYNCRIP bound to 3-UTR of *Ccr2* mRNA and stabilized its expression in DRG neurons. **Conclusions:** SYNCRIP contributes to the induction and maintenance of neuropathic pain likely through stabilizing expression of CCR2 in injured DRG. SYNCRIP may be a potential target for treating this disorder.

Disclosures: X. Feng: None. **H. Hu:** None. **Y. Tao:** None.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

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Program #/Poster #: PSTR401.20/E36

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant R01NS111553

Title: Nt-3 contributes to chemotherapy-induced neuropathic pain through trkc-mediated ccl2 elevation in drg neurons

Authors: *B. WANG;
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Abstract: AbstractCancer patients undergoing treatment with antineoplastic drugs often experience chemotherapy-induced neuropathic pain (CINP), and the therapeutic options for managing CINP are limited. Here, we show that systemic paclitaxel administration upregulates the expression of neurotrophin-3 (Nt3) mRNA and NT3 protein in the neurons of dorsal root ganglia (DRG), but not in the spinal cord. Blocking NT3 upregulation attenuates paclitaxel-induced mechanical, heat, and cold nociceptive hypersensitivities and spontaneous pain without altering acute pain and locomotor activity in male and female mice. Conversely, mimicking this increase produces enhanced responses to mechanical, heat, and cold stimuli and spontaneous pain in naive male and female mice. Mechanistically, NT3 triggers tropomyosin receptor kinase C (TrkC) activation and participates in the paclitaxel-induced increases of C-C chemokine ligand 2 (Ccl2) mRNA and CCL2 protein in the DRG. Given that CCL2 is an endogenous initiator of CINP and that Nt3 mRNA co-expresses with TrkC and Ccl2 mRNAs in DRG neurons, NT3 likely contributes to CINP through TrkC-mediated activation of the Ccl2 gene in DRG neurons. NT3 may be thus a potential target for CINP treatment.

Disclosures: B. Wang: None.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.21/E37

Topic: D.01. Somatosensation – Pain and Itch

Support: R01NS111553
RFNS113881

Title: Esrrg-controlled downregulation of kcnn1 in primary sensory neurons is required for nerve injury-induced neuropathic pain

Authors: *R. MA;
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Abstract: Peripheral nerve injury-caused neuronal hyperactivity in the dorsal root ganglion (DRG) participates in neuropathic pain. The calcium-activated potassium channel subfamily N member 1 (KCNN1) mediates action potential afterhyperpolarization (AHP) and gates neuronal excitability. However, whether DRG KCNN1 contributes to neuropathic pain remains elusive. we report here that chronic constriction injury (CCI) of the unilateral sciatic nerve or unilateral ligation of the fourth lumbar nerve resulted in the downregulation of Kcnn1 mRNA and KCNN1 protein in the injured DRG. This downregulation was partially attributed to a reduction in DRG estrogen-related receptor gamma (ESRRG; a transcription factor) because this reduction resulted in less binding to the Kcnn1 promoter. Rescuing this downregulation blocked the CCI-induced decreases in total Kv currents and AHP currents and increase in excitability in the injured DRG neurons and alleviated the CCI-induced development and maintenance of nociceptive hypersensitivities, without altering locomotor function and acute pain. Mimicking the CCI-induced DRG KCNN1 downregulation resulted in augmented responses to mechanical, heat, and cold stimuli in naïve mice. Our findings indicate that the ESRRG-controlled downregulation of DRG KCNN1 is likely required for the development and maintenance of neuropathic pain. KCNN1 may emerge as a potential target for the management of this disorder.

Disclosures: R. Ma: None.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant R01NS111553
NIH Grant R01NS117484
NIH Grant RFNS113881

Title: Transcription factor EBF1 mitigates neuropathic pain by rescuing Kv1.2 expression in primary sensory neurons

Authors: *Y. TAO[1], X. FENG[1], H. HU[2];
[1]Anesthesiol., Rutgers New Jersey Med. Sch., Newark, NJ; [2]Anesthesiol., NJMS, Newark, NJ

Abstract: Nerve injury-induced alternations of gene expression in primary sensory neurons of dorsal root ganglion (DRG) are molecular basis of neuropathic pain genesis. Transcription factors regulate gene expression. In this study, we examined whether early B cell factor 1 (EBF1), a transcription factor, in the DRG participated in neuropathic pain caused by chronic constriction injury (CCI) of the sciatic nerve. EBF1 was distributed exclusively in neuronal nucleus and co-expressed with Kv1.2 in the DRG neurons. The expression of *Ebf1* mRNA and protein was time-dependently downregulated in the ipsilateral lumbar (L) 3/4 DRGs after unilateral CCI. Rescuing this downregulation through microinjection of the adeno-associated virus 5 expressing full-length *Ebf1* mRNA into the ipsilateral L3/4 DRGs reversed the CCI-induced decrease of DRG Kv1.2 expression and alleviated the development and maintenance of mechanical, heat and cold hypersensitivities. Conversely, mimicking the downregulation of DRG EBF1 through microinjection of AAV5-expressing *Ebf1* shRNA into unilateral L3/4 DRGs produced a reduction of Kv1.2 expression in the ipsilateral L3/4 DRGs, spontaneous pain and the enhanced responses to mechanical, heat and cold stimuli in naive mice. Mechanistically, EBF1 not only bound to *Kcna2* gene (encoding Kv1.2) promoter but also directly activated its activity. CCI decreased the EBF1 binding to *Kcna2* promoter in the ipsilateral L3/4 DRGs. Our findings suggest that DRG EBF1 downregulation contributes to neuropathic pain likely by losing its binding to *Kcna2* promoter and subsequently silencing Kv1.2 expression in primary sensory neurons.

Disclosures: Y. Tao: None. **X. Feng:** None. **H. Hu:** None.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

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Topic: D.01. Somatosensation – Pain and Itch

Support: SDBRC-P&F P30AR079206
R01AR077691
P30AR079206

Title: Activation of G-qlinked G-Protein Coupled Receptors on Epidermal Keratinocytes Enhances Dorsal Root Ganglion Neuron Activity and Excitability

Authors: *A. BELMADANI[1], D. REN[1], N. D. JAYARAJ[2], C. G. VANOYE[1], A.-M. MALFAIT[3], A. L. GEORGE, JR[1], R. J. MILLER[1], D. M. MENICHELLA[2];
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Abstract: Abstract Epidermal Keratinocytes are closely juxtaposed to cutaneous nerve terminals, enabling bidirectional communication. To investigate potential mechanisms that mediate this communication, we genetically expressed stimulatory DREADDs (hM3Dq) into K14 basal keratinocytes (K14) as a tool for mimicking the activation of Gq-linked G protein-coupled receptors (GPCRs) in K14 expressing cells in mice. We observed that CNO activation of hM3Dq expressed in K14 cells induced an increase in depolarization induced $[Ca^{2+}]i$ responses in DRG neurons taken from these animals. We hypothesized that the increased $[Ca^{2+}]i$ responses reflected changes in voltage-gated ion channel function, including sodium channels, which are responsible for the initiation and the propagation of action potentials (APs) in excitable cells. We therefore performed an electrophysiological study to determine the mechanisms underlying any changes in cell excitability.
Whole-cell patch-clamp experiments were performed in DRG neurons acutely isolated from both CNO and vehicle-treated mice. All recordings, voltage-clamping and data acquisition were made using SynchroPatch 384 and the PatchLiner software (Nanion, Germany).
Analysis of the biophysical properties of Na_v channels showed that there is an increase in the current amplitude in CNO compared to vehicle treated mice. We next measured the normalized conductance amplitude and found that there is a significant shift in the depolarized direction of the midpoint voltage. We also observed larger peak currents as evidenced by measuring the time to peak in CNO group compared to vehicle. Furthermore, we found an increased percentage of DRG neurons in the CNO groups that displayed Na_v currents compared to vehicle treated group. Preliminary analysis of APs indicated that there was an increase in evoked AP generation in CNO compared to vehicle treated mice.These data indicate that activation of basal keratinocytes Gq linked GPCRs resulted in alterations in the biophysical properties of sodium channels in accompanying DRG neurons: in particular, we noted an increase in sodium current amplitude. Changes in Na_v function have been shown to be associated with excitability disorders including neuropathic pain. Our results indicate that keratinocytes can regulate the excitability of DRG neurons and suggest possible therapeutic effects of activating or blocking specific keratinocyte Gq linked GPCRs as a mechanism for modulating neuronal excitability in chronic disorders of pain and itch.

Disclosures: A. Belmadani: None. **D. Ren:** None. **N.D. Jayaraj:** None. **C.G. Vanoye:** None. **A. Malfait:** None. **A.L. George, Jr:** None. **R.J. Miller:** None. **D.M. Menichella:** None.

Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.24/E40

Topic: D.01. Somatosensation – Pain and Itch

Support: R01 NS070711 (CLS)
R37 NS108278 (CLS)

Title: Keratinocyte sensitization and signaling contributes substantially to traumatic nerve injury-induced mechanical hypersensitivity.

Authors: *C. M. MECCA[1], O. ISAEVA[2], A. MIKESELL[3], C. L. STUCKY[4];
[1]Cell. Biol. Neurobio. and Anat., Med. Col. of Wisconsin, Wauwatosa, WI; [2]Cell. Biol. Neurobio. and Anat., Med. Col. of Wisconsin, Milwaukee, WI; [3]Med. Col. of Wisconsin, Wauwatosa, WI, ; [4]Cell Biology, Neurobio. & Anat., Med. Col. of Wisconsin Neurosci. Doctoral Program, Milwaukee, WI

Abstract: Neuropathic pain, one of the most common and challenging types of chronic pain to manage effectively, is associated with severe cutaneous mechanical hypersensitivity. Much work has been dedicated to understanding the involvement of peripheral nerves in driving neuropathic pain, however, the role of non-neuronal cells that interact with injured nerves is under explored. Keratinocytes, which comprise the majority of the epidermis, communicate directly with sensory afferent fibers to convey mechanical information. The present study utilized the tibial spared nerve injury (tSNI) to determine whether keratinocytes contribute to touch evoked pain after traumatic nerve injury. First, brief optogenetic inhibition of keratinocytes completely alleviated the mechanical allodynia. Next, we asked whether keratinocytes become sensitized after tSNI. In whole cell patch clamp recordings of isolated keratinocytes, we found that tSNI keratinocytes were sensitized to mechanical stimulation - requiring a lower threshold of indentation to elicit inward current compared to sham controls. Second, in co-cultures of tSNI or sham keratinocytes with naïve DRG neurons, we found that tSNI keratinocytes increased the percentage of naïve neurons that exhibited spontaneous activity and decreased the neuronal resting membrane potential. Third, we injected media from cultured tSNI or sham keratinocytes into the hind paw of naïve mice. tSNI keratinocyte media, but not sham media, induced behavioral mechanical hypersensitivity in naïve mice. Together, our results suggest that after traumatic nerve injury, keratinocytes from the spared nerve territory exhibit sensitization and enhanced signaling to sensory neurons, and thereby contribute to the mechanical hypersensitivity after neuropathic injury. The targeting of peripheral keratinocytes in neuropathic pain may reveal novel peripheral analgesic targets that are void of central nervous system side effects.

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Poster

PSTR401

Peripheral Mechanisms of Neuropathic Pain and Cancer Pain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.25/F1

Topic: D.01. Somatosensation – Pain and Itch

Support: NRF Grant NRF2023R1A2C1008079
BK21 FOUR Grant 5199990614277

Title: Optogenetic inhibition of DRG glutamatergic neurons with flexible optic fiber attenuates DRG compression-induced chronic neuropathic pain in rat

Authors: *J. ISLAM[1], E. KC[2], M. RAHMAN[3], Y. PARK[4];
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Abstract: Glutamatergic neurons of the dorsal root ganglion (DRGg) exert a significant effect on peripheral nociceptive signal transmission. However, assessing the explicit modulatory effect of DRGg during chronic neuropathic pain (CNP) with neuromodulation techniques remains largely unexplored. Therefore, we inhibited DRGg by optogenetic stimulation and examined whether it could alleviate CNP and associated anxiety-like behaviors in a chronic compressed DRG (CCD) rat model. The CCD pain model was established by inserting an L-shaped rod into the lumbar 5 (L5) intervertebral foramen, and either AAV2-CaMKIIá-eNpHR3.0-EYFP or AAV2-CaMKIIá-EYFP was injected into the L5 DRG. Flexible optic fibers were implanted to direct yellow light into the L5 DRG. Pain and anxiety behavioral responses were assessed using mechanical threshold, mechanical latency, thermal latency, and open field tests. In vivo single-unit extracellular recording from the DRG and ventral posterolateral (VPL) thalamus was performed. CNP and anxiety behavioral responses along with increased neural firing activity of the DRG and VPL thalamus were observed in CCD animals. Enhanced expression of nociception-influencing molecules was found in the DRG and spinal dorsal horn (SDH). In contrast, during optogenetic stimulation, specific DRGg inhibition significantly alleviated the CNP responses and reduced the DRG and VPL thalamic neural hyperactivity in CCD animals. Inhibition of DRGg also reduced the active expression of nociceptive signal mediators in the DRG and SDH. Taken together, our findings suggest that CaMKIIá-NpHR-mediated optogenetic inhibition of DRGg can produce antinociceptive effects in CCD rats during peripheral nerve injury-induced CNP condition by altering peripheral nociceptive signal input in the spinothalamic tract.

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Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.01/F2

Topic: D.02. Somatosensation – Touch

Support: NINDS (NIH) R01 Grant 1RF1NS121911
NIH Grant R25NS080687

Title: Characterization of neuronal morphologies in Drosophila melanogaster

Authors: *O. DE PABLO CRESPO[1,2], S. HAMPEL[2], A. SEEDS[2];
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Abstract: Complex motor behaviors are produced through the sequential selection of different movements, but how the brain is organized at the level of neural circuits to drive behavioral sequences remains unclear. We study grooming in fruit flies, a complex motor behavior in which the legs are used to clean different body parts such as the antennae, eyes, and wings. Given that grooming consists of a stereotyped sequence, it serves as a behavioral model for understanding how neural circuits drive sequential movement selection and determining the neuronal mechanisms by which specific movements are initiated and controlled. To understand the neural mechanisms that drive grooming sequences, we seek to define how the individual grooming movements are elicited by neural circuits. Preliminary data indicates that individual grooming movements are elicited by their own dedicated circuits that consist of mechanosensory, inter-, and descending neurons. The interneurons include a lineage of morphologically distinct neurons that have been named brain neurons 2 (BN2), a subset of which we previously showed elicit grooming of the antennae. This led us to propose that each BN2 neuron in the hemilineage elicits a site-directed grooming movement in response to input from mechanosensory neurons from a specific body part to which they are connected. To test this hypothesis, we are defining the connections between the mechanosensory neurons from specific body parts and the BN2 neurons by analyzing an electron microscopy volume of the entire fruit fly brain. This is being done using the FlyWire.ai platform that enables the study of the connectivity of specific neural circuits. By defining the neural mechanisms that drive sequential movement selection, we will understand the basic principles of neural circuits and their organization to produce complex behaviors.

Disclosures: O. De Pablo Crespo: None. **S. Hampel:** None. **A. Seeds:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.02/F3

Topic: D.02. Somatosensation – Touch

Support: Swedish Research Council, grant to SM (2020-01085)

Title: Measures of mechanoreceptor's end organs deformation at threshold potential in vivo in Human

Authors: *B. DUVERNOY[1], E. JAROCKA[1], E. KINDSTRÖM[1], A. FRIDBERGER[2], S. MCINTYRE[1];
[1]Ctr. for Social and Affective Neurosci., Linköping Univ., Linköping, Sweden; [2]Linköping Univ., Linköping, Sweden

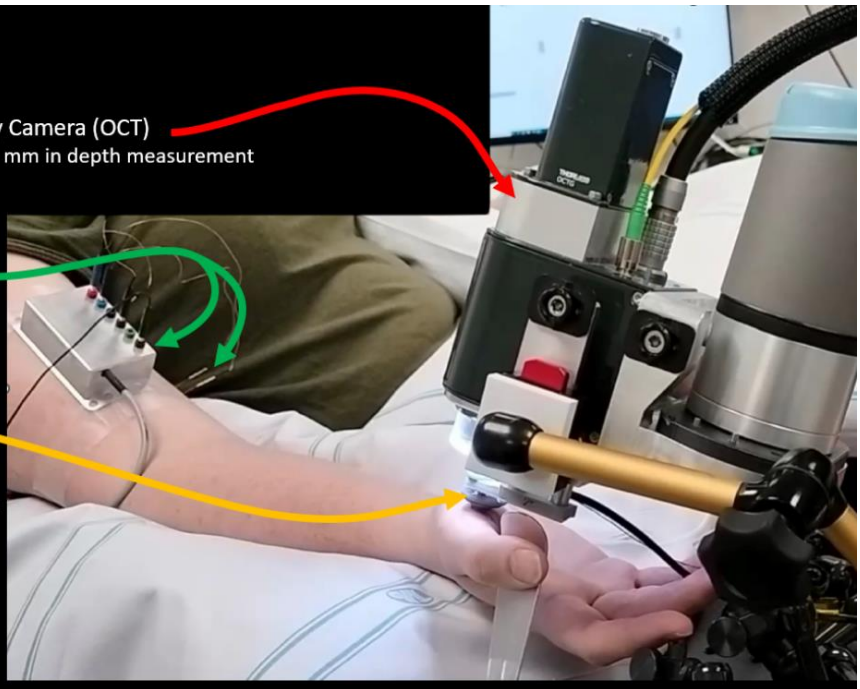
Abstract: Tactile mechanoreceptors consist of neurons whose afferents innervate end-organs embedded in the skin. The characteristics of human mechanoreceptors are usually inferred from the properties of stimuli acting on the skin surface in human studies. Alternatively, insights are gained from animal studies where end organs can be isolated from the skin for direct stimulation and observation. However, these approaches require procedures modifying both the end organ and the skin structure. In addition, they fall short in capturing how the stimulus is altered as it traverses the various layers of the skin, essential information to understand the role of the skin, the end-organs structures and their locations in the skin. In this work, we introduce an imaging technique that enables the tracking of skin deformations in-depth in vivo from hundred of micrometres to hundred of nanometres. By combining this method with microneurography, we aim to demonstrate the neuronal responses of tactile mechanoreceptors in relation to skin deformations at the locations of end organs in vivo in humans (see fig. 1). Hence, this novel approach enables the measurement of intact mechanoreceptors in human. Results give with higher precision the behaviour of end-organs in human, and highlight the relationship between skin mechanics, the end-organs structure and their locations in the skin (see fig. 2).

Fig. 1: Setup

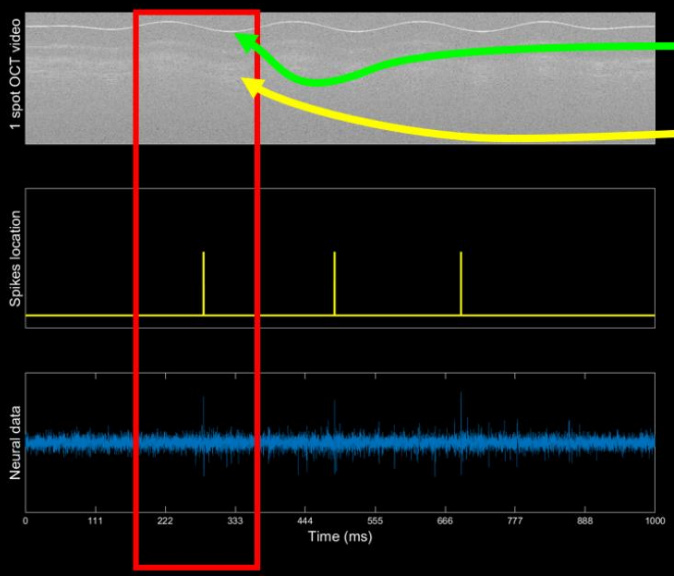
Optical Coherence Tomography Camera (OCT)
- 3.9x13 µm voxel size, up to 2.5 mm in depth measurement
- 10 kHz refresh rate

Microneurography Technique
- One neuron recording
- 10 kHz refresh rate

Vibrotactile actuator
- normal indentation
- 5–500 Hz range
- nm to mm displacement range



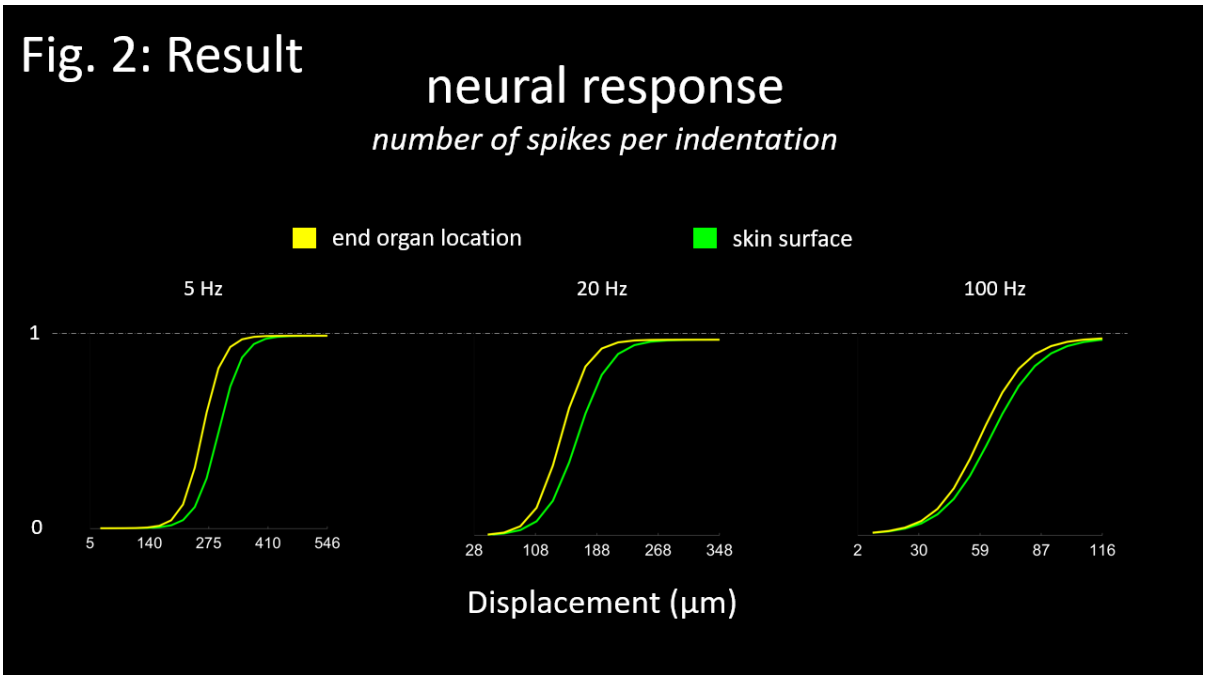
Acquired data



Surface of the skin

Location of the end organ

Stimulus parameters:
- Frequency = 5 Hz
- Amplitude at the surface = 350 µm



Disclosures: B. duvernoy: None. **E. Jarocka:** None. **E. Kindström:** None. **A. Fridberger:** None. **S. McIntyre:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: D.02. Somatosensation – Touch

Support: Swedish Research Council (K2007-63X-03548; K2010-62X-03548; 2021-02552)
European Commission (EU FP6 NANOBIOTACT 33287)
EU Horizon 2020 Marie Sklodowska-Curie Action Programme NEUTOUCH No 813713

Title: Real-time encoding of stimulus features in single tactile afferents in the human hand during passive touch

Authors: *V. A. LANG[1], H. B. WASLING[1], R. ACKERLEY[2,1], J. WESSBERG[1];
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Abstract: The human hand is densely innervated by fast-conducting low threshold mechanoreceptors that allow us to dexterously manipulate objects and to perceive a considerable number of different textures. We aimed to record the axonal firing activity of single mechanoreceptor afferent units in the fingers during the application of regular grated surfaces across their receptive fields in order to examine neural coding capabilities. The study was approved by a local ethics committee and the experiments were conducted in accordance with the Declaration of Helsinki, including obtaining written, informed consent. We used peripheral nerve microneurography of the median nerve to record from single afferents, while a robotic platform applied each grated surface in a sliding motion over the defined receptive field. Different normal forces (100 - 800 mN) and velocities (5 - 40 mm/s) were tested. The collection of grated surfaces varied by their surface spatial period (280 - 1920 μm). The exhaustive set of experimental parameters yielded 272 unique stimulus combinations, although it was not possible to test all per unit. Nerve recordings were analyzed from 18 afferent units: 9 FA-I (fast-adapting type I), 6 SA-I (slowly-adapting type I), 2 SA-II (slowly-adapting type II), and 1 FA-II (fast-adapting type II). We examined median instantaneous firing rates as a function of the stimulus parameters — normal force, sliding velocity, and surface spatial period — and found that unit activity is influenced by each parameter to varying extents. Multilevel regression models showed that FA-I afferent firing was driven by all three factors, while SA-I afferent firing was primarily guided by force and velocity. These results suggest that FA-Is fire linearly within the range of forces, velocities, and surface spatial periods tested, corroborating the current opinion on FA-I afferent function as encoders of stimulus intensity. Furthermore, SA-I afferents are understood to be force encoders and our results support this. Insufficient recordings were available to conduct similar analyses for SA-II and FA-II afferents. While the four classes of low-threshold mechanoreceptive afferents have distinct qualities that govern their neural responses, functional overlap is probable. Where one unit type may fail to reliably encode a stimulus feature, another unit type may be more dependable. Our analyses provide a base from which to build a framework for the description of maximal encoding for each mechanoreceptor afferent unit.

Disclosures: V.A. Lang: None. **H.B. Wasling:** None. **R. Ackerley:** None. **J. Wessberg:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.05/F5

Topic: D.02. Somatosensation – Touch

Support: Human Frontiers Science Program(HFSP)

Title: Exploring the Adaptability and Neural Correlates of Perceptual Biases in Working Memory

Authors: *Y. CHOPRA, M. DIAMOND;
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Abstract: Perceptual memory is a thriving area of research in cognitive neuroscience. However, existing studies usually focus on behavioral findings from a single paradigm, raising whether paradigm-specific mechanisms govern perceptual memories. To address this, our research group aims to develop a flexible framework capable of adapting to various behavioral tasks. To challenge this framework, we have created a new task called the One Back Memory task, which combines delayed comparison & binary classification tasks. Participants are presented with vibrotactile stimuli and are asked to judge whether the current stimulus is stronger or weaker than the stimulus of the previous trial. This task requires each percept to be used in dual functions: first it is judged in real-time and then it must be stored in memory and recalled as the reference for the subsequent trial.The stimuli are delivered in a Markov sequence, comprising nine intensities with unpredictable shifts between high and low-intensity clouds. This design allows us to test our hypothesis of two buffers: a short-term buffer (STB) representing the memory of the preceding stimulus and a long-term buffer (LTB) formed from a longer sequence of past inputs. Our findings indicate that perception is influenced by the context formed by recent stimuli, causing a bias in perception. However, this bias doesn't fundamentally alter the task's objective. Furthermore, our observations suggest a contraction bias toward the local means of the stimulus clouds.To understand the dynamics of the two buffers, we utilized a model that assumes that the information stored in the STB is attracted toward the LTB with the time constant **τ**;;STB while, simultaneously, the information stored in the LTB is attracted toward the STB with the time constant **τ**;;LTB. We discovered that, when the model is optimized to predict the choices of human subjects, **τ**;;STB has a mean of 12 seconds, while **τ**;;LTB has a mean of 33 seconds.Our goal is to further explore the neural locus of the decision boundary and the interaction between the two buffers (LTB & STB). The Subjects performed the same perceptual task while the EEG cap recorded the neural data. Preliminary results indicate that the perception of the stimulus as strong or weak can be determined by the response activity of subjects. The stimulus onset activity or stimulus encoding can easily distinguish the strength of the stimulus. We take this analysis to the Time frequency domain and study the network dynamics of the activity(Somatosensory Cortex, PFC & PPC) as the subject interacts with the stimulus during the onset, encoding, and response.

Disclosures: Y. Chopra: None. **M. Diamond:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.06/F6

Topic: D.02. Somatosensation – Touch

Title: Disentanglement of tactile latent variables in a hybrid supervised/self-supervised model of rodent whisker touch

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Abstract: Tactile perception routinely involves exploration with numerous active sensors, such as digits in humans and vibrissae in rodents. How the brain jointly represents information from across sensors is therefore critical to explaining tactile behaviors such as the discrimination of shape, texture, and other environmental features. Remarkably, recent studies have shown that mouse primary somatosensory cortex (S1) encodes contacts in roughly orthogonal or disentangled subspaces during a whisker-based shape discrimination task, balancing generalization with flexible behavior (Rodgers et al. 2021; Nogueira et al. 2023). In the present study, we explore how such a code might emerge by training artificial neural networks to perform various tasks on simulated whisker contact data.
Inspired by recent findings that disentangled representations arise naturally in feedforward artificial neural networks trained to perform a battery of tasks requiring simple integration of inputs (Johnston and Fusi 2023), we sought to bias compressed representations in a sparse autoencoder model of the rodent whisker system towards different geometries by encouraging it to further perform various tactile discriminations. In addition to a core architecture consisting of one hidden layer (ReLU units with L1-norm penalization for activity) trained to minimize reconstruction loss through gradient descent (ADAM, lr = 0.001, 500 epochs), we included auxiliary output neurons trained to read out tactile features such as object size, shape, distance, texture, speed, etc,. A hyperparameter was used to control the relative contributions of the different tasks to the weight updates.
We found disentangled representations emerged naturally when the autoencoder was only required to reconstruct the input and the hidden layer was small compared to the input. By contrast, when the hidden layer was large or biased towards tasks that required non-linear integration of whisker contacts, highly entangled representations were observed. Interestingly, when we augmented the training data with behaviorally irrelevant variables, the geometry of the representations also became more disentangled and better suited for generalization. Altogether these results indicate that the most convenient geometry of representations in areas like S1 might be shaped by the computational requirements of the task at hand, and that auxiliary loss terms like self-reconstruction can be used to balance the representational non-linearities that arise in complex tasks to maintain high levels of generalization.

Disclosures: D. Kato: None. **R. Nogueira:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

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Program #/Poster #: PSTR402.07/F7

Topic: D.02. Somatosensation – Touch

Support: ETRI Korea 24ZB1330
NRF Korea RS-2023-00302489

Title: Decoding of peripheral and cranial nerve spike signals in response to pressure stimuli on tactile receptors in rodents

Authors: *K.-H. PARK[1], Y. KANG[1], Y. CHOI[2], H. CHO[2], S. JUNG[2], S. LEE[3];
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Abstract: In order to implement virtual tactile technology that provides the same feeling as touching an object in virtual reality (VR), augmented reality (AR), or mixed reality (MR), wearable device technologies equipped with a mechanism unit that provides pressure or vibration to gloves are being developed. However, because these devices are heavy and uncomfortable to wear, it is required to develop a technology that allows users to feel pressure, vibration, and texture using electrical or micro displacement stimuli. Experimental research is needed to precisely decode neural spike firing signals in peripheral and cranial nerves while applying pressure stimulation to tactile receptors and to verify that similar neural spike signals are fired when similar patterns of electrical pulse stimulation are applied. To decode the response of mechanoreceptors to stimuli, electrophysiological experiments were conducted on the saphenous nerves of mice and rats under physical pressure conditions. C57bl/6 mice aged 8-10 weeks and SD rats aged 7 weeks were used in the experiments. A neural spike response was obtained by applying a force of 10 to 100 mN using a stimulus rod. To fit the exponential decay firing rate graph measured under the pressure stimulation, we performed the least square regression analysis using the exponentially attenuated curve. Based on this decoding model, we developed an electrical stimulation encoding model that corresponds to the intensity of pressure stimuli. Using this encoding model, we applied electrical spike trains of approximately 400uA~2mA to the SA mechanoreceptor nerve and confirmed a precise one-to-one matching spike firing results. To verify the idea of creating a virtual sense of pressure by applying an electrical pulse stimulation similar to the spike firing pattern that is generated during pressure stimulation, The spike firing signal was measured by inserting a needle-shaped electrode into the rat's VPL (ventral posterolateral) area in thalamic nuclei of brain using the stereotaxic method and applying pressure stimulation to the feet of rats. The brain nerve spike signals in the VPL were measured and analyzed in terms of firing rate while applying various pressure stimuli to the feet of anesthetized rats using von Frey and sticks that can apply constant pressure. In addition, various patterns of electric pulse stimulation were applied to the same foot area where the pressure stimulation was applied, and the neural spike firing patterns in the VPL were measured and analyzed compared with the result of applying pressure stimulation.

Disclosures: K. Park: None. **Y. Kang:** None. **Y. Choi:** None. **H. Cho:** None. **S. Jung:** None. **S. Lee:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.08/F8

Topic: D.02. Somatosensation – Touch

Title: Contribution of Piezo2 channels for the individual types of the sensory neurons in the mouse hairy skin

Authors: *Y. BABA[1], K. NGUYEN[2], E. A. LUMPKIN[3];
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Abstract: PIEZO2 channels are essential for detecting mechanical signal in various tissues, including the skin. In *Piezo2* conditional knock-out mice (CKO; $CDX2^{Cre}$; $Piezo2^{Fl/Fl}$), the absence of neural responses to low-threshold mechanical stimuli underscores the critical role of PIEZO2 channels in mediating this type of sensory perception. However, neural responses to high-threshold mechanical stimuli remain intact, suggesting the presence of additional, yet unidentified, mechanoreceptor channels.
Although sensory neurons in hairy skin are typically four types, these classifications are considered provisional. The contribution of PIEZO2 channels within these individual types remains unclear. In this study, we classified the myelinated mechanosensory neurons in hairy skin. Our analysis focused on delineating the contributions of PIEZO2 and other unidentified mechanosensory channels to the various classes of neuronal activity.
Using a variety of physiological characteristics, we identified 9 distinct types of sensory neurons. These characteristics include adaptation patterns, dynamic phase responses, end-organ specificity such as guard hair (GH), non-GH (nGH) hair and touch-dome (TD), optimal stimulation parameters, von Frey threshold and conduction velocity. Low-threshold sensory neurons were RA-GH, RA-nGH, SA-TD, SA-GH and SA-nGH. The high threshold (HT) sensory neurons included RA-skin, dynamic-SA-skin, and non-dynamic SA (ndy)-skin. About 95% of units belonged to one of these categories. The optimal stimulation for all HT neurons was skin indentation. The majority of nGH and some of the RA-skin classified neurons were Aδ-fibers while the others were Aβ-fibers.
Among these classifications, RA-skin, dySA-skin, and ndySA-skin type neurons were observed in *Piezo2* CKO mice, though the former two were rare (4 and 5 out of 119 units). Consequently, we focused our analysis on the ndySA-skin type neurons in both CKO and control (CONT) mice. No unique responses exclusive to the CKO group were observed. Additionally, statistical analyses revealed no significant differences in the minimum and average interspike intervals, coefficient of variation, indentation threshold, force response curves between CKO and CONT groups. These findings suggest that, at least in ndySA-skin neurons, PIEZO2 channels are either absent or have minimally contribution to mechanosensory reception. Additionally, while PIEZO2 channels are essential for the early detection of mechanical stimuli in low-threshold

neurons, they have no significant impact on high-threshold responses in knock-out models highlighting the role of unidentified channels in this process.

Disclosures: Y. Baba: None. **K. Nguyen:** None. **E.A. Lumpkin:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.09/F9

Topic: D.02. Somatosensation – Touch

Title: Vibrotactile Tuning of Excitatory-Inhibitory Circuits in Forepaw Primary Somatosensory Cortex

Authors: *M. DUHAIN[1,2], Y. LUO[3], K. H. WANG[4], M. GOMEZ-RAMIREZ[3];
[1]Univ. of Rochester Neurosci. Grad. Program, Rochester, NY; [2]Brain and Cognitive Sciences, University of Rochester, Rochester, NY; [3]Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY; [4]Dept. of Neurosci., Univ. of Rochester, Rochester, NY

Abstract: The glabrous skin in the hands and forepaws facilitates manipulation of objects in mammals by providing sensory feedback to the brain critical for maintaining a stable grasp of the object. Perception of vibrotactile frequency is fundamental during dynamic exploration of objects, and in static conditions when sensing movement in an environment. In mice, vibratory features that are presented to the forepaws are encoded by rapidly-adapting mechanoreceptors, and transmitted to primary somatosensory cortex (S1) where cortical representations of vibration selectivity emerge. Within cortical frequency-selective ensembles, GABAergic neurons have broad frequency tuning curves (relative to excitatory neurons), and exhibit robust correlations with other frequency-tuned neurons. However, it is unknown whether frequency selectivity is biased towards a specific inhibitory subtype, and how do different inhibitory subtype classes contribute to population-level representations of vibrotactile features. To address these questions, we performed two-photon imaging in forepaw S1 (fS1) of awake-behaving transgenic mice (PV-Cre-tdTom & SST-Cre-tdTom) that were presented with vibratory stimuli to their forepaw. The calcium sensors (jGCaMP7c or 8m) were expressed pan-neuronally to allow simultaneous imaging of putative excitatory and an inhibitory subtype through the GCaMP and tdTom overlapping fluorescence. Our imaging approach allowed us to investigate how parvalbumin-(PV) or somatostatin-expressing (SST) neurons contribute to the population coding of tactile vibration. We found that most PV and SST neurons in cortical layers 1-3 exhibit significant increases in GCaMP fluorescence in response to the vibratory stimuli, with many cells also showing selectivity for a vibration in a specific frequency range. Similar to orientation representations in mouse visual cortex, we found vibration selectivity to be broadly distributed in fS1 without apparent spatial clustering. Over time, through a period of 1-4 months we found the

tuning properties of some individually-tracked excitatory and inhibitory neurons fluctuated while others remained constant. As a causal manipulation we then deployed a classical-conditioning paradigm with reward-frequency pairing and found the distribution of frequency-representation in fS1 can be shifted to over-represent reward-coupled frequency ranges. Overall, this work provides evidence for integrated excitatory-inhibitory signaling, and reward-dependent modulation of vibratory coding patterns in fS1.

Disclosures: M. Duhain: None. **Y. luo:** None. **K.H. Wang:** None. **M. Gomez-Ramirez:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

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Support: National Key R&D Program of China (2021YFF0702200)
STI 2030-Major Projects (2021ZD0200401)
Key R&D Program of Zhejiang Province (2021C03001)
National Natural Science Foundation of China (U20A20221, 81961128029).

Title: Using 7T High-resolution fMRI to Reveal Frequency-Specific Activation of Human Sensorimotor Cortex in Vibrotactile Finger Stimulation

Authors: *M. YE[1,2], J. WANG[2,3], B. QU[1,2], Z. TANG[2,3], L. LAN[2,4], A. W. ROE[1,2,3,5], H.-Y. LAI[1,2,3,5];
[1]Col. of Biomed. Engin. & Instrument Science, Zhejiang Univ., Hangzhou, China; [2]Dept. of Neurol. of the Second Affiliated Hospital, Interdisciplinary Inst. of Neurosci. and Technology, Zhejiang Key Lab. of Rare Dis. for Precision Med. and Clin. Translation, Zhejiang Univ. Sch. of Med., Hangzhou, China; [3]Liangzhu Laboratory, MOE Frontier Sci. Ctr. for Brain Sci. and Brain-Machine Integration, State Key Lab. of Brain-machine Intelligence, Sch. of Brain Sci. and Brain Medicine, Zhejiang Univ., Hangzhou, China; [4]Dept. of Psychology and Behavior Science, Zhejiang Univ., Hangzhou, China; [5]Affiliated Mental Hlth. Ctr. & Hangzhou Seventh People's Hospital, Zhejiang Univ. Sch. of Medicine, Zhejiang Univ., Hangzhou, China

Abstract: Temporal frequency is crucial in encoding tactile information. Previous studies have shown that low- and high-frequency vibrotactile stimulation elicits distinct activation patterns in the primary (SI) and secondary (SII) somatosensory cortices. However, detailed representations of these frequencies in sensorimotor cortex subregions remain unclear due to limitations in functional magnetic resonance imaging (fMRI) resolution and tactile stimulator capabilities. In this study, we used 7T high-resolution fMRI (1 mm isotropy) and surface-based analysis to explore the functional organization of the sensorimotor cortex in ten individuals. We targeted

area 3b, area 1, area 2, SII, and the primary motor cortex (MI) contralateral to stimuli applied at frequencies of 1, 5, 50 and 100 Hz to the left index finger using an MRI-compatible multi-digit piezoelectric stimulator. Our findings revealed frequency-dependent effects in each region of interest (ROI), with different frequencies eliciting varied activation patterns. Notably, 50 Hz stimulation induced broader activations, suggesting simultaneous activation of RA and PC afferents, while other frequencies induced more localized responses. Similar effects in MI, reflecting those in area 3b, 1 and 2, indicate that MI neurons either directly receive sensory inputs or are influenced by SI, suggesting information flow between these areas. Furthermore, finger tactile-evoked fMRI responses across ROIs varied: Area 3b showed more concentrated activations, area 1 showed elongated patterns splitting in the lateral-medial direction, and area 2 had more dispersed responses, suggesting hierarchy architectures across SI subregions. Recent studies increasingly suggest that the organizational principle of SI extend beyond somatotopy. Our findings imply that the human somatosensory cortex may contain functional columns similar to those in visual cortex, with temporal frequency playing a role in their organization. This study provides valuable insights into cortical responses to vibrotactile stimuli at different frequencies, underscoring potential applications in brain-machine interface for rehabilitation therapy and prosthetics.

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Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.11/F11

Topic: D.02. Somatosensation – Touch

Support: NIH Grant 1R35NS122333
NIH Grant 5UH3NS107714

Title: Tactile response properties of human and macaque somatosensory cortex

Authors: *N. SHELCHKOVA[1], C. M. GREENSPON[2];
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Abstract: Rhesus macaques are considered the gold-standard for translational neuroscience due to their similarity with humans. This is especially true for touch as they are among a very small group of research animals that use their hands as we do. Consequently, substantial efforts have been made to characterize the response properties of neurons along the tactile pathway of macaques including the primary afferents, cuneate, thalamus, and cortex. To date, however, the similarity between the tactile response properties of macaques and humans has remained

untested. To address this gap, we delivered precise mechanical stimuli that have previously been used to characterize tactile responses to the fingers of a single human participant implanted with two microelectrode arrays in Brodmann's Area 1 of somatosensory cortex and two in motor cortex. In addition to this, during each stimulus we asked the participant to report the intensity of the stimulus such that we could relate the neural activity with their perception. First, we delivered trapezoidal stimuli and found a mixture of slowly adapting, rapidly adapting, and mixed responses with a significant over-representation of rapidly adapting responses. Surprisingly, we also observed a substantial number of motor channels that were modulated, though usually with a greater latency than those in S1. Second, we delivered sinusoidal vibrations between 5 and 500 Hz and used the evoked responses to measure the degree of afferent integration. Consistent with indentation responses, most units showed activation to a broad range of frequences implying significant integration. Third, spatiotemporal receptive fields and reverse correlation of responses to random dot patterns showed a multi-lobe structure. Consequently, tactile responses between macaques and humans are remarkably similar and demonstrates that macaque research is highly translational.

Disclosures: N. Shelchkova: None. **C.M. Greenspon:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

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Support: UNAM-DGAPA-PAPIIT Grant IN200822
CONAHCyT Grant FDC_1702
CONAHCyT 796537

Title: The representation of movement parameters in somatosensory cortical and striatal circuits

Authors: *A. HIDALGO-BALBUENA[1], C. I. PEREZ-DIAZ[2], P. E. RUEDA-OROZCO[3]; [1]NEUROBIOLOGÍA DEL DESARROLLO Y NEUROFISIOLOGÍA, Inst. DE NEUROBOLOGÍA, UNAM, Querétaro, Mexico; [2]Inst. of Neurobio., Univ. Nacional Autonoma de Mexico, Querétaro, Mexico; [3]Neurobiologia del Desarrollo y Neurofisiologia, Inst. de Neurobiologia, UNAM, Queretaro, Mexico

Abstract: In rodents, the dorsolateral striatum (DLS) hosts somatotopic representations of the animal's forelimbs and hindlimbs. During movement execution, the same region also contains representations of movement parameters such as speed and position. The exact relationship between both representations is not known, but we have previously described that the optogenetic activation/inactivation of sensory information to DLS bidirectionally biased the temporal component of the execution of a timely constrained sequence of movements, but

importantly, sparing speed control (Hidalgo-Balbuena, 2019). Therefore, we ask whether specific DLS neuronal representations of movement parameters are related to somatotopic inputs and whether the primary somatosensory cortex (S1), a major input to the DLS also represents movement parameters. To explore these possibilities, we recorded cortical and striatal multiunitary activity in freely moving rats, while executing an overtrained motor sequences. In accordance with the somatotopic organization of S1 and DLS, the activity of several neurons in both regions was correlated with the cyclic movement of forelimbs. These results were further confirmed with optogenetic manipulations of sensory pathways designed for offline classification of neurons into primary and secondary responders to VPL inputs. Then, consistent with previous observations, DLS neurons correlated with movement speed and position. On the other hand, S1 neurons were almost exclusively correlated with movement speed. Finally, S1 recordings in apprentice animals revealed a progressive development of speed representations. Our observations suggest that DLS speed representations maybe inherited from cortical S1.

Disclosures: A. Hidalgo-Balbuena: None. **C.I. Perez-Diaz:** None. **P.E. Rueda-Orozco:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.13/F13

Topic: D.02. Somatosensation – Touch

Support: NIH NINDS NS122333

Title: Kinetic and kinematic strategies of natural texture exploration

Authors: *N. DARABI, S. J. BENSMAIA, S. E. PALMER, A. R. SOBINOV;
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Abstract: Humans are endowed with an exquisite sense of touch, allowing us to perceive textures on objects we interact with every day with great keenness and reliability. Studies of the neural basis of texture perception often involve applying a moving texture to a single finger while recording neural activity. However, the exact kinetic and kinematic strategies used for actively exploring textures have not been measured and may differ from the values previously used in experiments. The discrepancy between the real-life and applied stimuli could impact the recorded neural signal, making them less representative of the natural behavior. To fill this gap and inform future studies, we asked healthy participants to explore textures hidden from their view with their right index finger and rate one of the qualities: roughness, hardness, or slipperiness. After exploring for as long as they wished, participants provided a rating on a self-specified scale. The trials were divided into blocks based on the quality, and each texture was presented three times within each block. The textures were attached to a six-dimensional force sensor recording at 2000 Hz, while movements were recorded using a high-speed camera at 120

frames per second. We used the video recordings to estimate the relative movement of the arm, hand, and finger during the exploration. We found that the specific feature being assessed had a stronger effect on the forces than the texture being explored. Trials focused on estimation of the hardness involved higher, more periodic force levels while roughness trials involved lower forces that did not fluctuate much throughout the trial. Kinematic analysis revealed that roughness trials involved periodic sweeps by the index finger whereas hardness trials involved a more stationary approach. The forces used during exploration were consistent across repeated trials with the same texture and specific to that texture. The 95% confidence interval of the mean force used across all participants was 2.06 N - 2.15 N, higher than forces used in previous neurophysiological experiments. The recorded movements and forces during active texture exploration can be directly applied to subjects' hands during future experiments to precisely replicate and control behavior during psychophysical tests. The quantification of these values provided here can inform future neurophysiological experiments to ensure the ecological relevance of the stimuli during measurements of the neural basis of texture perception.

Disclosures: N. Darabi: None. **S.J. Bensmaia:** None. **S.E. Palmer:** None. **A.R. Sobinov:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.14/F14

Topic: D.02. Somatosensation – Touch

Support: NIH R01 NS116277

Title: Understanding tactile sensing in whiskers and hands through biomechanical modeling

Authors: N. TUMMALA[1,2,3], K. J. KLECZKA[2,3], *M. HARTMANN[2,3];
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Abstract: Tactile sensation is initiated by mechanical inputs that excite peripheral sensory neurons. Therefore, it is crucial to quantify these mechanical and neural signals to understand tactile sensing both peripherally and centrally. However, this quantification is difficult to achieve through *in vivo* measurements due to experimental constraints and signal complexity. Recent improvements in biomechanical modeling of peripheral tactile sensing have begun to allow us to simulate the mechanical inputs and associated neural responses for rodent whiskers and primate hands, two widely studied model systems. Here, we focus on the rodent whisker system, leveraging the WHISKiT Physics simulation tool (Zweifel et. al., 2021) to model the mechanical signals generated during active whisking behavior. Specifically, we simulated a rat whisking against a cylindrical peg placed at 200 different locations relative to its snout. For each peg

location, the simulation generated twelve time-varying mechanical signals (three forces, three moments, and their temporal derivatives) at the base of each of the 54 whiskers in the array. We then used these mechanical signals to model the responses of both slowly and rapidly adapting primary sensory neurons in the trigeminal ganglion within the Nengo neural simulation framework (Bekolay et al., 2014). We demonstrate that this computational approach can simulate large populations of peripheral neurons that can serve as inputs to models of more central circuits in the whisker system. We then compare the results of our simulation to recent biomechanical models of peripheral tactile sensing developed for the human hand (Saal et al., 2017; Tummala et al., 2024). Finally, we describe promises and pitfalls of these simulation-based biomechanical approaches for understanding tactile sensation. This research contributes to a vision in which we can simulate the transformation and processing of peripheral tactile signals at increasingly central levels, essentially constructing the tactile system from the outside-in.

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Saal HP, Delhaye BP, Rayhaun BC, and Bensmaia SJ (2017). *PNAS*. 114(28). doi: 10.1073/pnas.1704856114
Tummala N, Reardon G, Dandu B, Shao Y, Saal HP, and Visell Y (2024). *bioRxiv*. doi: 10.1101/2023.11.10.565040
Zweifel NO, Bush NE, Abraham I, Murphey TD, and Hartmann, MJZ (2021). *PNAS*. 118(27). doi: 10.1073/pnas.2011905118

Disclosures: N. Tummala: None. **K.J. Kleczka:** None. **M. Hartmann:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.15/F15

Topic: D.02. Somatosensation – Touch

Title: Neural basis of coordinate transformations of tactile motion stimuli on the hand

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Abstract: Neural mechanisms that generate tactile motion perception play a key role in haptics by providing sensory feedback signals used to make grasp adjustments (e.g., signaling that an object is slipping). Understanding how proprioception transforms tactile representations of

objects that move on the skin is key to determining how the brain derives invariant representations of tactile motion. Previous studies show that neurons in the primary somatosensory cortex (SI) derive tactile motion representations by integrating different tactile cues of the object that impinge on the skin (e.g., speed, force, direction, and others), a mechanism known as the Full Vector Average model. This model was derived from studies that placed the hand in the same posture. However, recent human psychophysics studies from our lab show that the proprioceptive state of the arm modulates the perception of tactile motion on the hand. Here, we advance our understanding of how tactile motion representations are derived by studying where and how proprioceptive-dependent transforms of tactile motion emerge in the brain. We recorded single-unit activity from areas 3b and 1 of SI using Neuropixels arrays in an anesthetized monkey that was presented with tactile motion stimuli on the fingers. Stimuli were presented with the animal's hand placed in a supinated or pronated posture. We observed that a large number of neurons had strong responses, with many of these neurons having a preferred response to stimuli moving in a particular direction. Further, we found that the responses of a large fraction of motion-tuned neurons were modulated by proprioception. Specifically, proprioception phase-shifted and/or modulated the gain response of the tuning functions of neurons . These results suggest that proprioceptive information is integrated with tactile motion information as early as SI to derive invariant representations of tactile motion. Our data also show that tactile and proprioceptive integration mechanisms can be studied in non-human primate models under anesthetized preparations.

Disclosures: H. Ahuja: None. **A. Ignaco:** None. **M. Duhain:** None. **C. Li:** None. **G.C. DeAngelis:** None. **M. Gomez-Ramirez:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.16/F16

Topic: D.02. Somatosensation – Touch

Support: National Key R&D Program of China 2021YFF0702200
STI 2030-Major Projects 2021ZD0200401
Key R&D Program of Zhejiang Province 2021C03001

Title: Neural Mechanism of Tactile Spatiotemporal Integration in Area 3b

Authors: *Z. TANG[1,2,3], B. QU[4,5], H. WANG[4,5], M. YE[4,5], L. LAN[6,7], Z. LYU[6,3], H.-Y. LAI[6,3,5,8];
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Abstract: The perception of tactile-motion is contingent on the processing of spatiotemporal activation patterns across neuron populations in the somatosensory cortex. However, the interaction mechanisms between spatial (direction) and temporal (speed) information interact remain poorly understood. This study aims to investigate the variations in directional preferences at different speeds for individual digital and multi-digit. We used a lab-designed tactile stimulator to administer stimuli at speeds ranging from 5 to 320 mm/s and directions from 0° to 180° to two macaques. Stimulations were applied to individual digits, including index (D2) and middle (D3) fingers, and multi-digit (D2 and D3). Using a 16-channel microelectrode array implanted into area 3b, we recorded from 165 neurons, noting associations with single-digit stimuli in 121 neurons and multi-digit responses in 92. Our findings showed that most neurons (n=75) responded significantly to broad-bandwidth speeds, while fewer neurons (n=45) responded only to narrow-bandwidth speeds. Neurons sensitive to narrow-bandwidth speeds exhibited a consistent single directional preference. Conversely, neurons sensitive to broadband speeds displayed variable directional preferences as speed changed, including single- and multi-peaked directions. Multi-digit stimulation induced distinct neuronal activity patterns of excitation (n=53) and inhibition (n=39) compared to single-digit stimulation, suggesting complex interactions beyond simple additive responses. Notably, some neurons (n=65/92) changed the directional preference from signal-digit to multi-digit stimulation.
In conclusion, our study identified neurons with varied sensitivities to speed bandwidths and demonstrated that directional preferences change with speed in complex patterns. Additionally, the complex integration mechanisms for multi-digit tactile information in area 3b underscore the sophistication required in tactile processing for object perception.

Disclosures: Z. Tang: None. **B. Qu:** None. **H. Wang:** None. **M. Ye:** None. **L. Lan:** None. **Z. Lyu:** None. **H. Lai:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.17/F17

Topic: H.03. Decision Making

Support: Wellcome Trust

Title: Distinct signatures of perception in neuronal signals propagating through cortex and subcortex

Authors: *B. RUSSELL, M. B. LYNN, A. M. PACKER, A. LAK;
Univ. of Oxford, Oxford, United Kingdom

Abstract: Perception is inherently variable; the same external stimulus might be detected or missed in different instances. Previous studies have shown that as neuronal signals propagate from primary sensory cortices to higher cortical regions, they better reflect the trial-by-trial percept. In addition to sending projections to higher cortical areas, primary sensory regions also form substantial subcortical projections, for example to the thalamus or striatum. However, it remains unknown how the structure and timing of population codes underlying perception change as neuronal signals propagate from cortex to subcortical regions. To address this question, we recorded electrophysiological neuronal activity in a somatosensory optogenetic detection task. In this task, mice report optogenetic stimulation of primary somatosensory cortical neurons (S1), delivered using an LED through a cranial window, by turning a wheel to receive a water reward. Using Neuropixels probes, we have acquired high-temporal resolution neuronal data simultaneously from key S1 outputs, including secondary somatosensory cortex (S2), thalamus (ventral posteromedial nucleus, medial posterior nucleus) and dorsolateral striatum (DLS). Neuronal activity rapidly propagated from S1 to S2 and DLS with similar temporal delays, with a later response in thalamus observed ~100ms after S2 and DLS. All regions contained subpopulations of neurons that distinguished perceptual hits from misses by their stimulus-driven activity. However, S2 also contained a unique subpopulation of neurons that predicted upcoming perceptual hits from their baseline activity. Perceptual signals across all recorded regions were largely independent from motor signals related to choice or outcome. Together, these analyses suggest that subcortical and cortical regions receiving signals from S1 differ in how they represent neural signatures of perception. We are currently further analyzing the data to understand whether specific cortical-sub-cortical connections have a weighted role in perception, and whether the relative timing of these signals contribute to perception. Our results indicate that cortical and subcortical regions receiving signals from S1 exhibit differential timing and neural codes underlying perception.

Disclosures: B. Russell: None. **M.B. Lynn:** None. **A.M. Packer:** None. **A. Lak:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.18/F18

Topic: D.02. Somatosensation – Touch

Support: JST ERATO JPMJER1801
JSPS 22K21353
AMED CREST 22gm1510002h0002
JSPS 20K15926

Title: Brief light deprivation modulates rat somatosensory cortical activity of sole tactile sensation during natural walking

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Abstract: Multisensory integration plays a critical role in perception, but has primarily been studied in contexts that require focused attention on a task. However, it remains unclear whether similar sensory integration occurs for passive stimuli encountered during naturalistic rhythmic behaviors, such as walking, without explicit attentional demands. This study investigates how tactile representations in the primary somatosensory cortex (S1) of rats are modulated by sensory context during rhythmic walking movements.
Local field potentials (LFPs) were recorded from 32 electrodes spanning the forelimb and hindlimb regions of S1 in freely walking rats (n=11). Rats walked on a rotating disk with two different textured surfaces (coarse or smooth) under light (visual input) or dark (no visual input) conditions. Step-evoked tactile inputs were generated from the rats' forelimb contacts on the textured floor. Deep convolutional neural networks (CNNs) were trained to classify single-trial LFP responses.
Averaged LFPs revealed step-evoked potentials time-locked to forelimb contacts in forelimb S1. CNNs trained on both 2-class (texture or light/dark) and 4-class (texture and visual input discrimination) classification showed significantly above chance accuracy.
To extract features in LFPs, gradients were computed for the 4-class classification. The results showed that the most discriminative time points for classification were centered around the initial forelimb contact with the textured ground surface. Additionally, there were differences in the spatial distribution of the discriminative electrode channels, with those located in the forelimb S1 region showing a stronger influence compared to hindlimb channels.
Clustering analyses of tensor activations from intermediate CNN layers revealed that LFP response patterns were more discriminable between light and dark conditions than between the two ground textures. However, within the same visual context, the two texture classes showed higher discriminability in total darkness compared to the light condition.
Our results show that S1 dynamically represents passive tactile input from rhythmic walking movements based on the available multisensory context, even without focused attention. The improved texture decoding in darkness suggests that reduced cross-modal interference from vision may allow for distinct tactile representations. This provides novel insights into how the brain integrates and flexibly prioritizes sensory signals across modalities during naturalistic behaviors beyond task contexts involving directed attention.

Disclosures: K. Yamashiro: None. **S. Tanaka:** None. **N. Matsumoto:** None. **Y. Ikegaya:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.19/F19

Topic: D.02. Somatosensation – Touch

Support: R01 NS131549

Title: Two-photon imaging of proprioceptive information in dorsal column nuclei of mouse proprioceptive stimuli in gracile nucleus in mice

Authors: *R. IWAMOTO[1,2], E. STACY[3], S. TAMURA[4], A. MATUNIS[5], K. ABE[6], T. IMAI[7], T. HIKIDA[8], T. R. SATO[9];
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Abstract: It is generally known that gracile nucleus in medulla is activated in response to haptic and proprioceptive stimuli on lower body and hindlimbs. However, little is known about the spatial and functional properties of neurons that respond to these stimuli, especially proprioceptive stimuli. We therefore performed two-photon calcium imaging of neurons in gracile nucleus responding to tactile or proprioceptive stimuli by combining a system in which mechanical stimulation was applied to each toe of a mouse under anesthesia with another system in which the mouse was lifted up and moved its hindlimbs freely in the air. This allowed us to obtain spatial maps and activity time courses of each neuron that responded to each tactile stimulus site and to the movement and position of the hindlimb, showing significant differences. We also revealed that excitatory and inhibitory neurons show different activity properties, including spatial selectivity for tactile stimuli.

Disclosures: R. Iwamoto: None. **E. Stacy:** None. **S. Tamura:** None. **A. Matunis:** None. **K. Abe:** None. **T. Imai:** None. **T. Hikida:** None. **T.R. Sato:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.20/F20

Topic: D.02. Somatosensation – Touch

Title: Posture dependant changes in perceptual threshold during foot sole stimulation

Authors: *J. WATTS, F. MACRAE, S. PETERS;
Western Univ., London, ON, Canada

Abstract: Posture dependant changes in perceptual threshold during foot sole stimulationLight touch sensitivity of the foot sole is typically measured when individuals are seated or lying down; yet, a critical function of foot sole cutaneous feedback is to support standing and walking activities. Mildren et al found there to be a significant increase in vibratory perceptual threshold while standing compared to seated postures. To date, no one has examined the perceptual threshold of light touch in multiple postures which limits our understanding of how posture and perception may interact to modulate somatosensory information. The objective of this study was to evaluate the differences in how individuals perceive light touch stimulation across the foot sole when they are in different postures. To accomplish this, we measured the light touch perceptual thresholds in standing, seated, and supine postures in 25 healthy volunteers (9 males) ages 20-28 with a mean age of 23.8 years old (SD = ±2.19), using Semmes-Weinstein Monofilaments and a modified 4-2-1 stepping algorithm. Perceptual thresholds were calculated at three foot sole locations (1st metatarsal, lateral arch, and heel) in each posture. Significant differences in perceptual threshold were found at the 1st metatarsal between standing and both seated ($p = 0.016$) and supine ($p = 0.020$) postures, but not between seated and supine postures ($p = 1.0$). The same can be said about the lateral arch, with significant differences being found between both standing and seated ($p = 0.001$) and supine ($p = 0.001$) postures, but not between seated and supine postures ($p = 0.833$). Lastly, for the heel, significant differences were found between standing and both supine ($p = 0.001$) and seated ($p = 0.024$) postures, as well as between seated and supine postures (0.014). Our results demonstrate that postural changes significantly influence perception of light touch across the foot sole. Thus, performing perceptual threshold assessments on the foot sole while standing may offer more relevant insights into the capacity of foot sole cutaneous afferents to convey light touch information in conditions where such feedback plays a vital role in maintaining balance.

Disclosures: J. Watts: None. **F. MacRae:** None. **S. Peters:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.21/F22

Topic: D.02. Somatosensation – Touch

Support: NSERC Discovery Grant (LRB)
NSERC Postgraduate Scholarship - Doctoral (TS)

Title: Cutaneous reflexes evoked in abductor hallucis by stimulating various foot sole regions do not occur in a location dependent manner

Authors: *T. SHARMA[1], A. V. VANDERHAEGHE[1], A. A. KAPASI[2], L. C. MARRELLI[1], B. H. DALTON[3], L. R. BENT[1];
[1]Human Hlth. and Nutritional Sci., Univ. of Guelph, Guelph, ON, Canada; [2]Kinesiology, Univ. of Guelph-Humber, Toronto, ON, Canada; [3]Sch. of Hlth. and Exercise Sci., Univ. of British Columbia, Kelowna, BC, Canada

Abstract: The abductor hallucis (AH) is an intrinsic foot muscle that abducts and flexes the big toe and is active during postural tasks. Recent work has shown that AH's activity, morphological characteristics, and its contractile properties correlate with postural measures. Despite growing evidence for AH's role in posture, much remains unknown. For example, cutaneous reflexes, which contribute to modulating muscle activity for postural control, have yet to be characterized in the AH. In other postural muscles such as the soleus, the polarity (excitation or inhibition) of the middle-latency component of cutaneous reflexes (MLR) occurs in a location dependent manner to generate functional balance responses. Given AH's postural role, the MLR of its cutaneous reflexes may also be modified in a location-dependent manner; however, this has yet to be determined. The purpose of this study was to characterize the MLR in AH following stimulation of four-foot sole regions: big toe, fourth toe, metatarsal, and heel. While seated, twelve healthy, young participants (six female) performed AH contractions at 20% of maximal activation. During these contractions, stimuli were applied at a moderate-strong intensity as rated by the participant. We compared the amplitude and polarity of the MLR between stimulation sites. When MLRs were not obscured by artifacts, significant responses were evoked in 10/12 (big toe), 9/12 (fourth toe), 9/11 (metatarsal) and 6/9 (heel) participants. All reflexes evoked by big toe, metatarsal, and fourth toe stimuli were inhibitory, while 5/6 heel-evoked responses were inhibitory. The mean amplitude of evoked MLRs did not differ between stimulation sites ($p=0.076$). Our results indicate that cutaneous information from the foot sole can modulate AH muscle activity in most participants; however, the cutaneous-evoked MLR is not location-dependent, at least in a seated posture. This contrasts with other postural muscles that exhibit location-dependent MLRs in sitting. We speculate that for the AH, a seated posture may not provide the appropriate postural context to observe a location-dependent modification of its MLR. Our findings may also suggest that AH activity is modulated uniquely by cutaneous inputs, compared to other lower-limb postural muscles. This potential difference highlights the importance of additional research exploring the role of this intrinsic foot muscle in balance maintenance.

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Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.22/F23

Topic: D.02. Somatosensation – Touch

Support: P01NS057228

Title: A spinal circuit for mutual reinforcement of antagonist co-contraction

Authors: T. M. ROTTERMAN[1], A. S. DEARDORFF[2], ***P. NARDELLI**[3], T. C. COPE[4]; [1]Biol. Sci., Georgia Inst. of Technol., Atlanta, GA; [2]Dept. of Neurol., Boonshoft Sch. of Med., Dayton, OH; [3]Georgia Inst. of Technol., Atlanta, GA; [4]Biol. Scineces and Engin., Georgia Inst. of Technol., Atlanta, GA

Abstract: Everyday motor tasks could not be accomplished effectively, or perhaps even carried out at all, without the coordinated activation of muscles which generate opposing forces at skeletal joints, i.e., muscle antagonists. We propose a novel spinal reflex pathway that promotes co-activation of antagonist motor pools based on our unexpected finding that stretch of the pretibial flexor muscles tibialis anterior-extensor digitorum longus (TA-EDL) intermittently *facilitates* concomitant reflex contraction the ankle extensor medial gastrocnemius (MG). We tested our proposal using isoflurane-anesthetized rats to record synaptic potentials produced in motoneurons by naturalistic mechanical stretch of their antagonist muscles. We consistently found stretch-evoked excitatory postsynaptic potentials (strEPSPs) commonly with trisynaptic latency that was bidirectional, i.e., from TA-EDL onto MG (path A) or MG onto TA-EDL (path B) in every motoneuron in our sample. Average strEPSP amplitudes were similar (0.59 ± 0.07 mV, n=26 and 0.75 ± 0.16 mV, n=17 for path A and B, respectively) and suggestive of strong pathways, ones which brought at least 2 premotor interneurons to fire despite anesthesia. We also show that the strEPSPs were activated by IA afferents alone using high frequency muscle vibration, but were significantly larger when synchronously activated by IA, II and IB combined using muscle quick stretch. We conclude that these pathways constitute a bidirectional spinal circuit for feedforward excitation of antagonist motor pools. Recent reports identify potential neuron types including excitatory premotor interneurons that project to antagonist motor pools or ones that act through axo-axonic pathways. We speculate that mutual reinforcement of antagonist muscles may assist with managing limb inertia during rapid changes in direction, which is characteristic of scurrying locomotion exhibited by rodents.

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Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.23/F24

Topic: D.04. Interoception

Title: A mouse model for bimanual integration of proprioceptive information

Authors: *M. LIPTON, M. C. DADARLAT;
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Abstract: The sense of proprioception, or the brain's awareness of the body's position in space, is critical for tasks involving complex, multi-limb movements. Recent studies have highlighted the role of the mouse primary somatosensory (S1) and motor (M1) cortices during unilateral proprioceptive forelimb movements. These investigations suggest that neurons in sensorimotor cortex encode the direction and amplitude of the forelimb during movement. However, it remains unclear how proprioception of multiple limbs is represented in the sensorimotor cortex. To address this question, we developed a behavioral decision-making task to probe how mice perceive proprioceptive feedback during bilateral passive forelimb movements. We trained head-fixed mice (n=4) to grasp a handle with each front paw while a stepper motor passively displaced a single limb approximately 5 mm in the anterior direction. The mice reported which limb was passively displaced by licking right or left. Preliminary results indicate that mice learned to perform this task, achieving an average accuracy of 68%. Mice exhibited preferential responses to passive displacement of the right forelimb (89% accuracy) compared to 51% accuracy for left forelimb displacement trials. Additionally, mice were able to report correct decisions within 600 milliseconds on average after the start of the response window, further demonstrating accurate performance in this task. Using this behavior as a baseline, we will investigate the extent to which mice can discriminate between bilateral proprioceptive inputs. This novel behavioral task will serve as the foundation from which to study the neural mechanisms of bimanual proprioceptive processing in the sensorimotor cortex.

Disclosures: M. Lipton: None. **M.C. Dadarlat:** None.

Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.24/F25

Topic: D.02. Somatosensation – Touch

Support: R01NS110823
GRANT12635723
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Eugene McDermott Graduate Fellowship 202108

Title: Exploring the Effects of Chronic Microelectrode Array Implantations on ICMS-Evoked Perception Thresholds and Recording Performance

Authors: *T. J. SMITH[1], B. S. STURGILL[2], Y. WU[3], L. R. VILLAFRANCA[4], S. L. VARGAS[5], M. BENDEA[1], S. F. COGAN[2], A. G. HERNANDEZ-REYNOSO[2], J. J. PANCRAZIO[2];
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Abstract: Chronic intracortical microstimulation (ICMS) within the somatosensory cortex offers a promising avenue for studying neural dynamics and restoring vibrotactile sensations. While the recording performance of these devices is known to deteriorate with chronic use, the relationship between changes in recording performance and the efficacy/stability of ICMS-evoked sensory thresholds is not well characterized. In this study, we aimed to investigate this relationship by examining the impact of chronically implanted intracortical 12-shank microwire arrays on ICMS-evoked perceptual stability and neural recording performance, focusing on changes in both putative inhibitory and excitatory neurons across superficial, middle, and deep cortical layers over a 30-week post-implantation period. Five arrays were implanted in rat somatosensory cortex, totaling 60 electrode sites (20 superficial, 30 middle, and 10 deep). Of those sites, 50 were stimulated 4 days/week in an established Go/No-Go behavioral task for ICMS-evoked sensory threshold estimation. Linear regression analysis assessed long-term changes in recording performance. Our findings revealed significant changes in unit activity, particularly among middle-layer fast-repolarizing putative inhibitory neurons. Linear regression analyses of these units' properties exhibited significantly positive slopes from zero ($p < 0.001$), showing increases in mean firing rate of 0.02 spikes/sec/week, peak-to-peak voltage of 0.69 µV/week, and average number of units per electrode of 0.01 units/week. Furthermore, the overall average active-electrode-yield only reduced by 30% from an initial level of ~80%, indicating robust recording performance. Simultaneous multi-channel stimulation demonstrated that a minimal per-site charge per phase of ~1 nC reliably evoked stable sensory percepts in rats over the chronic period. Interestingly, an early-phase threshold reduction of ~2 nC was observed from an initial naïve estimation over the first 10 weeks. Despite continued changes in neural recording performance, our analysis indicates that the stability of stimulation-evoked sensory percepts remained largely unaffected. This suggests that while ICMS may induce alterations in neural activity, it does not necessarily compromise the ability to reliably evoke sensory percepts. Following, we will further examine differences between stimulated and non-stimulated sites. While these findings underscore the robustness of microwire arrays for both long-term neural recordings and ICMS applications, further studies are needed to better understand why the early-phase perception threshold changes occur.

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Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.25/F26

Topic: D.02. Somatosensation – Touch

Support: DARPA HR001120C0120

Title: Subthreshold intracortical microstimulation variably impacts the detection of somatosensory afferent inputs

Authors: *V. ARRIOLA[1,2], L. OSBORN[3], B. CHRISTIE[4], M. S. FIFER[5], F. TENORE[6], S. J. BENSMAIA[7], J. M. YAU[8], C. M. GREENSPON[9];
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Abstract: The conscious experience of touch is predicated upon the activation of the somatosensory cortex, as evidenced by the fact that such an experience can be evoked by electrically activating neurons in the somatosensory cortex (S1). In the present study, we investigated how natural, bottom-up tactile signals evoked through vibrotactile stimulation of the skin interacted with artificial tactile signals directly injected into S1 via intracortical microstimulation (ICMS). To this end, we trained two Rhesus macaques to perform a detection task in which they reported whether or not a vibration was delivered to their hand. After they had reached asymptotic performance on the task, we then had them perform the task in the presence or absence of an ICMS stimulus delivered through chronically implanted Utah arrays. We then compared the animals' ability to detect the presence of the stimulus in the presence or absence of the ICMS. We found that, on a subset of electrodes, concurrent ICMS increased sensitivity for detecting mechanical stimulation, as evidenced by a decrease in the detection threshold. This effect was present only when the ICMS was subthreshold. We also investigated the extent to which this effect depends on a variety of stimulus parameters. We conclude that, under certain circumstances, ICMS improves sensitivity to natural sensory inputs.

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Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.26/F27

Topic: D.02. Somatosensation – Touch

Support: NINDS Award Number UH3NS100541

Title: Effect of stimulation parameters on percepts evoked by epidural spinal cord stimulation in people with lower-limb amputation

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Abstract: Sensory feedback is crucial for balance and gait. People with lower-limb amputation lack sensory feedback from the missing limb, which contributes to impaired balance and increased risk of falling. We aim to examine the feasibility of spinal cord stimulation (SCS) to restore sensations in the missing limb. Ideally, restored sensations should be perceived as naturalistic, similar to what an intact nervous system would generate in an able-bodied individual. Past studies with cortical and peripheral nerve stimulation have shown that modulating stimulation parameters (amplitude, frequency) can affect the naturalness of the stimulation-evoked sensation. Our goal is to characterize the effect of SCS parameters on the quality and perceived naturalness of evoked sensations.We implanted two or three 8- or 16-contact SCS leads in the epidural space near the lumbar spinal cord in three human subjects with transtibial amputation over 28 days (for Subject 1 and 2) and 84 days (for Subject 3). We delivered 1-sec long stimulation trains through monopolar and multipolar combination of contacts over a range of stimulation parameters (amplitude, frequency) and recorded the location of the evoked sensations. We also recorded the perceived naturalness score using a visual-analog scale from 0 to 10. We performed psychophysical experiments using a 2-alternative force choice task to estimate sensory thresholds. We also measured the relationship between stimulation amplitude and frequency with the perceived intensity of the stimulation. In all subjects, SCS elicited sensations were localized to the ankle and foot of the missing limb, as well as the residual limb. The threshold for evoking sensations in the missing limb was always higher than the residual limb but decreased over time. For all three subjects, the perceived quality of each SCS-evoked sensation was described with a combination of natural and paresthetic descriptors. We found that the sensory magnitude could be systematically manipulated by varying the

stimulation amplitude but did not alter the perceived quality. The area of the evoked sensation also increased linearly with the stimulation amplitude. Increasing stimulation frequency increased the perceived intensity up to 100Hz. There was no consistent effect of stimulation frequency on the location and perceived quality of the evoked sensations. Both stimulation amplitude and frequency did not have an effect on the perceived naturalness of the evoked sensation. Overall, these results demonstrate the effect of SCS parameters in modulating the evoked percepts and will guide the design of SCS paradigms to deliver effective sensory feedback during functional tasks.

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Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.27/F28

Topic: D.02. Somatosensation – Touch

Support: NIH NINDS Award Number UH3NS100541

Title: A patient-specific model of lateral spinal cord stimulation for the delivery of sensory feedback from prosthetic limbs

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Abstract: Sensory feedback delivered via electrical stimulation of the nervous system can be an effective technique for improving functional outcomes for lower-limb prosthesis users, from increasing walking speed to improving balance and reducing phantom limb pain. Much previous work has examined peripheral nerve stimulation for the delivery of sensory feedback. Recent work in our lab has leveraged spinal cord stimulation technology to deliver focal sensory feedback that appears to originate in the missing limb. Spinal cord stimulation is well established as a therapeutic technology in widespread use for managing chronic intractable pain. Therefore, leveraging this technology for somatosensory neuroprosthetic applications can enable fast future translation of somatosensory neuroprostheses. To target focal regions of the limb, we employ a lateral spinal cord stimulation (LSCS) approach. In this approach, electrodes are placed laterally to target neural responses in the dorsal rootlets, which are expected to innervate smaller regions

of the limb. Previous work in our lab has shown that this approach can evoke sensations that appear to emanate from the missing limb, but little is known about the underlying recruitment of neural structures. Further, the process of finding stimulation parameters to evoke desired sensations can be costly and time consuming. This project uses a computational modeling approach to elucidate the recruitment properties of LSCS. We use manual segmentation of MR and CT images from a participant in an ongoing LSCS study to create a patient-specific computational model representing the realistic anatomy of the participant's spinal cord. Tissues are modeled using electrical properties from literature. We equip the model with realistic electrodes placed according to the electrode locations in the participant, derived from imaging, and populate the model with axons built in the NEURON simulation environment. Using this model, we explore the recruitment properties of dorsal root and spinal cord neurons in response to LSCS and aim to understand how those neural responses map to the perceptual outputs subjects experience during stimulation. This model will ultimately enable an *in silico* platform for testing electrode placements and tuning stimulation parameters, thereby accelerating translation by reducing the time-cost for initial device calibration for research participants and consumers.

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Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

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Topic: D.02. Somatosensation – Touch

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Title: Exploring selective stimulation in the lumbar spinal cord: insights for neuroprosthetic electrode design

Authors: *M. DURAN[1], G. ANSAH[1], M. DEL BROCCO[2], R. BOSE[3], D. J. WEBER[4], M. K. JANTZ[3], S. F. LEMPKA[5], L. E. FISHER[2], C. GOPINATH[6];
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Abstract: Current lower-limb prosthetics lack the capability to restore somatosensory feedback. Our lab has shown that lateral spinal cord stimulation (SCS) can generate focal sensations in the distal limbs of people with lower-limb amputation, however sensations were also felt on proximal regions of the residual limb, creating unwanted distractors. To improve the focality of evoked sensations, in this project, we developed and test novel paddle electrodes with the objective of assessing their efficacy in eliciting targeted sensations within specific and localized areas of the distal limb, using varying stimulation configurations. Experiments were conducted on six cats. After placing nerve cuffs on twelve branches of the sciatic and femoral nerve, a lumbosacral laminectomy was performed custom paddle electrodes were placed on the epidural surface of the spinal cord. Three paddles were used, each consisting of a 16x2 array of contacts, with contact diameters of 1 mm, 0.5 mm, or 0.15 mm. The paddle placement was varied from animal to animal, spanning the L4-L7 spinal cord. Both monopolar and bipolar stimulation configurations were used using a current ranging from 15 - 350 µA. Stimulus trains consisted of 250 biphasic pulses with a 66 µs pulse width per phase, and a 33 µs interval between phases. Compound action potentials (CAPs) evoked by SCS were recorded via the nerve cuffs placed on the sciatic and femoral branches. Selectivity was measured by our ability to evoke activity in a single distal nerve branch. We characterized selectivity at threshold (i.e., the minimum current amplitude required to elicit any nerve response). We also measured dynamic range, the range of stimulation amplitude over which selectivity could be maintained before a second nerve was recruited. At L6, L5 and L4 the percentage of selectively recruited nerves were 73%, 54%, and 65% respectively. The average thresholds of selectively recruited nerves were 52.98 µA, 43.29 µA and 36.29 µA for electrode paddle sizes 1 mm, 0.5 mm, and 0.15 mm respectively. The median dynamic range was 21.43 µA, 13.93 µA, and 21.12 µA for monopolar stimulation with 1 mm, 0.5 mm, and 0.15 mm electrodes respectively, and 12.57 µA, 10.7 µA, and 14.73 µA for the bipolar configuration. These results will guide the next steps in optimizing electrode designs to restore focal somatosensory feedback via SCS.

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Poster

PSTR402

Somatosensory and Proprioceptive Stimuli: Receptive Fields, Responses, and Coding

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.29/F30

Topic: D.02. Somatosensation – Touch

Support: NSF GRFP Grant No. 1937968

Title: The strength-duration curve can efficiently define a multi-dimensional dynamic intensity range for peripheral nerve stimulation somatosensation

Authors: *R. S. JAKES, B. ALEXANDER, V. MARCU, L. ROLDAN, B. AJIBOYE, D. J. TYLER;
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Abstract: Electrical peripheral nerve stimulation (PNS) can restore the sense of touch for people with limb loss, providing valuable environmental information. Tactile percept characteristics such as sensation intensity, location, and quality arise from axon recruitment patterns evoked via the manipulation of PNS parameters. However, the PNS parameter-percept space is interdependent and broad, limiting the efficacy and efficiency of single-parameter perceptual modulation exploration. A multivariable alternative is Weiss' strength-duration (SD) curve, which relates two PNS parameters, pulse duration and pulse amplitude, to tissue excitation. In sensory PNS, perceptual thresholds have been shown to follow an SD curve. In this study, we hypothesized SD curves can also describe equal intensity contours across the perceptual dynamic intensity range and can be treated as solvable systems to efficiently map a multi-dimensional parameter space for more diverse neural activation. Experiments were conducted through implanted cuff electrodes on the median nerves of two upper extremity limb loss subjects. Equal intensity contours were generated at five intensities via the method of adjustment, with each contour defined by at least nine points. Participants blindly modulated the pulse duration of a trial stimulus until it matched the intensity of a reference stimulus of a predefined magnitude. Each equal intensity contour fit the Weiss SD curve with a coefficient of determination greater than 0.95 ($R^2 = 0.98 +/- 0.01$). To determine if the SD curve could efficiently estimate equal intensity contours, the two points at the maximum and minimum pulse amplitudes for each contour were applied to the SD curve formula as a system of equations. The fit of this determined system curve to the original contour data was then evaluated ($R^2 = 0.97 +/- 0.02$). A paired t-test showed no significant difference between the R^2 values of the SD curve fit from the full dataset and the two-point determined solution. The application of SD curves to equal intensity contours moves PNS research toward a model that considers the differential impact of pulse amplitude and duration on neural activation and therefore on other percept characteristics. In a case study with one limb loss participant, stimulating a single electrode contact at opposite extrema of the same intensity SD curve resulted in percepts with minimally overlapping locations. Overall, the efficient characterization of a two-dimensional perceptual intensity range in as few as four points increases the accessibility of multi-parameter PNS modulation for more diverse neural activation patterning and rich tactile information production.

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Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.01/F31

Topic: D.05. Auditory and Vestibular Systems

Support: JSPS KAKENHI 20H03794
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Title: The entrainment to rapid auditory input modulated by vagus nerve stimulation

Authors: *S. KUMAGAI[1,2], T. I. SHIRAMATSU[2], K. OSHIMA[2], K. KAWAI[1], H. TAKAHASHI[2];
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Abstract: Vagus nerve stimulation (VNS) modulates neural activities in the sensory cortex. Previous studies suggest that VNS can enhance feedforward (FF) auditory processing from the lower to higher-order area. However, it remains unclear how VNS modulates auditory steady-state response (ASSR), a neural response entrained to rapid and periodic sound, which reflects FF processing. In this study, we hypothesized that VNS enhances ASSR in the cerebral cortex receiving the FF thalamocortical input. We investigated the effect of VNS using electrocorticography that covered the auditory and insular cortex of 11 Wistar rats under isoflurane anesthesia. Electrophysiological recordings were performed after implantation of the VNS system. The auditory cortex and insular auditory field were characterized by auditory-evoked potentials while presenting click stimuli. We presented rapid click trains in a session containing 300 trials before and more than 3 hours after VNS. Click trains were 500ms in duration at rates of 20- and 40-Hz (20 and 40 clicks/s). The intertrain interval was 500ms. ASSR was estimated using inter-trial phase clustering (ITPC) at the rate of presented click trains. We assessed, using analysis of variance, how VNS modulates ITPC. ITPC increases were found at 20 Hz in the auditory cortex during 20-Hz click trains and 40 Hz in the auditory and insular cortex during 40-Hz click trains. VNS significantly enhanced ITPC, corresponding to the rate of click trains in the auditory cortex and insular auditory field. On the other hand, VNS did not change ITPC in spontaneous activities in the intertrain intervals. These results suggest that VNS can enhance ASSR, consistent with its effect on FF processing in previous studies. VNS may strengthen the entrainment of cortical oscillations induced by repetitive auditory stimulation.

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Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.02/F32

Topic: D.05. Auditory and Vestibular Systems

Support: NIH Grant K01DC018310B

Title: Auditory discrimination deficits in a rat model of autism

Authors: *D. W. GAUTHIER[1,2,3,4], N. JAMES[4,3,1], B. D. AUERBACH[4,3,2,1];
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Abstract: Atypical sensory processing, particularly in the auditory domain, is one of the most common and quality-of-life effecting symptoms seen in Autism Spectrum Disorders (ASD). ASD individuals often exhibit altered sound sensitivity and feature discrimination, contributing to sensory overload and disrupted language comprehension. Fragile X Syndrome (FXS) is the leading inherited cause of ASD and a majority of FXS individuals present with these auditory processing alterations. We have shown previously that a Fmr1 KO rat model of FXS exhibits altered sound sensitivity that coincides with abnormal perceptual integration of sound duration and frequency. Here we further characterized auditory processing deficits in Fmr1 KO rats using an operant conditioning tone discrimination assay and in-vivo electrophysiology recordings from the auditory cortex (ACx) and inferior colliculus (IC). We found that Fmr1 KO rats exhibit poorer frequency resolution, which corresponded with sound-evoked hyperactivity and broader frequency tuning in auditory cortical but not collicular neurons. These findings suggest that cortical hyperexcitability may account for a range of auditory behavioral phenotypes in FXS, providing a potential locus for development of novel biomarker and treatment strategies that could extend to other forms of ASD.

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Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

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Topic: D.05. Auditory and Vestibular Systems

Support: Beijing Natural Science Foundation Grant IS23074
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Title: Statistical structure of marmoset vocal acoustics and adaptive coding in the auditory cortex

Authors: *E. X. HAN[1], Y. XU[1], Z. PAN[2], J. TSUNADA[1];
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Abstract: Exploring the adaptation of the brain to the statistical properties of natural sensory inputs is crucial for understanding the evolutionary shaping of sensory systems and for the development of bio-inspired algorithms in artificial intelligence. In group-living primates, species-specific vocalizations play a pivotal role in communication, and the auditory cortex may have adapted to process these sounds efficiently. While previous research has demonstrated that the marmoset monkey auditory cortex exhibits both temporally synchronized responses (temporal coding) and non-synchronized rate-coding responses to time-varying auditory stimuli, the optimization of these coding strategies for processing the statistical characteristics of monkey vocalizations has yet to be elucidated. Our study first involved a detailed analysis of the structure and acoustic properties of marmoset vocalizations recorded in a colony setting. We focused on three primary types of social calls—Phee, Trillphee, and Trill—and measured key parameters such as fundamental frequency (f0), f0 contour, modulation frequency and depth, and frequency modulation (FM) transition time. Utilizing these metrics, we synthesized vocalizations that either conformed to or deviated from the natural parameter range. Subsequently, we investigated how these synthesized sounds are represented in the auditory cortex. Our preliminary analysis revealed that neurons showing synchronized response to FM-rich trill calls depended upon the modulation frequency and depth, as well as f0 contour, with no clear preference for acoustics in the natural range. Consistent with the opponent model, rate-coding neurons typically exhibited monotonically changing firing rates depending upon modulation frequency. However, a subset of neurons showed specificity for acoustic features falling within the natural parameter range. These findings suggest that the marmoset auditory cortex employs specific adaptations to process auditory features in their species-specific vocalizations.

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Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

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Program #/Poster #: PSTR403.04/F34

Topic: D.05. Auditory and Vestibular Systems

Support: NIH NIBIB U24EB028998

Title: Identifying cell-type-specific alterations underlying schizophrenia-related deficits in auditory steady-state response and aperiodic spectral activity: insights from a multiscale model of auditory thalamocortical circuits

Authors: *S. MCELROY[1], S. A. NEYMOTIN[2], D. C. JAVITT[2], D. D'SOUZA[3,5], R. RADHAKRISHNAN[4,5], C. METZNER[6], S. DURA-BERNAL[7];
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Abstract: Individuals with schizophrenia exhibit a variety of symptoms categorized as positive, negative, and cognitive. A cognitive symptom extensively studied using electroencephalography (EEG), is sensory processing deficits, particularly in the auditory system. These deficits manifest as abnormalities in event-related potentials and cortical oscillations. In particular, this work focuses on the reduced 40 Hz Auditory Steady State Response (ASSR) and alterations in spontaneous 1/f slope, which are thought to reflect impaired inhibitory interneuron activity and gamma oscillations. We have extended our previously developed model of auditory thalamocortical circuits to better reproduce and investigate the source of these schizophrenia-related EEG biomarkers. This model simulates a cortical column containing over 12k neurons and 30M synapses. Neuron densities, laminar locations, morphology and biophysics, and connectivity at the long-range, local, and dendritic scale were derived from published experimental data. Auditory stimulus-related inputs to the thalamus were simulated using a phenomenological model of the cochlea. The model reproduced in vivo cell type and layer-specific firing rates, local field potentials, and EEG signals consistent with controls. Changes made to the model to reproduce schizophrenia patient EEG biomarkers were informed using data from positron emission tomography imaging, genetics, and transcriptomics specific to schizophrenia patients. Specifically, we have employed experimental findings such as layer-specific reductions in somatostatin (SST) and parvalbumin (PV) expression in interneurons, reduced NMDA efficacy at synapses on PV interneurons, and feedforward circuit-specific connectivity changes to explore mechanistic explanations for EEG biomarkers of schizophrenia. We found that disturbances of PV, SST, and NMDA in isolation affected firing rates in a layer- and cell-type-specific way, significantly altering superficial and deep layers. Furthermore, in EEG recordings, they altered the 1/f slope, with differential effects in lower frequencies compared to gamma oscillations. Application of these changes in concert resulted in decreased gamma band activity in response to 40 Hz click trains compared to control, consistent with experimental ASSR results in schizophrenia patients. This work aims to bridge the gap between experimentally determined molecular and genetic changes associated with schizophrenia and the resulting circuit and network behavior that give rise to robust EEG biomarkers.

Disclosures: S. McElroy: None.

Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.05/F35

Topic: D.05. Auditory and Vestibular Systems

Support: NIDCD Grant # R01DC013073
NIDCD Grant # R01DC016599

Title: The spectrotemporal features of sound are mapped through a novel cellular organization on the dorsal cortex of the mouse inferior colliculus.

Authors: *B. IBRAHIM, A. DOUGLAS, Y. SHINAGAWA, G. XIAO, A. R. ASILADOR, D. LLANO;
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Abstract: The inferior colliculus (IC) is an information processing hub that receives widespread convergent auditory projections. While the dorsal cortex (DC) - the non-lemniscal division of the IC- receives major auditory cortical projections, some reports showed that the DC is a tonotopic structure, which indicates the structure's ability to integrate the basic spectral features of sound to process the complex auditory information. However, it is unclear if the DC has another level of mapping to integrate the different spectral and temporal features of complex sounds across different sound levels. Therefore, the two-photon imaging of the calcium signals was used to track the neuronal response of the DC to sounds of different degrees of spectral and temporal complexity such as pure tones (PT), unmodulated noise (UN), and amplitude modulated noise (AMN). In addition to the tonotopic map, the DC showed a periodotopic organization whereby the cells of a medial rostrocaudal area were best tuned to UN separating medial and lateral regions where the cells were best tuned to AMN. Analyzing the neuronal response to each tested sound was used to generate spectral and temporal indices for each neuron, which were then used to map the DC based on the dynamics of the neuronal responses across different sound amplitudes. The DC showed a cellular organization that mapped the DC surface into two main regions: dorsomedial (DMC) and dorsolateral (DLC) cortices. At the lowest tested sound level (40 dB SPL), the DMC was more responsive to simple tones (i.e. PT) and less responsive to complex sounds (i.e. UN and AMN) compared to the DLC. Although increasing the sound level increased the percentage of responsive cells in both DMC and DLC, it dynamically modulated the cells of the DMC to be more responsive mostly to UN without changing the response profile of the DLC. These maps were consistent across males and females at different estrous phases. These data suggest that the DC is mapped to process the different spectrotemporal features of sound based on the sound intensity to enhance the segregation of different sound sources.

Disclosures: B. Ibrahim: None. **A. Douglas:** None. **Y. Shinagawa:** None. **G. Xiao:** None. **A.R. Asilador:** None. **D. Llano:** None.

Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.06/F36

Topic: D.05. Auditory and Vestibular Systems

Support: Department of Science and Technology (DST) Ministry of Science and Technology, India Grant DST/INT/CZ/P-04/2020 and DST/CSRI/2021/340

Title: Processing of regular and irregular sound streams in the mouse auditory cortex.

Authors: *A. MICHEAL[1], S. BANDYOPADHYAY[2];
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Abstract: Our capability of discriminating between patterned and irregular sound sequences is derived from the sensitivity of auditory neurons to regularity and violations of regularity. We use multiple regular and irregular sound streams to ask if neurons are inherently selective to one over the other and if so, do they generalize to different such patterns? We record the responses of single units in the mouse auditory cortex (ACX) to the above sets of periodic and aperiodic sound streams (PER and APR) to answer the questions above. To understand the role of local micro-networks of neurons in the discrimination of PER and APR sounds, we performed chronic 2-photon Ca^{2+} imaging of single Thy-1+ excitatory neurons in the ACX of awake mice. Sound sequences with 30 ms tokens, F1 or F2 (2 different tones) were used. The sequences either had a period of 2, 3 or 4 segments. Each segment consisted of 1 token (F1/F2) followed by a silent gap of 70 ms. We used another set of stimuli, with periodicity 3 as above, with 4 different gaps following each token - 70, 120, 170 or 270 ms. For each set of stimuli with a certain gap, we used 2 PER and 2 APR stimuli. Data from Layer II/III ACX single-units (n=307, 5 mice) and from 1885 significant responsive neurons out of 2,319 neurons imaged using 2-photon Ca^{2+} imaging in awake mice, n=3, over multiple days). Approximately 40% of neurons are selective to either PER or to APR when we consider responses of the entire sequence, including the silent gap periods. The proportion of such units is significantly reduced when not considering the response of the entire stream, using only the response in sound tokens' duration. The above suggests that the stream as a whole is represented in single neurons as opposed to the combination of the distinct tokens. In terms of generalization, we however find that there is very little overlap in the populations of neurons selective to PER or APR stimuli of the different types - periodicity 2, 3 or 4, and gaps 70, 120, 170 or 270 ms. Thus the neurons are intrinsically not selective to PER or APR as 2 classes and do not generalize. On analysing responses over successive segments cumulatively, we find that PER selective units adapted to APR stimuli over the duration of the stimulus while they either did not adapt or adapted ~30-50% less for PER stimuli. Similarly, APR selective neurons showed the opposite behaviour. The degree of difference between the responses to the 2 types of stimuli by each set of neurons increased with increasing gap. Thus our results show how the regular or irregular structures in auditory stimuli get integrated differentially by different populations of neurons and could underlie the formation of percept of auditory object streams.

Disclosures: A. Micheal: None. **S. Bandyopadhyay:** None.

Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.07/F37

Topic: D.05. Auditory and Vestibular Systems

Title: Effects of apomorphine and MK801 on different aspects of information processing in the three-tone auditory oddball paradigm

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Abstract: Introduction In our environment only a few of the sensory stimuli are behaviorally relevant. This distinction between relevant and irrelevant information is impaired in certain neuropsychiatric disorders characterized by disturbed information processing as schizophrenia. Injection of the dopamine receptor agonist apomorphine and the glutamate NMDA receptor antagonist MK801 is used experimentally as model for these disorders in rats. In this study, we investigated the effect of apomorphine and MK801 on behavior in the auditory three-tone oddball paradigm, which allows to investigate the processing of behaviorally relevant auditory events.
Mehtods Male Sprague-Dawley rats (n=11) were trained in the auditory three-tone oddball paradigm, in which they had to respond by nose poking to a rare target tone (5000 Hz, rewarded with a casein pellet), while ignoring a rare distractor (1500 Hz) and frequent standard tone (3000 Hz). After reaching a pre-defined success criterion of correct response to the target tone and correct rejection of the standard and distractor tones (80%, each), rats were injected with different doses of either apomorphine (vehicle, 0.0625, 0.125 and 0.250 mg/kg) or MK801 (vehicle, 0.05, 0.1, and 0.15, and 0.2mg/kg) and then behaviorally tested in the oddball paradigm.
Results Both, apomorphine and MK801 impaired performance in a dose-dependent manner. After apomorphine, rats stopped responding to all stimuli, resulting in a reduced hit rate to the target tone, combined with ignoring both standard and distractor tones ($p<0.05$). In contrast, rats injected with low doses of MK801 still responded correctly to the target tone but also made more false responses to the distractor and standard tones, which was combined with more impulsive hits in the inter-trial intervals ($p<0.05$).
Conclusion Although both neuroactive compounds impair performance in the oddball paradigm, low doses of dopamine receptor agonists reduce responses to all stimuli, whereas NMDA receptor antagonists enhance false responses to standard and distractor tones. Together, apomorphine and MK801 address different aspects of disturbed information processing seen in certain neuropsychiatric disorder.

Disclosures: M. Oelerich: None. **F. Decker:** None. **J. Krauss:** None. **K. Schwabe:** None.

Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.08/G1

Topic: D.05. Auditory and Vestibular Systems

Support: Simons Foundation Society of Fellows - Junior Fellow Award 965382
NIH Grant 2U19NS107616

Title: Longitudinal monitoring of developmental plasticity in the mouse auditory cortex

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Abstract: The postnatally developing brain undergoes tremendous structural as well as functional changes, including in how individual and populations of neurons in the cortex respond to environmental stimuli (Katz and Shatz Science 1996; Froemke Annu Rev Neurosci 2015). Such developmental changes in sensory coding have been difficult to measure and quantify due to challenges in applying *in vivo* recording methods to small and physically growing brains. Here, we present results from longitudinal two-photon calcium imaging of hundreds of excitatory layer 2-4 neurons in the developing primary auditory cortex of mouse pups (N=9, mean=351 neurons per animal), starting from just after ear-opening at postnatal day 12 (P12) into adulthood (up to P60) in the same animals. Methods were adapted from Che et al. (Neuron 2018). Auditory cortical neurons transition from highly-correlated, sound-independent activity patterns to decorrelated, sound-evoked activity in response to pure sine-wave tones, as well as frequency-modulated sweeps, at P13-14. We tracked individual neurons over days (minimum: 9 days) to weeks (maximum: 7 weeks) of postnatal development. We found that superficial cortical neurons initially have a clear tonotopic organization, but that best frequencies can shift considerably over days. This plasticity did not seem random, but instead predominantly progressed towards higher frequency representations in many neurons, as the tonotopic map expanded to encompass a broader range of frequencies in our imaging fields of view. Finally, we observed that neuronal responses to playbacks of ultrasonic pup vocalizations (USVs) typically emerged a few days after auditory response onset, at P16-18. These responses were initially correlated with high-frequency spectral tuning, which contrasts with the organization of vocalization responses in the adult auditory cortex (Galindo-Leon et al. Neuron 2009; Marlin et al. Nature 2015). USV responses then seemed to disperse across the whole of the imaging field, so that local best frequencies were uncorrelated with vocalization responses in older animals. Altogether, these data reveal how single-cell sensory-evoked activity in the auditory cortex - including to a spectrotemporally complex and naturalistic stimulus, USVs - emerges and changes across postnatal development.

Disclosures: M.A. Kirchgessner: None. **M. Vaze:** None. **R.C. Froemke:** None.

Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.09/G2

Topic: D.05. Auditory and Vestibular Systems

Support: NIH R01

Title: Information-theoretic analysis on cross-species EEG signals reveals consistent features within neural oscillations in response to temporally varying sounds.

Authors: *A. MUKESH[1], V. M. ATHREYA[1], M. PATRA[2], M. G. HEINZ[3];
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Abstract: Hearing loss affects ~15% of American adults, with age being the strongest predictor of sensorineural hearing loss (SNHL); however, the neural correlates of the perceptual deficits caused by SNHL remain unclear. Previous work has shown that sensitivity to spectro-temporal modulation is predictive of speech-in-noise intelligibility. However, the underlying neural basis of this reduced sensitivity and impairment is under-studied because of the challenges in relating neural coding deficits in pre-clinical SNHL animal models and perception in human listeners with hearing loss. We performed 32-channel EEG recordings in both humans and chinchillas and evaluated the correspondence between the response properties of both species. Chinchillas are known to be able to detect short-duration gaps behaviorally. Hence, we used a gap detection stimulus paradigm to investigate how varying gap values are encoded in multi-channel EEG responses. In addition to this we also used a series of coherent and incoherent tonal frequencies that can be used to study auditory object perception. We used young normal hearing and moderately noise-exposed chinchillas to study how neural representation is impaired in the case of temporary-threshold-shift induced cochlear synaptopathy. We used information theoretic tools to quantify the degree of encoding in various bands in the EEG signal in response to changing gap values and varying spectro-temporal information Our analyses revealed that the change in spectral and temporal content of stimuli is reflected in specific bands of the EEG signal. We also found the presence of synergistic and redundant interplay between the energy content of different bands. Furthermore, we found that certain neural response features that encode temporal information are dynamic in long-time scales. By comparing our results across chinchilla and human, we found certain measures of dependence that are consistent across species. Overall, our study represents an early attempt to draw similarities between the neural encoding of perceptually relevant spectro-temporal modulated stimuli in human and chinchillas through

information theoretic measures and to use this correspondence to expand the use of the chinchilla animal model as a useful platform to study SNHL.

Disclosures: A. Mukesh: None. **V.M. Athreya:** None. **M. Patra:** None. **M.G. Heinz:** None.

Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: MCP Hall A

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Program #/Poster #: PSTR403.10/G3

Topic: D.05. Auditory and Vestibular Systems

Support: NRF Grant 2022R1A2C3008991

Title: Emergence of functional maps from neuronal interference in auditory cortex

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Abstract: Pitch perception is a fundamental auditory function for processing natural sounds, such as vocal speech and musical tones (McPherson, 2018). These natural sounds are harmonic, which have periodic waveforms. At the spectral level, harmonic sounds are decomposed into integer multiples of fundamental frequencies by cochlear mechanical filtering. Interestingly, the auditory systems of humans and animals consistently perceive harmonic sounds as a single tone, a phenomenon often referred to as pitch perception. However, how the brain extracts the periodicity of harmonic sound remains unclear. Recent studies have reported that the brain utilizes a periodotopic map, a spatially graduated tuning of periodicity, to extract pitch from a sound (Langner, 2009; Baumann, 2011; Barton, 2012). It raises a question that how periodotopic map develops within the tonotopically arranged auditory pathway. Here, we propose that a periodotopic map can develop spontaneously from the neuronal interference between tonotopic maps from the ipsilateral and contralateral auditory pathways. We focused that the periodotopic map appears behind anatomical chiasms at the brainstem, such as the inferior colliculus and primary auditory cortex (A1). We assumed that bilateral tonotopic maps crosses with tilting, which can generate wiring of two areas with different characteristic frequencies. The superposition of two waveforms with different frequencies generates interference, which modulates the periodicity of the envelope. We simulated the chiasm of tonotopic maps with tilting and calculated the carrier and beat frequency at each position. We modeled the tonotopic map using carrier frequency, and the periodotopic map using beat frequency. We observed that the emerged periodotopic map shows a wide range of periodicity coding, while the tonotopic map maintains the frequency gradient. Next, to support our model, we also analyzed tonotopic and periodotopic maps from the optical recording data of the A1 in cats (Langner, 2009). Our simulated model shows a significant two-dimensional correlation compared to the maps in A1,

both tonotopy and periodotopy. Specifically, our model predicts that the high-pitch area, which is achieved by wiring a large frequency difference, should prefer high frequencies in the tonotopic map. We validated this tendency in map data of A1 as predicted in model maps. Overall, these results suggest a scenario of how our brain consistently extracts pitch from spectrally decomposed tonotopic representation. Moreover, our model can explain how a periodotopic map simply develops through interference within a tonotopically organized auditory pathway.

Disclosures: J. Cheon: None. **S. Paik:** None.

Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.11/G4

Topic: D.05. Auditory and Vestibular Systems

Support: DFG Grant 453200017

Title: Neurons of the intermediate nucleus of the lateral lemniscus build a vast molecular and biophysical continuum breaking with tonotopy for cross-frequency integration

Authors: *K. D. WICKE[1], N. KLADISIOS[1], K. KATTLER-LACKES[2], F. FELMY[1];
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Abstract: In the auditory brainstem pathways sound frequencies are mainly represented tonotopically. However, strict frequency channels limit the available information and cross-frequency integration is considered to extract crucial information about the acoustic environment. Neurons in the intermediate nucleus of the lateral lemniscus (INLL) have been suggested to be involved in early cross-frequency integration, but their biophysical, synaptic and morphological features remain largely uncharacterized so far. We use *in-vivo* single unit recordings to detect frequency integration. The biophysical characteristics, synaptic inputs and morphological features were extracted via *in-vitro* whole cell recordings and transcriptomic expression patterns were identified from PatchSeq samples of preciously recorded INLL neurons. When presented with pure and paired tones *in-vivo*, the large majority of INLL neurons show a shift in their frequency tuning, demonstrating their ability to integrate information between frequency-channels. On the cellular level, the population of INLL neurons build a large continuum of membrane time constants (τ_{mem}) ranging from a sub-milliseconds to more than 100 ms. These differences in τ_{mem} translate to correlating active features, such as the temporal properties of action potential generation and firing pattern. Furthermore, the decay time and paired pulse ratio of synaptic inputs match the biophysical phenotype of the postsynaptic cell, and in turn further enhance the dynamic range of integrational properties. The continuum of biophysical phenotypes does not exhibit an organisation along a spacial map. Moreover, the

dendritic structure and cell soma size do not correlate with τ_{mem}. Thus, cell location and morphology are no determinants of the biophysical behaviour of INLL neurons. The vast heterogeneity is driven by gradual and overlapping differences in gene expression patterns following the continuum. Furthermore the transcriptomic data reveals a small subpopulation of inhibitory neurons spread across the continuum. This inhibitory subpopulation might indicate an interneuronal network within the INLL that could establish a population based coding strategy. Taken together our data shows that the INLL is composed of a heterogeneous neuronal population that breaks with the strict tonotopic organisational principle of the auditory brainstem. This population rather provides a substrate of continuous temporal integration times, serving as a temporal filterbank across the range of perceived frequencies.

Disclosures: K.D. Wicke: A. Employment/Salary (full or part-time):; Institute of Zoology, University of Veterinary Medicine, Hanover. **N. Kladisios:** A. Employment/Salary (full or part-time):; Institute of Zoology, University of Veterinary Medicine, Hanover. **K. Kattler-Lackes:** A. Employment/Salary (full or part-time):; Department of Genetics/Epigenetics, Saarland University. **F. Felmy:** A. Employment/Salary (full or part-time):; Institute of Zoology, University of Veterinary Medicine, Hanover.

Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.12/G5

Topic: D.05. Auditory and Vestibular Systems

Title: Eeg-based source localization of the neural response at the fundamental frequency of speech

Authors: *J. AUERNHEIMER, T. REICHENBACH;
Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

Abstract: Neural processing of auditory stimuli such as pure tones and vowels can occur at processing rates up to a few hundred Hertz due to phase-locking of neurons to specific stimulus frequencies, a phenomenon termed frequency-following response (FFR). In natural speech, voiced parts oscillate at the speaker's fundamental frequency (f0) and higher harmonics. This harmonic structure elicits a neural response at the fundamental frequency (speech-FFR) which can be measured from noninvasive electroencephalography (EEG) or magnetoencephalography (MEG). While studies with EEG have found a major subcortical origin of the speech-FFR, studies with MEG have recently emphasized cortical involvement as well. Here we investigated how the different neural contributions to the speech-FFR can be disentangled using source reconstruction on a template MRI with EEG data. We therefore analysed EEG recordings from 13 subjects who listened to audiobooks read by a single male speaker. Two stimuli were

extracted from the speech signal, 1) the carrier signal filtered around the fundamental frequency and 2) the high gamma (75 - 150 Hertz) modulation in the envelopes of higher harmonics. For the second stimulus, we used three different processing strategies to compute the high-frequency spectra: A gammatone filter bank to approximate cochlear filtering, a group of band-pass filters with constant bandwidth and an inner ear model reflecting early stages of auditory peripheral processing. We subsequently used ridge regression to fit the EEG to both features simultaneously and applied inverse source analysis on the resulting coefficients. For all methodologies, the band-pass, gammatone and inner ear model, source localization yielded major midbrain activity to both features with weaker brainstem contributions. For the band-pass and inner ear model, later cortical contributions were identified as well, both for the fundamental waveform and the envelope modulations. Interestingly, the envelope modulations in the band-pass and inner ear model exhibited higher spectral power at lower frequencies consistent with the lower phase-locking limit of cortical neurons. Our results show that both subcortical and cortical sources contribute to the EEG-measured speech-FFR. However, the response is still dominated by subcortical activity from the midbrain and brainstem.

Disclosures: J. Auernheimer: None. **T. Reichenbach:** None.

Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.13/G6

Topic: D.05. Auditory and Vestibular Systems

Support: DC017078

Title: Thalamic encoding of temporal patterns

Authors: *W. YANG, D. B. POLLEY;
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Abstract: Neurons in the central auditory pathway offer temporal processing *par excellence* in the brain. The intrinsic, synaptic, and circuit specializations that support high-fidelity, high-speed feature extraction in subcortical auditory centers have been extensively characterized. The other end of the temporal processing spectrum - encoding slowly varying temporal features - is an essential building block for the perception of important features in speech (e.g., prosody), music (e.g., rhythm), and segregation of auditory foreground objects from background sounds but has received far less attention. A recent study from our lab demonstrated an inverse hierarchy for specialized processing of rapid and slowly modulated sound features (Asokan et al., Curr. Biol 2021). This study identified a tradeoff between the inferior colliculus (IC) and primary auditory cortex (A1), with the IC offering excellent resolution of local features and poor sensitivity to

slowly emerging features and A1 offering the opposite specialization. Recordings from the ventral subdivision of the medial geniculate body (MGBv) were somewhere in between, offering weak sensitivity to slow rhythmic patterns and middling resolution of rapidly modulated features. Here, we have revisited temporal processing in the auditory thalamus with an expanded emphasis on higher-order thalamic subdivisions, which are known to be the among the first sites of time-to-rate conversions for encoding envelope modulation rate and could therefore feature sensitivity to slowly emerging temporal patterns the matches or even exceeds the auditory cortex (Bartlett and Wang, J. Neurophys 2011). We performed simultaneous recordings from single units in the MGBv and higher-order auditory thalamus in unanesthetized head-fixed mice while presenting noise bursts separated by inter-burst intervals (IBIs) at different lengths to generate burst trains, we found that thalamic neurons exploited different encoding strategies to represent burst trains with short or long IBIs. Moreover, by arranging the IBIs separating consecutive noise bursts randomly or in a pattern (i.e., a rhythm), we revealed the activity of thalamic neurons emerged during the establishment of temporal patterns. Our findings open a door for further investigating the function of the auditory thalamus in processing complex temporal patterns.

Disclosures: W. Yang: None. **D.B. Polley:** None.

Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: MCP Hall A

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Program #/Poster #: PSTR403.14/G7

Topic: D.05. Auditory and Vestibular Systems

Support: NIH Grant DC003180
Kavli NDI distinguished postdoc fellowship

Title: Tonotopic organization of auditory cortex in awake marmosets revealed by multi-modal wide-field optical imaging

Authors: *X. SONG[1], Y. GUO[2], C. CHEN[3], J. LEE[4], X. WANG[5];
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Abstract: Tonotopic organization of the auditory cortex has been extensively studied in many mammalian species using various methodologies and physiological preparations. Tonotopy mapping in primates, however, is more limited due to constraints such as cortical folding, use of anesthetized subjects, and mapping methodology. Here we applied a combination of through-skull and through-window intrinsic optical signal imaging, wide-field calcium imaging, and neural probe recording techniques in awake marmosets (*Callithrix jacchus*), a New World

monkey with most of its auditory cortex located on a flat brain surface. Coarse tonotopic gradients, including a recently described rostral-temporal (RT) to parabelt gradient, were revealed by the through-skull imaging of intrinsic optical signals and were subsequently validated by single-unit recording. Furthermore, these tonotopic gradients were observed with more detail through chronically implanted cranial windows with additional verifications on the experimental design. Moreover, the tonotopy mapped by the intrinsic-signal imaging methods was verified by wide-field calcium imaging in an AAV-GCaMP labeled subject. After these validations and with further effort to expand the field of view more rostrally in both windowed and through-skull subjects, an additional putative tonotopic gradient was observed more rostrally to the area RT, which has not been previously described by the standard model of tonotopic organization of the primate auditory cortex. Together, these results provide the most comprehensive data of tonotopy mapping in an awake primate species with unprecedented coverage and details in the rostral proportion and support a caudal-rostrally arranged mesoscale organization of at least three repeats of functional gradients in the primate auditory cortex, similar to the ventral stream of primate visual cortex.

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Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: MCP Hall A

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Program #/Poster #: PSTR403.15/G8

Topic: D.05. Auditory and Vestibular Systems

Title: Steady-state response deficits to multimodal sensory stimuli in juvenile myoclonic epilepsy; a magnetoencephalography study

Authors: *R. MITOMA[1], S. TAMURA[2], T. MITSUDO[3], Y. SUNG[4], T. MUKAINO[5], K. MATSUMOTO[6], H. SHIGETO[5,7], Y. HIRANO[2,8,9], S. HIRANO[8], N. ISOBE[5], T. NAKAO[8]; [1]Dept. of Neuropsychiatry, Kyushu University: Kyushu Daigaku, Fukuoka, Japan; [2]Psychiatry, Univ. of Miyazaki, Miyazaki, Japan; [3]Div. of Clin. Res., Hizen Psychiatric Ctr., Saga, Japan; [4]Hlth. Sci., [5]Neurol., [7]Div. of Med. Technol., [8]Neuropsychiatry, [6]Kyushu Univ., Fukuoka, Japan; [9]Inst. of Industrial Sci., The Univ. of Tokyo, Tokyo, Japan

Abstract: Repetitive sensory tasks with modality-specific frequency stimuli elicit neural oscillatory activity, referred to as the steady-state response <SSR>, within the sensory cortex. The SSR is supposed to reflect sensory integration sensitivity, tied to the excitatory/inhibitory balance of neural networks, and is anticipated as a potential neurophysiological biomarker for aberrant cognitive processes in neuropsychiatric disorders. Despite the acknowledged presence of cognitive deficits in epilepsy, the literature on SSR in epilepsy remains limited compared to that on psychiatric disorders. Our study is the first to examine SSR in juvenile myoclonic

epilepsy <JME>, the most typical type of genetic generalized epilepsy characterized by distinctive seizures and cognitive dysfunction related to emotions and sensory perception. We measured 40 Hz auditory SSR <ASSR> and 20 Hz somatosensory SSR <SSSR> using a 306-channel magnetoencephalography in patients with JME and compared the evoked power and the degree of phase synchronization <phase locking factor: PLF> in each primary sensory cortex with age- and sex-matched healthy controls <HCs>. The ASSR task consisted of 40 Hz chirp sounds presented binaurally, while the SSSR task comprised 20 Hz vibrotactile stimuli applied to the right index finger pad. Each task was presented 150 times with a duration of 500 ms and an inter-trial interval of 600 ms. Analysis of data from 12 JME patients and 12 HCs indicated potential impairments in entrainments for both 40 Hz ASSR <evoked power: $p = .025$; PLF: $p = .027$> and 20 Hz SSSR <PLF: $p = .004$>. These findings would provide neurophysiological evidence of sensory processing impairments in JME. The aberrant SSR entrainments in both auditory and somatosensory domains suggest that the underlying cause may stem from common pathways in these sensory processing mechanisms. Given the structural and functional abnormalities of the thalamus, which serves as a communication hub in the sensory network, in JME, our results may signify thalamic dysfunction contributing to deficits in multimodal sensory processing. Furthermore, our findings also emphasize the importance of the thalamus in the unspecified mechanisms underlying SSR generation.

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Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.16/G9

Topic: D.05. Auditory and Vestibular Systems

Support: DoD CDMRP Hearing Restoration Research Program

Title: Perceptual Consequences of Noise-Induced Hidden Hearing Loss

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Abstract: Many individuals with clinically normal hearing thresholds nonetheless report significant hearing difficulties under real-world listening conditions, such as with speech perception in noisy environments. Recent animal studies have shown that aging or noise

exposure can cause permanent damage to auditory nerve fibers without affecting hearing thresholds. This "hidden" hearing loss (HHL) may explain struggles in noisy settings despite normal thresholds. However, the perceptual impact of HHL is not fully understood due to the challenges of diagnosing auditory nerve dysfunction in humans and the scarcity of relevant animal behavioral studies. To address this, we developed a rat model for noise-induced HHL. We used an operant conditioning task to assess the rats' ability to perceive tones in both quiet and noisy environments and detect gaps within a continuous sound. After training, the animals were exposed to 109 dB noise at frequencies of 8-16 kHz for two hours to induce HHL. We evaluated auditory brainstem responses (ABR) before exposure and one day, two weeks, and six weeks post-exposure, using wave I as an indicator of neural output from the periphery to the central nervous system. At one day post-exposure, ABR thresholds increased and wave I amplitudes were significantly decreased. While ABR thresholds returned to pre-exposure levels by 2 weeks, wave I amplitudes remained lower for up to 6 weeks post-exposure, consistent with prolonged peripheral damage in the absence permanent thresholds shifts that is associated with HHL. Interestingly, this exposure did not affect tone detection thresholds in quiet or noisy conditions. However, thresholds for gap detection were significantly worsened, suggesting that HHL may impact perception of time-related signals that are crucial for speech perception and communication. Our results indicate that disrupted temporal processing is a mechanism by which HHL effects real-world hearing abilities, providing new potential diagnostic tools and insight for future protection and treatment strategies.

Disclosures: X. Liu: None. **N. James:** None. **Z. Sun:** None. **S. Kumar:** None. **B.D. Auerbach:** None.

Poster

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Auditory Processing: Temporal, Frequency, and Spectral Processing

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Program #/Poster #: PSTR403.17/G10

Topic: D.05. Auditory and Vestibular Systems

Support: Department of Defense HRRP W81XWH-21-1-0602 (ELB, AP)

Title: Short-term longitudinal changes in peripheral and subcortical auditory processes in response to small arms fire-like noise exposure

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Abstract: Auditory thresholds, distortion product otoacoustic emissions (DPOAEs), and auditory brainstem responses (ABRs) are widely used to assess hearing loss in patients, whether trauma, age, or noise induced. Their broad application often leads to a one-size-fits-all treatment approach, primarily addressing loss of hearing sensitivity (increased thresholds). While these

methods are helpful, determining underlying causes of a patient's hearing loss is crucial for selecting optimal treatments for each individual. For example, blast exposure, aging, and noise exposure all result in increased thresholds, yet are likely to possess distinct diagnostic profiles. In 2019, Altschuler et al. identified threshold changes along with persistent damage to outer hair cells (OHCs) and a reduction in cochlear synapses following exposure to noise resembling small arms fire (SAF). However, little is known about how this type of damage influences early (<10 weeks post-exposure) longitudinal adaptations of peripheral and subcortical pathways. During these early periods, dynamic, region-specific changes in gain may occur that change diagnostic profiles. To evaluate peripheral (cochlear/auditory nerve) versus central changes, ratios of ABR waves can be compared (including wave 1/wave 5 (W1/W5)), which may demonstrate shifting gain in different portions of the subcortical auditory pathways even as thresholds recover. The purpose of this study is to reevaluate traditional diagnostic methods in the context of SAF to identify biomarkers and suggest mechanisms of progressive stages of noise induced hearing loss (NIHL). We exposed rodent subjects to moderate SAF noise (50 biphasic 0.3 ms pulses at 120 dB peak SPL, 1 every 3 s) and analyzed thresholds, DPOAEs, and ABRs over 8 weeks to map longitudinal changes and identify characteristics most sensitive to SAF NIHL. Following SAF noise exposure, DPOAEs demonstrated a persistent decrease in OHC function (greatest sensitivity at 8 kHz). Thresholds increased approx. 20 dB before declining to a 10 dB increase, though this was not accompanied by DPOAE recovery. The ABR amplitudes of W1 and W5 decreased after exposure. W1 decreased to a lesser extent, which is reflected in a temporary increase in W1/W5, contradicting central gain as a resulting adaptation and further adding evidence of a distinct diagnostic profile for SAF NIHL. Interestingly, we observed 20-40 dB change in the sound level needed to generate "equivalent" waveform amplitudes on post-exposure days compared to baseline. Future work involves imaging cochlear and brain tissues to determine anatomical correlations to the reported functional changes.

Disclosures: M. Ziliak: None. **E. Bell:** None. **A. Navarro:** None. **E.L. Bartlett:** None.

Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

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Topic: D.05. Auditory and Vestibular Systems

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Title: Humans detect rising and falling pitch using spectrotemporal correlations and opponency

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Abstract: Motion detection is fundamental to a wide range of adaptive behaviors. Critically, motion detection is not just a visual function - it is also performed by other sensory systems. For example, in audition, motion is detected both spatially and in the frequency (spectral) domain. Our study addresses two key questions about the auditory system's ability to perceive spectral motion. First, at the algorithmic level, do we perform similar computations for detecting pitch motion as we do for visual motion? And second, do frequency-tuned neural populations perform computationally analogous motion detection operations as visual regions, such as MT? To answer these questions, we adapted a stimulus used to study visual motion detection to develop new correlated noise auditory stimuli that possess no persistent features across frequency or time but do possess either positive or negative spectrotemporal correlations at specific offsets in frequency and time. Across multiple psychophysics experiments, we found robust evidence that humans are sensitive to both positive and negative spectrotemporal correlations and use them to judge pitch direction. This novel result is directly analogous to illusory reverse-phi visual motion percepts, which have been reported across many species. We then turned to naturalistic stimuli - databases of English and Mandarin speech recordings - and found robust positive and negative correlation cues in those stimuli as well. Lastly, building on our psychophysical results, we reasoned that human auditory cortex might possess a localized region for performing opponent subtraction of rising versus falling pitch responses. An fMRI experiment supported this result, showing a region in the superior temporal gyrus that displayed MT-like signatures of opponency. Overall, our results point to a conserved correlation-sensitive algorithm for motion detection that persists across modalities (vision and audition) and dimensions (space and pitch).

Disclosures: P.A. Vaziri: None. **S.D. McDougle:** None. **D. Clark:** None.

Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.19/G12

Topic: D.05. Auditory and Vestibular Systems

Support: R01DC020097

Title: Predicting spectro-temporal selectivity and auditory midbrain responses to natural sounds using a spiking Gabor receptive field model with contrast adaptation

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Abstract: Spectro-temporal receptive fields (STRFs) are widely used in auditory neuroscience to model the time-frequency sensitivity of auditory neurons. Often, STRFs are derived using

unbiased synthetic stimuli, which can be estimated using spike-triggered averaging. When natural sounds are used, however, decorrelation and regularization techniques are needed to remove residual stimulus correlations that can distort the estimated STRFs. Furthermore, nonlinearities and non-stationarities (such as adaptation) make it difficult to predict neural responses to natural sounds.
We obtained neural recordings from the inferior colliculus of unanesthetized rabbits in response to natural sounds, dynamic moving ripples, and speech in varying background noises. We developed a model-based approach for deriving auditory STRFs and predicting single trial spike trains to these sounds. The model consists of a nine parameter Gabor STRF (gSTRF; Qiu et al. 2003), which accounts for the neuron's spectro-temporal integration and a nine parameter contrast STRF which dynamically changes the threshold of the neuron. Additionally, a four-parameter nonlinear integrate-and- fire compartment incorporates intrinsic noise, cell membrane integration, and nonlinear thresholding to generate simulated output spikes and a four parameter gain control component accounts for adaptation of the neuron. We used Bayesian optimization to fit neural data and derive optimal model parameters by maximizing the model's log-likelihood. To validate our spiking gSTRF model, we compared optimal gSTRFs to more common approaches such as a generalized linear model. We found that STRFs derived via regression were spectrally smeared, indicating that stimulus correlations were not effectively removed, despite implementation of decorrelation techniques. In comparison, our gSTRF was compact and provided biologically feasible estimates of the parameters, such as the neuron's best frequency, delay, and best temporal and spectral modulation frequency. We also carried out comparisons with simulated data where the "ground truth" STRF and spiking activity were known a priori. For the simulations, we demonstrate our gSTRF converges to the original simulation parameters and replicates the spiking activity from the original simulations with a millisecond precision. Collectively, this new approach allows one to derive auditory STRFs and predict neural spiking activity to natural sounds using functionally interpretable basis functions. The small number of parameters make exploration of nonlinear and non-stationary effects due to natural sound statistics more feasible.

Disclosures: J. Blain: None. **M.A. Escabi:** None. **I.H. Stevenson:** None.

Poster

PSTR403

Auditory Processing: Temporal, Frequency, and Spectral Processing

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Topic: D.05. Auditory and Vestibular Systems

Support: NIH R01DC020097

Title: The influence of spectrum and modulation cues on the neural representation of vocalizations in natural background sounds

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Abstract: Humans and animals are challenged when real-world sounds occur in background noise. These scenarios also challenge the hearing impaired and speech recognition systems. Perceptual studies have shown how spectrum and modulation statistics of a background sound influence the perception of a foreground target, yet how the brain separates sound mixtures is poorly understood. We recorded multi-unit population activity from the inferior colliculus of head-fixed unanesthetized rabbits via linear 64-channel arrays. Speech sentences or zebra finch song motif foregrounds were presented in the presence of seven natural backgrounds at multiple signal-to-noise ratios (SNRs). These backgrounds included speech babble, bird babble and construction noise. The backgrounds were delivered in the original unmodified (OR) or the perturbed phase randomized (PR) or spectrum equalized (SE) conditions. PR preserves the original spectrum but distorts (whitens) the original modulations, whereas SE distorts the spectrum but not the modulations. Via a shuffled spectrum estimation, we separated the foreground- and background-driven neural responses for each sound mixture and condition (OR, PR and SE), which allowed us to separately compute the foreground- and background-driven power spectra. To assess the fidelity of neural encoding for each background and condition, we estimated the neural SNR by dividing the foreground by the background-driven spectra. Results show that neural SNRs depend on the background and its spectrum and modulations. Compared to original backgrounds, PR backgrounds enhance or reduce the neural SNR depending on the background, which implies that the original background modulations improve or hurt the foreground representation. Similarly, SE backgrounds enhance or reduce the neural SNR, suggesting that the original background spectra could beneficially or detrimentally impact foreground encoding. For some backgrounds, the spectrum dominates the neural SNR, while for others the modulations have a greater impact. We also demonstrate how spectrum or modulation interference is most prominent for modulation frequencies < 10 Hz, overlapping the temporal fluctuations for individual words or syllables in the song motif. Finally, preliminary comparisons to human perceptual data using the same backgrounds in speech recognition tasks suggest that neural SNR correlates with recognition accuracy. Collectively, the findings suggest that the spectra and modulations of backgrounds influence and interfere with the representation of foreground vocalizations, suggesting that these statistics critically underlie masking of real-world natural sounds.

Disclosures: J. Dion: None. **I.H. Stevenson:** None. **M.A. Escabi:** None.

Poster

PSTR404

Properties of Thalamic Nuclei in Visual Pathways

Location: MCP Hall A

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Topic: D.06. Vision

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Title: Unveiling the Diversity of Extracellular Signals and Functional responses in Awake Monkey LGN

Authors: *S. SUN[1,2], J. S. PEZARIS[1,2], N. J. KILLIAN[3];
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Abstract: The primate LGN, with its distinct divisions (magnocellular, M; parvocellular, P; and koniocellular, K), has been extensively characterized through electrophysiological recordings. While much of our understanding has relied on single-channel electrode recordings of extracellular spikes (or extracellular action potentials), recent advancements in dense multi-electrode arrays and spike-sorting algorithms have revealed a broader spectrum in the variation of spike waveform shapes reported across multiple species and brain regions that are atypical and poorly understood. Consequently, crucial populations may have been overlooked in previous studies, warranting a thorough exploration of LGN extracellular signals to help understand the LGN's role in the visual pathway's processing. Here, we present a comprehensive survey of the extracellular space in the LGN of rhesus macaques (n = 3), utilizing a variety of stimulus and electrode properties. For every LGN unit recorded (n = 303), we identified its receptive field (RF) class (M, 45%; P, 23%; or K, 7%) and extracellular spike waveform class (Narrow, 35%; Broad, 22%; Triphasic, 13%; or Positive, 30%), alongside various response metrics (spike-rate, response latency, etc.). We observed that each extracellular spike waveform class had distinguishable functional characteristics, indicating certain relationships between a cell's extracellular spike shape and possible neuronal type. We also observed a set of units without an estimated RF (non-RF, 25%) that are not regularly reported, with most of these units consistently responsive to the visually presented stimulus, although at a lower and more sustained response rate than units with an RF. The presence of non-RF cells hints that the LGN may require understanding beyond the classification into M, P, and K responses, and further insight into these nuances could reveal computations in the visual system that have yet to be discovered. Overall, these findings have significant implications for understanding early visual processing mechanisms and interpreting extracellular signals in neural circuits.

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Poster

PSTR404

Properties of Thalamic Nuclei in Visual Pathways

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.02/G15

Topic: D.06. Vision

Title: Inferior pulvinar projections to the amygdala in macaque monkeys.

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Abstract: Visual information flowing from the superior colliculus through the pulvinar to the amygdala is thought to mediate fast neural responses to threatening stimuli. The exact pathway of this information, and whether it is conserved across commonly studied animal models such as rodents and primates is not well understood. Previous reports suggest that only the medial pulvinar, a portion of the pulvinar often thought to be a primate specialization, projects to the amygdala in macaque monkeys. However, in non-primate species such as tree shrews and rodents, the main source of amygdala input is from a portion of the pulvinar that is homologous to the inferior pulvinar of primates. These findings suggest that either there was a major shift in this subcortical pathway to the amygdala during primate evolution or, alternatively, projections from homologous pulvinar structures in macaques have been overlooked. To address this issue, we placed anatomical tracer injections of cholera toxin subunit B (CTB) as well as dextran amines into the lateral amygdala of macaque monkeys. After tracer transport, tissue was processed to allow visualization of retrogradely labeled cells and stained for acetylcholine esterase (AChE) and vesicular glutamate transporter type 2 (VLGUT2) to reveal anatomical borders within the pulvinar. Tracer study results were aligned with anatomical borders. Retrogradely labeled cells were observed within the medial pulvinar as well as within the posterior inferior (PIp) and the central medial inferior (PIcm) pulvinar. These divisions of the pulvinar are known to receive projections from superficial layers of the superior colliculus and are thought to be homologous to the dorsal pulvinar of tree shrews, and the caudal pulvinar of rodents. Our findings reveal that macaques possess a subcortical visual pathway to the amygdala that is observed in rodents and tree shrews in addition to a projection from the medial pulvinar, which is likely unique to primates. Future studies should address whether one or both pathways serve as a source of fast unconscious visual input to the amygdala.

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Poster

PSTR404

Properties of Thalamic Nuclei in Visual Pathways

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Title: Resolving Single Cone Receptive Field Centers in Macaque LGN Neurons

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Abstract: There is ample evidence from retinal anatomy and human psychophysics that visual acuity is limited by cone photoreceptor size and spacing. This implies neurons in the retina and lateral geniculate nucleus (LGN) may have receptive field centers composed of only one cone for cells representing vision in and near the fovea. To date, such single cone receptive field centers have not been mapped directly. To identify these small fields, we compared measured receptive field sizes from spike-triggered averages (STA) to expected sizes from a light capture model (Meadway & Sincich 2018). We recorded extracellularly from 57 neurons in LGN parvocellular layers in 3 anesthetized macaques with receptive fields located 0.43°-4.1° from the fovea. An adaptive optics scanning laser ophthalmoscope was used with infrared (842±25 nm) light to image the retina, while red (711±12 nm) and green (543±11 nm) channels delivered binarized white noise movies. Each color was modulated independently over a 0.32° stimulus field. A movie pixel subtended 0.6 arcmin (~2-3 μm), just smaller than most cones. STAs were generated to map the receptive field of each cell and fit with an elliptical 2D Gaussian to estimate the size of the receptive field center. The signal-to-noise ratio (SNR) of each STA was computed from the center amplitude and the root-mean-square values of pixels outside the receptive field. Cone spacing was used to infer cone size. To compute cone spacing at each retinal recording site, we used radial averaging around a mean cone image generated from cones manually selected within 0.15° of the stimulus location. We used a Monte Carlo simulation of light capture to determine if the STA receptive field centers originated from 1, 2, or 3 cones. Simulation variables included placement of movies relative to the cone mosaic, delivery jitter, and STA noise level. Of the cells recorded, 34 had receptive fields fully within the movie stimuli and high enough SNR to be analyzed. We found that STA receptive field center diameters averaged 4.06±0.9 μm for cells responding best to red, and 4.38±1.2 μm for cells most responsive to green. We compared these diameters to the Monte Carlo simulations by measuring the Mahalanobis distance of each cell against the theoretical range of 1, 2, or 3 cone field sizes. To classify the STAs, we computed the silhouette coefficients for each comparison, and found that 22 cells (65%) matched the 1-cone simulation, 7 matched the 2-cone, and 5 matched the 3-cone. Our results suggest that high spatial frequency noise can be used to map single cone receptive field centers in LGN neurons, making these neurons capable of mediating visual acuity at the limit of cone photoreceptor spacing.

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Poster

PSTR404

Properties of Thalamic Nuclei in Visual Pathways

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Title: Functional Topography of the Murine Lateral Geniculate Nucleus

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Abstract: Visual stimuli are first received and processed in the retina and transmitted along the form vision pathway through the dorsal lateral geniculate nucleus of the thalamus (dLGN). Across species, there are regional differences in retina that are adapted for natural habitat and accompanying ecological pressure (Heukamp et al., 2020; Kerschensteiner, 2022). For example, most retinal ganglion cells (RGCs) in highly visual animals have stereotyped center-surround receptive fields that are smallest in the center of gaze, forming a zone of high acuity (e.g., fovea in primate, area centralis in carnivore), and that grow larger towards the periphery. Receptive field structure and the center-periphery gradient in size are preserved in dLGN. By contrast, murine retina differs from primate and carnivore in meaningful ways. Receptive field sizes do not scale with eccentricity, there are many more types of RGCs, and the distribution of these different cell classes varies asymmetrically across the dorsoventral and nasotemporal axes of retina.
Here, we ask how the nonuniform organization of distinct RGC types in mouse translates to dLGN (Piscopo et al., 2013). The answer is not straightforward because not all types of RGCs project to the form vision pathway, with the remainder favoring sensorimotor structures such as the superior colliculus or other non-image forming sites (Hammer et al., 2014; Kerschensteiner and Feller, 2024). Moreover, multiple RGCs converge on single cells in mouse dLGN, affording the emergence of novel response properties in thalamus. By using multichannel multishank electrodes, we are able to simultaneously record many cells at both local and remote sites across dLGN. Registration of the anatomical position of each recording site onto the Allen Mouse Brain Common Coordinate Framework provides a means to organize data across experiments.
We use our growing dataset to compare the visuotopic distribution of receptive field types and response properties in dLGN with that in retina to highlight features of particular importance to form vision in mouse, as well as commonalities with other species. At a finer grain, it is possible

to determine whether there are different functional groupings of relay cells at different retinotopic locations (e.g. the binocular zone) and, by means of cross-correlation analysis, if cells share common retina input. Future work will include companion maps for the two main sources of inhibition in dLGN, local interneurons and cells in the thalamic reticular nucleus. Thus, we will estimate functional topographies in visual thalamus and explore their potential roles in form vision.

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Poster

PSTR404

Properties of Thalamic Nuclei in Visual Pathways

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Title: Characterizing pulvinar neurons in the alert, fixating macaque

Authors: *H. CHEN, T. KIM, S. BEAUFRAND, A. K. PASUPATHY;
Dept. of Biol. Structure and Washington Natl. Primate Res. Ctr., Univ. of Washington, Seattle, WA

Abstract: Interactions between area V4, a midlevel visual form processing stage, and ventrolateral prefrontal cortex (vlPFC) maybe critical for attention-demanding vision tasks, such as the perception of the Dalmatian dog illusion. While V4 may interact with the vlPFC via the inferotemporal cortex, another possibility is that this communication is routed via the visual pulvinar (Pul) as a “bypass”. To facilitate simultaneous investigation of V4, vlPFC and Pul during an attention-demanding behavior, here we attempted an unconventional diagonal approach to the Pul, from a recording chamber optimized for targeting V4. This chamber (ID: 19 mm) was positioned to target the gyrus between the lunate sulcus (ls), superior temporal sulcus (sts) and the inferior occipital sulcus (ios), with an anterior tilt. This allows access to dorsal V4 between the ls and sts superficially, and Pul deep with a penetration at or anterior to the sts. Based on anatomical MRI scans, we anticipate reaching Pul ~15-20mm from the surface within the current chamber. In our pilot experiments, we characterized the responses of neurons in the Pul in an alert and fixating macaque with tungsten electrodes. During each recording session, we

first obtained a rough estimate of the receptive field (RF) of each neuron with mouse-controlled stimuli as the monkey fixated a white spot (diameter: 0.1°) within a 1° window. Then, centered on this RF-estimate, we conducted an automated RF mapping procedure by presenting a circular dynamic texture patch (diameter: 1.4°) on a grid of positions separated by 1°; stimuli were presented for 250 ms at each position separated by 250 ms inter-stimulus interval. Among 15 well-isolated neurons that displayed visual response (t-test, $p < 0.01$), 10 showed mappable RFs with a median response latency of 66.5ms. The RF centers were mostly in the contralateral lower visual field, with eccentricity ranging from 2.9° to 12.06°. RF diameter increased with eccentricity and was slightly larger than eccentricity, ranging from 4.01° to 11.62°. Current physiological evidence suggests that these neurons are in the Pul, although histology is needed for confirmation. As a next step, Neuropixels long probes will be adopted to access this area.

Disclosures: H. Chen: None. **T. Kim:** None. **S. Beaufrand:** None. **A.K. Pasupathy:** None.

Poster

PSTR404

Properties of Thalamic Nuclei in Visual Pathways

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.06/G19

Topic: D.06. Vision

Support: The E. Matilda Ziegler Foundation for the Blind

Title: Coordination of local and global responses in individual thalamic local interneurons

Authors: W. HU, K. WANG, C. LIU, Y. FEI, C. LI, ***L. LIANG**;
Neurosci., Yale Univ., New Haven, CT

Abstract: GABAergic synaptic inhibition plays critical roles in visual processing in the retina and visual cortex. However, the visual properties and function of GABAergic local interneurons in the primary visual thalamus, or the dorsolateral geniculate nucleus (dLGN), are not fully understood. These local interneurons extend their dendrites across a large portion of the dLGN and have both retinal input sites and GABA release sites throughout their dendrites. Yet, it remains unclear how distinct parts of dendrites within the same local interneuron may be functionally related and how they contribute to the overall responses of the neuron. Using chronic two-photon calcium imaging to record visual responses in dLGN local interneurons of awake head-restrained mice, we observed surprisingly diverse and often sharp visual responses in the somas of these interneurons. We developed an algorithm to automatically extract dendritic structures from recorded videos and classify them into individual neurons. This allowed us to compare visual response properties among dendrites belonging to the same neuron. We found that different dendrites within a single local interneuron showed different location preferences in a way that is consistent with their retinotopic locations. Moreover, each dendritic compartment displayed feature-selective responses that were coarsely consistent across the dendrites of the

same neuron and with the global response of the interneuron. These results demonstrated a high degree of functional consistency among local and global computation within individual local interneurons. Such coordination may allow local interneurons to coordinate feature-selective inhibition across the dLGN.

Disclosures: W. Hu: None. **K. Wang:** None. **C. Liu:** None. **Y. Fei:** None. **C. Li:** None. **L. Liang:** None.

Poster

PSTR404

Properties of Thalamic Nuclei in Visual Pathways

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Topic: D.06. Vision

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Wellcome Fundation 219561/Z/19/Z

Title: Visual cortex instructs learnt suppression of fear responses

Authors: *S. MEDEROS[1], P. BLAKELY[1], N. VISSERS[1], C. CLOPATH[2], S. B. HOFER[1]; [1]Univ. Col. London (UCL), London, United Kingdom; [2]Imperial Col. London, London, United Kingdom

Abstract: In a changing and uncertain world, choosing appropriate responses to environmental stimuli is central to adaptive behaviour. Our brains integrate sensory information with prior knowledge to guide our decisions and generate flexible behavioural outcomes. However, through which neural pathways prior experience can adapt behavioural responses is unclear. Avoidance behaviors in response to predators, specifically escaping from visual threats, are manifestations of crucial decision-making mechanisms vital for survival. The perceived threat level of environmental stimuli can change over time as animals acquire information about potential dangers in their environment and adapt their behavior. We hypothesized that visual cortex, in particular higher visual areas (HVAs), would be well-positioned to integrate sensory evidence, prior experience, and context to facilitate adaptive behavior. To test this, we investigated how mice learn to suppress fear responses to innate threats, and explored the role of HVAs in this learning process. We used a paradigm in which mice escape from an innately threatening visual stimulus, a dark overhead expanding disk. With repeated exposure, mice cease to escape as they learn that this stimulus does not pose danger. We find that posterolateral HVAs play a pivotal role in learning to suppress fear responses via a corticofugal pathway through the ventral lateral geniculate nucleus (vLGN). Inactivating either HVAs or their projections to vLGN during the learning process prevented suppression of fear responses, and mice continued to escape. Interestingly, inactivating HVAs after learning had no impact on behavior. Chronic

electrophysiological single-unit recordings revealed that vLGN neurons receiving input from HVAs increased their activity during learning. Importantly, these neurons were crucial for learning, and suppression of vLGN activity reversed the behavioral learning effect. This indicates that while HVAs instruct the learning process, the memory of the visual stimulus's threat level is likely encoded within specific vLGN populations. We investigated the synaptic mechanism underlying this memory formation and found that it involves endocannabinoid-mediated, depolarization-induced suppression of inhibitory synapses onto those vLGN neurons that receive input from HVAs. In summary, we determined the detailed neural mechanisms underlying the experience-dependent adaptation of ethologically-relevant behavior, and discovered a novel corticofugal pathway with a central role in this form of learning.

Disclosures: S. Mederos: None. **P. Blakely:** None. **N. Vissers:** None. **C. Clopath:** None. **S.B. Hofer:** None.

Poster

PSTR404

Properties of Thalamic Nuclei in Visual Pathways

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Topic: D.06. Vision

Support: German Research Foundation (DFG) grant KR 5138/1 - 1 & KR 5138/3 - 1 (Heisenberg Professorship)
Wellcome Trust Strategic Award 101092/Z/13/Z
German Research Foundation (DFG) - Project number 500900173

Title: Pulvinar projections to dorsal and ventral subdivisions of area LIP in the macaque

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Abstract: The pulvinar is a large subcortical structure in the primate brain. Its exact functional role is not fully understood, but it appears to participate in the integration and modulation of sensory information between cortical areas. There is an abundance of anatomical connections between the pulvinar and visual and visuomotor cortices, including the lateral intraparietal area (LIP). The dorsal and ventral subdivisions of LIP (LIPd and LIPv, respectively) are structurally and functionally distinct from each other, with LIPd having been linked to visuospatial processing and LIPv to saccade planning. However, it is unknown whether the anatomical connectivity between pulvinar and LIP is differentially related to these subdivisions. To address this question, small volumes (ca. 80nl) of CholeraToxinB (CTB low salt, List Biological Labs Inc) tracer were injected into either LIPd or LIPv of 2 male and 2 female, anesthetized rhesus macaques, using structural MRIs under Brainsight (Rogue Research Inc) control. After 86-234 h,

animals were perfused transcardially with 4% paraformaldehyde. Parasagittal sections were cut at 50 µm and the pulvinar was examined for retrograde neuronal body labeling. Confirming previous studies, labeled cells were found almost exclusively within dorsal parts of the pulvinar. This pattern was observed following both LIPd and LIPv injections. However, injection of retrograde tracer into LIPd resulted in more numerous and widespread labeling, which was determined to be in the medial pulvinar and the dorsal medial division of lateral pulvinar, unlike for injections into LIPv, which resulted in sparser labeling of a small number of cell clusters mostly confined to the medial pulvinar. In stark contrast to retrograde tracer injections into earlier, dorsal visual area V5/MT, we found no label in the inferior pulvinar or the LGN, underlining the importance of these connections for purely visual processing. These anatomical connections provide the structural basis for the involvement of the pulvinar in visuo-motor processes. While similar regions of the pulvinar project to LIPd and LIPv, the data also indicate potential asymmetries in the extent to which pulvinar contributes to individual processes associated with these different subdivisions of LIP.

Disclosures: S. Ziegler: None. **B. Ahmed:** None. **A.J. Parker:** None. **K. Krug:** None.

Poster

PSTR404

Properties of Thalamic Nuclei in Visual Pathways

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Program #/Poster #: PSTR404.09/G22

Topic: D.06. Vision

Support: NIH R01 MH131317
NIH R01 EY028657
NIH 5 T32 EY 021462 12

Title: Thalamic burst and tonic firing modes are disrupted in Fragile X

Authors: *R. T. O'SHEA[1], N. J. PRIEBE[2], D. H. BRAGER[3];
[1]Psychology, Univ. of Texas At Austin, Austin, TX; [2]Neurosci., Univ. Texas, Austin, Austin, TX; [3]Sch. of Life Sci., Univ. of Nevada, Las Vegas, Las Vegas, NV

Abstract: The thalamus controls what information gets relayed from the periphery to sensory cortex. In Fragile X syndrome (FX), sensory processing is dysregulated. We hypothesize that disruption of thalamic sensory relay in FX contributes to some sensory impairments. Thalamic relay neurons are characterized by two distinct firing modes- burst and tonic. In burst mode, neurons fire a volley of action potentials (APs) in quick succession following a relatively long period of silence. This firing pattern is due, in part, to the activation of T-type Ca^{2+} channels. At more hyperpolarized membrane potentials, the T-type Ca^{2+} current (I_T) generates a depolarizing envelope known as a low-threshold spike (LTS) which underlies a burst of Na^{+}-dependent APs. At more depolarized membrane potentials, I_T is inactivated, and depolarizing inputs trigger a

train of APs in the tonic mode. These two firing modes are hypothesized to relay distinct sensory information to cortex.We densely sampled spontaneous and visually-evoked neural activity in the lateral geniculate nucleus (LGN) and found that the pattern of burst and tonic firing is disrupted in *Fmr1* knockout mice as compared to wild type (WT) mice. Burst firing comprised a smaller proportion of each unit's total spikes in FX than in WT LGN (FX mean = 1.71 +/- 2.69 %, WT mean=7.95 +/- 10.0 %, p<.001 rank sum test). The frequency of bursts was also lower in FX than WT LGN (FX mean = .02 +/- .04 bursts/second, WT mean=.16 +/- .26 bursts/second, p<.001 rank sum test). Bursts from FX neurons contained fewer APs than bursts from WT neurons (FX mean= 2.46 +/-.81 APs/burst, WT mean= 2.94 +/-1.02 APs/burst, p<.001 rank sum test).In parallel, we performed *in vitro* whole cell recordings from thalamic neurons in WT and FX mice. FX neurons fired more action potentials in response to current injection compared to WT. This was because FX thalamic neurons fired predominately in tonic mode while WT thalamic neurons fired in burst mode (n=5, 6; p<0.001 Two-way ANOVA). To explore the mechanisms that underlie the attenuation of burst firing in FX thalamus, we recorded the LTS in the presence of TTX. While the LTS was elicited in WT neurons at membrane potentials below -60 mV, FX neurons had to be more hyperpolarized to observe the LTS. This indicates that although FX thalamic neurons are capable of generating LTS events, and thus burst firing, they are only able to after a large hyperpolarization transitions them out of the tonic firing mode. Our results indicate that the relay of sensory information to cortex is disrupted in FX, which may contribute to the abnormal sensory and cognitive phenotype of individuals with FX.

Disclosures: R.T. O'Shea: None. **N.J. Priebe:** None. **D.H. Brager:** None.

Poster

PSTR404

Properties of Thalamic Nuclei in Visual Pathways

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Program #/Poster #: PSTR404.10/G23

Topic: D.06. Vision

Support: NIH EY027888
William M. Wood Foundation
NIMA Foundation

Title: Wide-band field potential response fields in LGN

Authors: K. T. RENSHAW[1], ***J. PEZARIS**[2,3];
[1]Tufts Univ. Sch. of Med., Boston, MA; [2]Massachusetts Gen. Hosp., Boston, MA; [3]Harvard Medical School, Boston, MA

Abstract: The role of the lateral geniculate nucleus (LGN) in visual image processing is largely, but incompletely understood. In this experiment, we attempted to better characterize how the local architecture of LGN might influence the responses of individual relay cells. Traditional

measurement of response properties in LGN correlates evoked spike trains against animated spatiotemporal mapping stimuli. However, the spiking of individual neurons is often accompanied by electrical activity from the immediate environment. Here, we used the entirety of raw recordings, rather than sorted spiking activity alone, to calculate wide-band field potential (WFP) response fields (RFs) in the LGN. We hypothesized that adjacent neurons help refine WFP RFs by enhancing the signal or suppressing background. We recorded spiking activity in the LGN of three alert Rhesus Macaques using multi-channel electrodes while presenting white noise stimuli. Consistently, we observed higher signal-to-noise ratios in WFP-based RFs compared to spike-based RFs. Additionally, we observed a strong similarity between the two RF types, indicating local homogeneity in response. To better discern the role of low-amplitude spiking activity, we selectively removed action potentials of varying amplitudes from the raw signal and recomputed WFP RFs, using spike amplitude as a proxy for distance from the electrode. Notably, eliminating lower amplitude spikes led to increased RF noise, suggesting their involvement in suppressing extra-receptive-field input and thus sharpening the WFP RF. Further investigation involved overlaying spiking activity from one recording onto baseline electrical activity from another, effectively altering the low-level electrical signal to one no-longer relevant to the stimulus that evoked the spikes. This manipulation attenuated the effect associated with low amplitude spikes, lending support to their role in background suppression. Our findings underscore the significance of low amplitude spiking activity in LGN recordings, proposing its involvement in refining WFP RFs by mitigating extra-receptive-field noise. Mechanisms to explain the cooperativity of adjacent neurons on WFP RFs include the proposed presence of gap junctions in LGN, which help synchronize high-threshold bursting characteristic of LGN (Hughes et al., 2004), as well as indirect contribution from thalamic interneurons (Dubin & Cleland, 1977). Such insights contribute to a deeper understanding of LGN neuron RF structure and the functional implications of cooperativity among neighboring neurons.

Disclosures: K.T. Renshaw: None. **J. Pezaris:** None.

Poster

PSTR404

Properties of Thalamic Nuclei in Visual Pathways

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Topic: D.06. Vision

Support: NIH Grant NS123912
HHMI
Kavli Institute for Fundamental Neuroscience

Title: Functional and Molecular Convergence and Divergence of Retinal Ganglion Cells in the Visual Thalamus

Authors: *X. WEI[1], X. DUAN[2], M. SCANZIANI[3];
[1]Physiol., [2]Ophthalmology, UC San Francisco, San Francisco, CA; [3]HHMI Dept. of Physiol., Univ. of California, San Francisco, San Francisco, CA

Abstract: The mammalian dorsal lateral geniculate nucleus (dLGN) of the thalamus is the main conduit of visual information from the retina to the cortex. At least 40 subtypes of retinal ganglion cells (RGCs) convey distinct features of the visual scene to the dLGN. The traditional view that dLGN neurons act simply as relays for visual information is challenged by emerging anatomical and functional studies indicating that RGCs conveying different features can converge onto individual dLGN neurons. We are addressing this issue using anatomical, molecular and functional approaches to obtain a comprehensive connectivity profile of the retina to the dLGN. We first optimized a genetically-encoded wheat germ agglutinin (WGA)-mCherry with high anterograde trans-synaptic trafficking efficiency. We could conditionally express AAV2-Flex-WGA-mCherry in specific RGCs-Cre lines to trans-synaptically label recipient neurons in the dLGN. Second, we implemented a **Trans-MERFISH** platform which combines the mWGA-mCherry tracer with a spatial transcriptomic platform MERFISH. With this platform we can establish the transcriptomic identity and spatial location of dLGN neurons receiving inputs from specific RGCs-Cre lines. We found that one subtype of non-direction-selective RGCs (αRGCs, tagged by KCNG4-Cre line) synapse on the dLGN neurons located in the ventromedial region (dLGN core) while another subtype of RGCs, On-Off direction-selective ganglion cells (ooDSGCs), synapse on the dLGN neurons located in the superficial dorsolateral region (dLGN shell). Among dLGN core neurons those receiving αRGCs input have specific transcriptomic profiles. We confirmed this connectivity pattern using monosynaptic G-deleted rabies virus tracing. To investigate whether core dLGN neurons that receive input from αRGCs also receive inputs from other RGC subtypes we implemented a functional approach. In KCNG4-Cre mouse, we used the Cre_ON and Cre_OFF strategy to express red-shifted channelrhodopsin (ReaChR) in αRGCs and channelrhodopsin-2 (ChR2) in all other RGCs (non-αRGCs). Using this dual optogenetic approach we performed whole cell recordings from dLGN neurons in acute brain slices and compared the amplitude of opto-evoked excitatory postsynaptic currents (oEPSCs) by either amber or blue LED light respectively. We discovered that core dLGN neurons receive strong inputs (i.e. large EPSCs) from either αRGCs or non-αRGCs, but not from both. Taken together, dLGN neurons in the core and in the shell receive inputs from distinct RGC subtypes and that, even though several subtypes project to the core, individual dLGN core neurons are dominated by the input of one specific RGC subtype.

Disclosures: X. Wei: None. **X. Duan:** None. **M. Scanziani:** None.

Poster

PSTR404

Properties of Thalamic Nuclei in Visual Pathways

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Topic: D.06. Vision

Support: NIH Grant EY033528
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Title: A high-resolution transcriptomic atlas of cell types in the lateral geniculate nucleus

Authors: *K. STEBBINS[1], M. JALIL[2], J. SU[3], J. CAMPBELL[4], M. A. FOX[5];
[1]Virginia Technol., Roanoke, VA; [2]Biol., Univ. of Virgina, Charlottesville, VA; [3]Virginia Technol. Carilion Res. Inst., Roanoke, VA; [4]Biol., Univ. of Virginia, Charlottesville, VA; [5]Univ. of Massacheusetts, Amherst, Amherst, MA

Abstract: Axons from over 40 subtypes of retinal ganglion cells convey visual information from the outside world to distinct brain regions. Understanding how this information is processed requires a detailed understanding of the cell types and associated circuits in these regions. In some retinorecipient nuclei, the lack of detailed characterization of these target cells has hampered our ability to study the circuits underlying visual functions and behaviors. In this study, we use state-of-the-art single-nucleus sequencing to identify a comprehensive list of cells in the mouse visual thalamus, including a list of the cell types in the ventral lateral geniculate nucleus (vLGN), which represents the third largest retinorecipient region in rodents but for which we know little about the cells and circuits that process visual information. Additionally, we employed the same technique to study cell types in the neighboring dLGN which has been thoroughly characterized in terms of cytoarchitecture and neuronal morphology, but for which transcriptomic identity has not been identified for some of these cell types. Systematic analysis of the neuronal, non-neuronal, and immature neuronal cell types across LGN reveals nearly 20 potential subtypes of vLGN neurons, including a previously undetected population of GABAergic cells in a hidden layer of the vLGNe the first-ever characterization of excitatory neurons in vLGN. The results also suggest the identity of unique features of cell type organization in dLGN; in particular, the identity of over a dozen potential subtypes of dLGN neurons including multiple types of GABAergic interneurons. This comprehensive transcriptomic atlas of the mouse LGN establishes a benchmark reference atlas and a foundational resource for deep and integrative investigations of cell type and circuit function, development, and evolution of an essential nucleus of the mammalian visual system.

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Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

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EMBO grant ALTF 712-2021

Title: Electrophysiology with identified cell types in layer 5 pyramidal neurons of awake behaving mice

Authors: *M. SHINN[1], B. ZHOU[2], I. PRANKERD[2], C. A. MAAT[2], M. BOURDENX[2], D. NICOLOUTSOPOULOS[2], R. TILBURY[2], P. SHUKER[2], S. BUGEON[3], K. D. HARRIS[2]; [1]UCL, London, United Kingdom; [2]Univ. Col. London, London, United Kingdom; [3]Inst. de Neurobiologie de la Méditerranée, Marseille, France

Abstract: The brain contains an enormous diversity of neurons that differ widely in structure and function. An explosion of innovation in the last few years has increased our ability to identify individual cell types using techniques such as fluorescent labeling, immunohistochemistry, and spatial transcriptomics. At slow timescales, neurons can be recorded in vivo with two-photon imaging and spatially matched to these in situ techniques for cell typing, providing a link between neuron structure and function. However, fast-timescale activity is critical to understanding neural circuits. Due to methodological limitations, the role of different neuron types in vivo at fast timescales is largely unknown.

Here, we develop a method to perform cell typing on electrophysiological recordings from high-density Neuropixels probes in awake behaving mice. First, we use a functional fingerprinting approach to link neurons in primary visual cortex (V1) from electrophysiology to two-photon imaging. Then, cells recorded in two-photon imaging can be spatially matched to in situ methods for cell type identification. We focus on deep layer 5 excitatory neurons due to their high firing rates.

Combined with the methods from our companion poster, "Identifying transcriptomic subtypes of layer 5 neurons after functional recording" (Zhou et al), we can identify transcriptomic neuron types in large scale electrophysiology recordings. This allows the short-timescale properties of fine transcriptomic cell types to be identified simultaneously, elucidating the role of cell types within the neural circuits of vision.

Methods: We first performed two-photon calcium imaging in V1 on mice expressing GCaMP in layer 5 excitatory neurons. After imaging, we replaced the imaging coverslip with a coverslip containing a small hole, protected by silicone gel, and recorded using Neuropixels probes inserted through the hole.

We developed a video stimulus based on thresholded pink noise that elicited highly reliable responses among V1 neurons. Mice were shown this stimulus during two-photon recordings, and subsequently during electrophysiology recordings. The pairwise correlation of neurons' response to this stimulus from each modality, combined with the probe's spatial location, allowed identifying neurons between the two modalities.

Disclosures: M. Shinn: None. **B. Zhou:** None. **I. Prankerd:** None. **C.A. Maat:** None. **M. Bourdenx:** None. **D. Nicoloutsopoulos:** None. **R. Tilbury:** None. **P. Shuker:** None. **S. Bugeon:** None. **K.D. Harris:** None.

Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

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BBSRC grant BB/W015293/1
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Title: Identifying transcriptomic subtypes of layer 5 neurons after functional recording

Authors: *B. ZHOU, M. SHINN, I. PRANKERD, C. A. MAAT, M. BOURDENX, D. NICOLOUTSOPOULOS, R. TILBURY, P. SHUKER, S. BUGEON, M. CARANDINI, K. D. HARRIS;
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Abstract: The diversity of cortical neurons was noted by their anatomical features over a century ago, but we still do not understand how these cell types work together to process information. Neurons can be classified according to many different features, such as their connectivity and gene expression profile. For example, neocortical excitatory cells constitute ~80% of neurons and can be divided into coarse classes with similar axonal projection patterns, physiology, and gene expression. However, fine details of cortical circuits are not captured by coarse top-level classifications. Thanks to development of single-cell RNA sequencing, which measures the expression of all genes in dissociated cells, we are now able to classify the neurons into much finer subtypes. Moreover, novel spatial transcriptomics tools can localize gene expression in situ, without any disruption of the microenvironment. The next question is how can we relate these subtypes to their activity patterns in the living brain. To study this question, we recorded from layer 5 excitatory neurons in mouse primary visual cortex (V1) and identified their fine subtype using spatial transcriptomics. Our tool, Coppafish3D, allows detection of mRNA on brain tissue with subcellular resolution in thick 50 μm sections. With spatial information preserved, the ex vivo sequenced neurons can be aligned to their in vivo activity recorded using two-photon imaging. We used a predecessor of this method to explore the relationship between transcriptomic subtypes and activity of layer 1-3 inhibitory neurons in V1 (Bugeon et al., 2022, Nature). Here, we focused on layer 5 neurons of V1 due to the diversity of fine transcriptomic subtypes. We used mice that genetically express calcium reporter GCaMP6m across layer 5 excitatory neurons for functional recording, and virally-injected sparse fluorescent labels in superficial layers to improve in vivo to ex vivo alignment. After functional imaging, we perfused, sliced the brain into 50 μm sections, and used Coppafish3D to detect cell types using 120 genes. These identified cells can then be matched to cells from functional imaging. This method allows us to identify the cell types of simultaneously recorded neural populations deep in

cortex, providing a clearer understanding of how information is processed and routed through cortical circuits. When combined with the methods from the companion poster "Electrophysiology with identified cell types in layer 5 pyramidal neurons of awake behaving mice" (Shinn et al., 2024), we can further link these cells to electrophysiological recordings.

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Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

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Topic: D.06. Vision

Support: NIH Grant R01 EY031009
KTEF Competetive Renewal Grant

Title: Locomotion-induced enhancement of visual response is mediated by nicotinic activation of deep layer Somatostatin interneurons in mouse visual cortex

Authors: *T.-J. CHEN[1], J. S. RICEBERG[2], Y. GARKUN[3], K. YOSHITAKE[3], T. NISHIOKA[3], T. MANKOUSKAYA[3], M. SADAHIRO[5], H. MORISHITA[4];
[1]Ichan medical school at Mount Sinai, New York, NY; [2]Neurosci., Mount Sinai & Albany Med. Col., New York, NY; [4]Psychiatry, Neuroscience, Ophthalmology, [3]Icahn Sch. of Med. at Mount Sinai, New York, NY; [5]Dept. of Mol. and Cell Biol., Univ. of California, Berkeley, CA

Abstract: Locomotion-induced enhancement of visual response is mediated by nicotinic activation of deep layer Somatostatin interneurons in mouse visual cortex Ting-Jiun Chen, Justin Riceberg, Yury Garkun, Kohei Yoshitake, Tadaaki Nishioka, Tatsiana Mankouskaya, Masato Sadahiro, Hirofumi MorishitaLocomotion enhances the visual-evoked responses of excitatory pyramidal neurons in the primary visual cortex (V1) of awake mice. Previous studies showed that this effect results from the release of acetylcholine (ACh) during locomotion in V1, however, the molecular and circuit mechanisms remain poorly understood. In this study, we investigated the contribution of deep-layer somatostatin (SST) interneurons expressing α2 subunit-containing nicotinic acetylcholine receptors (nAChRα2) in V1 to the modulation of visual responses induced by locomotion. We performed fiber photometry imaging to monitor either ACh or calcium levels in V1 nAChRα2+SST interneurons of awake mice while allowing them to run on a head-fixed running disc. We found that locomotion increased ACh release onto V1 deep layer SST interneurons and enhanced the visual response of this cell-type. Additionally, we evaluated the effects of either enhancing (using hypersensitive nAChR α2 knock-in mice) or reducing (via CRISPR-based knockdown of nAChRs) nACh signaling in deep-layer SST

interneurons on the activity of GCaMP-expressing pyramidal neurons during visual stimulation with drifting gratings, when the mice are stationary or running on the disc. Notably, enhancing nicotinic signaling in deep layer SST interneurons was sufficient to enhance visual response of V1 pyramidal neurons, while dampening nAChR signaling in the SST interneurons reduced the locomotion-induced enhancement of visual responses in V1 pyramidal neurons. These results suggest that nAChR signaling in deep-layer SST interneurons plays a crucial role in enhancing visual responses during locomotion. Given that deep-layer SST interneurons provide inhibitory monosynaptic input to Parvalbumin (PV) expressing interneurons, our study also highlights SST->PV disinhibitory pathway as a potential circuit-level mechanism for modulating visual responses induced by locomotion.

Disclosures: T. Chen: None. **J.S. Riceberg:** None. **Y. Garkun:** None. **K. Yoshitake:** None. **T. Nishioka:** None. **T. Mankouskaya:** None. **M. Sadahiro:** None. **H. Morishita:** None.

Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR405.04/G29

Topic: D.06. Vision

Support: R01 Ey31716
F32 EY034013

Title: Cholinergic modulation of somatostatin expressing interneurons depends on behavioral state

Authors: *C. CAMMARATA[1], T. HAWLEY[2], S. S. LIM[1], M. STONE[2], M. R. TADROSS[3], L. L. GLICKFELD[4];
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Abstract: The visual cortex can process identical stimuli differently depending on the context in which they are seen. For example, in mice, behavioral variables such as locomotion or arousal increase the magnitude and specificity of neural responses to visual stimuli. The neuromodulator acetylcholine (ACh) is implicated in context-dependent processing and acts on the diverse inhibitory interneurons in cortical circuits. However, it remains uncertain how cholinergic modulation of distinct interneuron classes shapes visual cortex activity across contexts. Here we dissect the cell-type specific effects of ACh within the mouse primary visual cortex, focusing on the interplay between somatostatin (SST) and vasointestinal peptide (VIP) expressing interneurons. The competitive antagonism of these interneurons influences context-dependent visual processing and is implicated in mediating the effects of locomotion on cortical responses. In addition, M1-type muscarinic ACh receptors (mAChRs) are robustly expressed on both cell

types, and we find a robust increase in their excitability upon application of muscarine *in vitro*. We employ the innovative Drugs Acutely Restricted by Tethering technology to selectively block mAChRs on SST or VIP interneurons. We find that blocking mAChRs on SST cells decreases their visual responses when the mice are stationary. This is consistent with their proposed excitatory impact, and argues that ACh provides substantial modulatory drive in the absence of locomotion. Surprisingly, we find that the effect of blocking mAChRs on SST cells depends on the behavioral context: while this manipulation suppresses activity during stationary epochs, it facilitates responses during locomotion. Despite the context-dependent effects of mAChRs on SST cells, the responses of pyramidal cells are suppressed across states, and they are less modulated by locomotion. Conversely, preliminary experiments blocking mAChRs on VIP cells show minimal influence on VIP responses to visual stimuli and no effect on pyramidal cell locomotion modulation. Our findings underscore the nuanced role of ACh in modulating visual cortex across cell types, and reveal that cholinergic modulation not only contributes to context-dependent processing but is itself influenced by behavioral context. Finally, an existing model proposes that ACh induces locomotion modulation by increasing VIP inhibition of SST cells, in turn disinhibiting the pyramidal population. In future experiments we will probe this directly by antagonizing gamma-aminobutyric acid receptors on SST cells and examining locomotion modulation of SST and pyramidal cells.

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Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

Location: MCP Hall A

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Program #/Poster #: PSTR405.05/G30

Topic: D.06. Vision

Support: George E. Hewitt Foundation for Medical Research Postdoctoral Fellowship
Fiona and Sanjay Jha Chair in Neuroscience

Title: Spontaneous high firing rate events in primate cortex

Authors: *P. JENDRITZA, N. DOTSON, J. H. REYNOLDS;
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Abstract: Recent advances in high-density neural recordings have made it possible to track the activity of large populations of neurons at an unprecedented scale. This opens up the possibility to study neuronal phenomena that are difficult to observe because they occur infrequently. We used Neuropixels probes to record from thousands of neurons in the parietal and prefrontal cortex of awake marmosets, and reliably find spontaneous high firing rate events (>200 spikes/s)

that occur in one or a few localized single units and last several hundred milliseconds. These events do not appear to be directly related to external sensory stimulation or eye movements and occur only approximately once per 20 neurons per hour of recording. We performed several analyses to determine whether these events could be explained by probe movement in the brain tissue. Neurons that participated in these events exhibited normal and comparable firing rates before and after the event. Furthermore, events occurred even in the absence of any detectable movement, suggesting that they might not be due to damage of the cell membrane caused by probe movement.

Disclosures: P. Jendritza: None. **N. Dotson:** None. **J.H. Reynolds:** None.

Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

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Program #/Poster #: PSTR405.06/G31

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R21 NS127299
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Kempner Institute Graduate Fellowship

Title: Reliable Non-Stimulus Driven Signaling in Visual Cortex across Species and Conditions

Authors: *D. HIDALGO[1], G. DELLAFERRERA[2], W. XIAO[3], M. PAPADOPOULI[4], S. M. SMIRNAKIS[5], G. KREIMAN[6];
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Abstract: Neural activity in visual cortex is commonly analyzed as trial averages to mitigate stimulus-irrelevant variability, or "noise". This noise, however, can convey valuable non-sensory information such as top-down and cross-modal modulations influencing visual perception. We investigated trial-by-trial inter-areal interactions using Ridge regression to predict the activity in one area from that in another, encompassing both stimulus- and non-stimulus-driven inter-areal communication. We analyzed datasets from mice (V1 layer 4 and layers 2/3, calcium imaging) and macaques (V1 and V4, extracellular electrophysiology). In both species, visual areas predict each other's activity in response to multiple visual stimuli, repetitions of the same stimulus, and even in the absence of a stimulus (spontaneous conditions). Examining the neuronal properties that influence inter-areal predictability, we found that in macaque, V1 and V4 neural sites with high receptive field (RF) overlap result in better inter-cortical predictions compared to neural

sites with low RF overlap. This increased predictability was also present during spontaneous conditions, suggesting the enhanced functional connectivity in high RF overlap subpopulations is an intrinsic property of inter-cortical interactions, and not stimulus-dependent. To quantify how predictability depends on stimulus or non-stimulus driven activity, we showed that trial repeat shuffling reduced (but did not eliminate) predictability in both datasets, suggesting that the reliable information between cortical areas/layers only partly depend on strict stimulus-driven effects. Our findings show the prevalence of both visual stimulus- and non-stimulus-related components of shared information between visual areas and highlight the importance of non-visual variability shared between visual regions in both mice and macaques.

Disclosures: D. Hidalgo: None. **G. Dellaferrera:** None. **W. Xiao:** None. **M. Papadopouli:** None. **S.M. Smirnakis:** None. **G. Kreiman:** None.

Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

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Program #/Poster #: PSTR405.07/G32

Topic: D.06. Vision

Support: R01NS123778

Title: Exploring the influence of binocular anti-coactivation on ocular dominance column formation beyond primary visual cortex

Authors: *S. NAJAFIAN[1], V. K. BEREZOVSKII[1], M. J. ARCARO[2], M. S. LIVINGSTONE[1]; [1]Neurobio., Harvard Med. Sch., Boston, MA; [2]Psychology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: It is widely accepted that neurons from both eyes that fire together wire together through Hebbian mechanisms. As a result, columnar structures comprising neurons from both the left and right eyes, known as ocular dominance columns, emerge in layer 4 of the macaque primary visual cortex (V1), the geniculate input layer. Beyond primate V1, visual neurons are predominantly binocular . Notable research includes studies on tadpoles with transplanted third eyes, displaying abnormal ocular dominance despite frogs typically lacking binocular overlap (Constantine-Paton et al. 1978). Inspired by this study, here we use innovative non-invasive manipulations of early visual experience in infant macaques to dramatically alter the normal relationship of binocular vision. We raised two macaques from birth for one year wearing helmets with liquid crystal lenses. The lens shutter opened and closed in alternation at 0.1HZ. The primary question guiding this study is whether anti-correlated activity between the two eyes throughout development could induce the formation of ocular dominance columns beyond V1. This could provide insights into feature domain development and self-organization in higher visual areas like the inferior temporal (IT) cortex. To visualize the ocular dominance domains,

one eye was injected with tetrodotoxin for 3 weeks prior to euthanasia. The cortex was post fixed, and sectioned tangentially and the tissue processed for cytochrome oxidase. Approximately 250 sagittal sections, 70 microns thick, of each entire hemisphere were obtained. All slices were scanned using a VS120 Whole Slide Scanner. We developed to reconstruct 3D volumes of CO staining and visualize at various laminar depths. The pipeline registers the neighboring histology sections using a linear then non-linear registration process. Variations in staining intensity were normalized across the slices to minimize the impact of artifactual fluctuations that could arise from the staining procedure. The non-linear part of the pipeline involves the manual selection of landmark points on the inner and outer areas of the gray matter between the slice and the reference slice to ensure accurate registration, which is crucial for aligning columns. The resulting 3D histological volume is then registered to a reference MRI using Advanced Normalization Tools (ANTs). By comparing the ocular dominance columns in these animals with those in a control monkey, we aim to investigate the extension of ocular dominance columns beyond V1. Preliminary findings indicate successful reconstruction of these columns in the primary visual cortex, demonstrating the effectiveness of our pipeline.

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Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR405.08/G33

Topic: D.06. Vision

Support: funded by the Max Planck Society and University of Tübingen

Title: Visual illusions from V1 by removing top-down feedback to aid seeing through an information bottleneck

Authors: *L. ZHAOPING[1,2];
[1]Max Planck Inst. For Biol. Cybernetics, Tuebingen, Germany; [2]Univ. of Tübingen, Tübingen, Germany

Abstract: It has been proposed that vision is more vulnerable to illusions in the peripheral rather than the central visual field, because central vision should have more top-down feedback (from late to early processing stages along the visual pathway) to query for extra information to aid seeing and thus vetoing illusions. This proposal is by the Central-peripheral Dichotomy (CPD) theory (Zhaoping 2019) motivated by brain's information bottleneck. This study reports the following findings supporting this theory: (1) illusions visible to peripheral but not central vision are created based on the known neural responses from the primary visual cortex (V1) to retinal inputs and on the assumption that the bottleneck starts from V1 along the visual pathway, (2) an

example of such illusions is the reversed depth illusion (Zhaoping & Ackermann 2018), and this illusion becomes visible in central vision when the top-down feedback is compromised using visual backward masking.
The CPD theory hypothesizes that peripheral and central vision are mainly for looking and seeing, respectively. Looking selects a tiny fraction of sensory information into the bottleneck by shifting gaze and attention to selected inputs, and seeing recognizes the selected inputs. Since sensory information is progressively impoverished by the bottleneck from early to late processing stages along the visual pathway, the theory additionally proposes that to aid seeing, later stages send feedback to earlier stages to query for extra information to disambiguate between, confirm, or veto potential perceptual outcomes, and that this feedback query is mainly in central vision.
The reversed depth illusion is from random-dot stereograms (RDSs). A RDS normally evokes percepts of surfaces in three-dimensional (3D) scenes by images presented to left and right eyes that comprise interocularly corresponding random black and white dots. The spatial disparities between the corresponding dots determine the depths of the random dots on surfaces in 3D. If a black dot in one eye corresponds to a white dot in the other, the RDS is nonsensical, but V1 neurons respond as if the depth, near versus far, is reversed, causing the reversed depth illusion if not vetoed by the feedback query. As predicted, this illusion is normally only visible in peripheral vision which lacks the feedback query, it becomes visible in central vision using dynamic RDSs in which each RDS is quickly replaced by the next RDS for the same disparities (and sensicality), but with the random dots independently generated. In dynamic RDSs, a subsequent RDS masks the details (about, e.g., if the correspondence is sensical) in the preceding RDS, impairing the feedback veto.

Disclosures: L. Zhaoping: None.

Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: D.06. Vision

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P30EY007551
Kavli Foundation
University of Houston Higher Education Assistance Fund

Title: Arousal modulates neural responses in the primate primary visual cortex.

Authors: M. MUSTANSIR[1], S. COLE[2], Z. W. DAVIS[3], *L. NURMINEN[1];
[1]Vision Science, Biomed. Engin., [2]Vision Sci., Univ. of Houston, Houston, TX; [3]Ophthalmology and Visual Sci., Univ. of Utah, Salt Lake City, UT

Abstract: Behavioral states affect cortical processing of sensory signals in a variety of species. Arousal is one of the strong modulators of sensory processing, and moment-to-moment fluctuations in arousal can switch the cortex from a desynchronized state with enhanced neural responses during high arousal to a synchronized state with lower-magnitude neural responses during low arousal. The mechanisms that underlie the effects of arousal on neural processing in the primary visual cortex have been mostly studied in rodents, but less is known about the cellular-level effects of arousal on early visual processing in primates. To better understand how arousal affects processing in visual cortical circuits in primates, we performed extra-cellular neural recordings using high-density laminar probes (Neuropixels 1.0 NHP) that were vertically inserted into the primary visual cortex of awake, head-fixed marmoset monkeys (Callithrix Jacchus). To estimate moment-to-moment fluctuations in arousal, we measured the pupil diameter with a video-based eye tracker. Blinks were removed from the pupil diameter traces with an algorithm that detected deviations larger than 3SD in the temporal derivative of the pupil diameter and marked 200 milliseconds around the detected deviation as a blink. The few remaining blinks were manually marked. The pupil diameter data was linearly interpolated around the blinks and normalized to 0 and 1. The animals were required to maintain fixation on a centrally presented fixation target (0.8 x 0.8° monkey face) while natural images from the Berkeley Segmentation Dataset were flashed on a screen. The natural images were windowed into a 16° diameter window, and a 1.5° diameter region of the image around the fixation target was masked with a mean gray circle. We analyzed the responses of 27 visually responsive units (evoked response at least 5 spikes higher than the baseline). Our analysis showed that the mean firing rate was statistically significantly (t-test $p < 0.01$) higher in those trials in which the pupil diameter was larger than the median pupil diameter compared to the trials in which the pupil diameter was smaller than the median pupil diameter (mean firing-rate ± SE 53.4±1.9Hz vs 46.8±1.5Hz). These preliminary results show that arousal modulates the responses of neurons in the primate primary visual cortex.

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Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR405.10/G35

Topic: D.06. Vision

Support: NIH Grant R35NS097287

Title: Compositionality of latent dynamics on multiple timescales underlies mesoscopic neural activity during behavior

Authors: E. D. VICKERS[1], ***M. B. JOHNSON**[1], L. MAZZUCATO[1,2], D. A. MCCORMICK[1,2]; [1]Inst. of Neurosci., [2]Dept. of Biol., Univ. of Oregon, Eugene, OR

Abstract: Neural activity unfolds as sequences of population activity patterns reflecting the animal's fluctuating movements, arousal state, and goals. To understand these brain-wide activity patterns, we focused on the concept of compositionality, which asserts that higher-order neural patterns representing behavioral *motifs* (e.g. grooming, balancing) are generated via superposition of simpler neural patterns, or factors, representing behavioral *primitives* (e.g. pupil diameter, whisking). *We tested the hypotheses that 1) Neural activity is compositional and spatially distributed across dorsolateral cortex, and 2) Cortical neurons exhibit mixed selectivity, such that the encoding of different patterns of behavior across multiple timescales can occur in spatially overlapping neural populations.*

We recorded mesoscale cortical activity via 2-photon (2p) Ca^{2+} imaging in two novel *in vivo* preparations that allow access to the entire mouse dorsolateral cortex (~10k cells; 52 sessions; 20-90 min / session; N=8; CaMKII-tTA x tetO-GCaMP6s mice, 8-50 weeks old)[1]. To quantify the distributed compositionality of brain-wide patterns, we deployed a dynamical systems framework based on a factorial hidden Markov model (fHMM).[3] In the fHMM, several independent Markov chains (factors) generate latent discrete dynamics, with each factor representing the dynamics of brain-wide patterns encoding a combination of behavioral primitives and motifs. We found that the time course of neural activity can be explained by the dynamical superposition or "overlap" of a small number of brain-wide neural activity patterns corresponding to the fHMM factors, each with its own kinetics corresponding to different levels of the behavioral hierarchy.

Our results suggest that the neural code employs differential compositionality across behaviorally relevant timescales. This implies that neural dynamics are organized to take advantage of a robustness/flexibility trade-off whereby a high degree of compositionality at specific timescales, in designated combinations of cortical areas, enables primitive patterns to encode a combinatorially large number of higher-order representations, *thus describing a novel fundamental principle underlying flexible cognitive control.* At the same time, more spatially distributed representations at timescales with less compositionality might endow other aspects of the neural code with robustness against perturbations, irrelevant contextual changes, and/or unnecessary learning.

Refs: [1]Vickers & McCormick, *eLife*, 2024. [2]Stringer et al, *bioRxiv*. 2023. [3]Ghahramani & Jordan, *Advances in neural information processing systems* 8 (1995).

Disclosures: E.D. Vickers: None. **M.B. Johnson:** None. **L. Mazzucato:** None. **D.A. McCormick:** None.

Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

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Topic: D.06. Vision

Support: NIH grant 1R01EY031059,
NSF grant 822598

Title: Encoding manifolds constructed from grating responses organize responses to natural scenes in primary and higher visual cortical areas

Authors: *M. STRYKER[1], L. DYBALLA[2], G. D. FIELD[4], S. W. ZUCKER[3];
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Abstract: We have created “encoding manifolds” to reveal the overall responses of a brain area to a variety of stimuli. Encoding manifolds organize response properties globally: Each point on an encoding manifold is a neuron, and nearby neurons respond similarly to the stimulus ensemble in time. We previously found using a large stimulus ensemble including optic flows that encoding manifolds for the retina were highly clustered, with each cluster corresponding to a different ganglion cell type. In contrast, the topology of the V1 manifold was continuous (PMID: 38227668). Now, using responses of individual neuronal to drifting gratings from the Allen Institute Visual Coding-Neuropixels dataset in the mouse (PMID: 31844315), we infer encoding manifolds for V1 and for five higher cortical visual areas (VISam, VISal, VISpm, VISl, VISrl). We show here that the encoding manifold topology computed only from grating data is also continuous, not only for V1 but also for the higher visual areas, with smooth coordinates spanning it that include, among others, orientation selectivity and firing-rate magnitude. Surprisingly, the encoding manifold for gratings also provides information about natural scene responses. To investigate whether neurons respond more strongly to gratings or natural scenes, we plot the log ratio of natural scene responses to grating responses (mean firing rates) on the encoding manifold. This reveals a global coordinate axis organizing neurons' preferences between these two stimuli. This coordinate is orthogonal (i.e., uncorrelated) to that organizing firing rate magnitudes. This holds for all visual cortical areas. Analyzing layer responses, a preference for gratings is concentrated in layer 6, whereas preference for natural scenes tends to be higher in layers 2/3 and 4. We also find that preference for natural scenes dominates the responses of neurons that prefer low (0.02 cpd) and high (0.32 cpd) spatial frequencies, rather than intermediate ones (0.04 to 0.16 cpd). Conclusion: while gratings seem limited and natural scenes unconstrained, machine learning techniques can reveal subtle relationships between them beyond linear techniques.

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Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

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Program #/Poster #: PSTR405.12/G37

Topic: D.06. Vision

Support: NIH Grant 1U19NS107613-01

Title: Quantification of nonsense-free correlation uncovers the interaction between top-down and bottom-up sources of behavioral correlation in mouse V1

Authors: *P. YU[1], H. YOON[2], N. JI[3], B. DOIRON[1];
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Abstract: Brain-wide neuromodulation by behavioral variables, such as locomotion, pupil area, and face motion, have been observed in mice (Stringer et al., 2019; Musall et al., 2019). To study this mechanism at higher spatial resolution, we used two-photon imaging that allows recording of individual neuronal and synaptic bouton activity in mouse primary visual cortex (V1), while the animal's face was simultaneously videotaped.We aim to understand how facial motion is related to the population activity of cortical neurons and their lateral geniculate nucleus (LGN) afferents, both during spontaneous and stimuli evoked periods.While standard correlation analysis could yield spurious, 'nonsense correlations' due to slow, continuous drift over time and limited number of trials (Harris KD, 2020).We considered the session permutation method to assess the nonsense correlation in our recordings (Harris KD, 2020), and compared the difference between the standardized cross-validated regression results.We observed a robust correlation between facial motion and neuronal population activity, which is higher for visually evoked response compared to spontaneous activity.In contrast, LGN bouton activity does not correlate with facial motion during spontaneous periods, but surprisingly becomes significantly correlated for visually evoked responses.To explain this last observation we show that LGN boutons are almost silent during the spontaneous period, in contrast to their high activity during evoked states, implying that the LGN is subthreshold during the spontaneous period and cannot transfer any received information about facial motion. This prompts the hypothesis that the improved encoding of facial motion variables in V1 cortical neurons when visually evoked is mainly due to the onset of facial motion correlated bottom-up visually evoked LGN inputs, rather than stronger top-down movement-related cortical inputs.In total, our work gives an unprecedented analysis of the circuit pathways that underlie the recent observations that mouse V1 activity is related, in part, to non-visual inputs.

Disclosures: P. Yu: None. **H. Yoon:** None. **N. Ji:** None. **B. Doiron:** None.

Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

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Program #/Poster #: PSTR405.13/H1

Topic: D.06. Vision

Support: ERC VarPL Grant agreement No. 802482

Title: The functional hierarchy of orientation processing along the ventral stream in macaque visual cortex

Authors: *B. KARAMI[1,2,4], C. M. SCHWIEDRZIK[3,1];
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Abstract: The primate visual system is a complex network of brain areas that efficiently process retinal inputs to generate a rich percept across multiple stages of processing. The organization and precise nature of transformations occurring between these stages are not fully understood, owing to the challenges of recording activity across entire visual hierarchy simultaneously. Here, we investigate how one cardinal visual feature, orientation, is represented and successively transformed across visual cortex, using functional magnetic resonance imaging (fMRI) in awake macaque monkeys.We acquired blood oxygenation level dependent (BOLD) signals at 3T in two male adult monkeys (monkey R, and P) while being presented with a full-field Gabor gratings of 2 orthogonal orientations (45°, 135°). We decoded orientation information, with varying accuracy, from low- and high-level areas of the visual ventral stream, as well as control regions in the dorsal stream using Support Vector Machines (SVM) trained on BOLD activity patterns (V1: *0.655(0.057)*, V2: *0.625 (0.057)*, V3: *0.606 (0.056)*, V4: *0.648 (0.055)*, TEO: *0.617 (0.058)*, TE: *0.589 (0.061)*, MT: *0.498 (0.057)*, LIP: *0.536 (0.059)*). We then characterized the spatial layout of orientation representations in early (V1, V2), mid (V3, V4) and late (TEO, TE) processing stages: we determined the amount of clustering of voxels with the strongest orientation preferences. Here, we observed a decrease in cluster number along with an increase in cluster size along the hierarchy (early: N=38, size=*10.2*, mid: N=*27.5*, size=*14.2*, late: N=*20.5*, size=*17.6*). Next, we determined whether transformations of orientation information between successive stages in the ventral stream entailed qualitatively distinct representations. To this end, we trained SVMs on the most orientation preferring voxels in a given upstream region and tested for generalization of the classifier weights to the corresponding downstream region. We found generalization between V1 and V2 ($p<10^{-5}$ in monkey R, and $p=0.002$ in monkey P, permutation test), but not any of the other region pairs. Together, these findings suggest that orientation information is represented throughout the visual hierarchy, including in areas TEO and TE. As orientation information traverses the hierarchy, it gets successively more clustered into discrete patches, consistent with meso-scale encoding reported for complex object representations like faces in high-level visual cortex. Moreover, we find evidence of a direct information transfer from V1 to V2, supporting the existence of a communication subspace between these regions that is not replicated for other region pairs.

Disclosures: B. Karami: None. **C.M. Schwiedrzik:** None.

Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR405.14/H2

Topic: D.06. Vision

Support: ZIAMH002032

Title: Encoding of visual stimulus identity and reward size in inferotemporal, perirhinal, and orbitofrontal cortex of rhesus monkeys

Authors: *P. RODRIGUEZ[1], B. LI[2], M. A. ELDRIDGE[3], W. WANG[4], B. J. RICHMOND[5]; [1]NIH, Natl. Inst. of Mental Hlth. (NIMH), Bethesda, MD; [2]Natl. Inst. of Mental Hlth., NIMH, Bethesda, MD; [3]Lab. of Neuropsychology, NIMH, Bethesda, MD; [4]Natl. Inst. of Mental Hlth., Bethesda, MD; [5]NIMH, Bethesda, MD

Abstract: Evaluation of choice options based on visual cues is an essential component of human and other primate behaviors (e.g., whether to eat the green, yellow, or brown banana). Using aspiration lesions and chemogenetic techniques in nonhuman primates, our group has previously established that the inferotemporal cortex (including two subregions, areas TEO and TE), perirhinal cortex (PRh), and orbitofrontal cortex (OFC) form a circuit through which the value of visual stimuli may be estimated. However, the computations performed in the individual regions of this circuit are not clear yet.

To investigate region-specific stimulus-reward encoding, we implanted multi-electrode Utah arrays in PRh, OFC, TEO, and TE in two rhesus macaques. Neuronal activity was recorded while subjects performed a behavioral task in which four images were each associated with a different size of liquid reward. Subjects pressed a bar to initiate a trial. A red dot appeared at the center of the screen. A cue image, indicating the reward size available, appeared behind the red dot. The red dot turned green after 500-1500ms ('go' cue) indicating that the bar could be released to obtain the liquid reward. Bar releases before the red dot turned green or more than 1s after the go cue were counted as errors. After error trials, the same cue image would be presented in the next trial. We evaluated stimulus-reward association at the behavior level by counting the error rate for each reward size. Error rates were lower in trials featuring larger rewards, indicating that subjects learned the stimulus-reward association.

To differentiate reward-selective and image-selective responses, the mapping of images to reward sizes changed between blocks of trials. Subjects were able to learn the new stimulus-reward association in each block. The selectivity of individual cells in each region was evaluated by examining their firing rate after image onset. Cells showing significantly elevated activity for a particular image across blocks, regardless of mapping to reward size, were determined to be image selective. Similarly, cells showing a sensitivity to reward size regardless of cue mapping were determined to be reward selective. The time course of information was evaluated by examining activity across regions over the period following cue onset. We found image-selective neurons in all four regions studied but, reward-selective neurons have most often been seen in

OFC. Analysis of the time course of information shows a visual response first in area TEO and then later in TE, PRh, and OFC. The activity of TEO peaks later than in other regions, suggesting that it may receive feedback from downstream areas.

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Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR405.15/H3

Topic: D.06. Vision

Support: Award from National Eye Institute of the National Institutes of Health R00EY031795
McDonnell Center for Systems Neuroscience Grant
Sofina & Boël Master's level research Fellowship from the B.A.E.F

Title: Shadow-discounted lightness signals in primate visual cortex

Authors: *F. DIDEHVAR[1], R. VANSTIPHOUT[3,2], P. CAVANAGH[4], T. P. FRANKEN[1]; [1]Washington Univ. in St.Louis, SAINT LOUIS, MO; [2]Washington Univ. in St.Louis, Saint louis, MO; [3]KU Leuven, Leuven, Belgium; [4]Psychological and Brain Sci., Glendon Col., Toronto, ON, Canada

Abstract: In some situations, a black object in sunlight may reflect more light than a white object in shadow, nevertheless we easily perceive the object reflectance as its lightness (how white/grey/black an object is) - vision discounts the effect of shadows to infer the true reflectance of objects. It is not well understood how this works. Here we investigate whether cortical area V4 contains shadow-discounted, lightness signals. Our choice to record from V4 is motivated by previous findings that this area contains both neurons tuned for surface features (e.g. color) and neurons tuned for cues that may help to identify shadows (blur, illumination vectors). Moreover, it is known to take features of illumination into account, e.g. its spectral content. We used Neuropixels to record from single units in V4 of the awake behaving macaque while displaying variations of Adelson's checkershadow stimulus, after confirming in psychophysics experiments that the monkey demonstrates some degree of lightness constancy in this stimulus. We used checkerboards that were partially covered by shadow (shadow boards), as well as paint boards, in which the pixels of each square are set to the average luminance of that square in the shadow boards, and shuffled boards, in which the position of the squares of the paint board were randomly permuted. The scenes were displayed such that the measured classical receptive fields (cRFs) were centered on a specific square in the left half of the board. We trained a random forest decoder on spike counts to the paint boards to predict the luminance

of the square centered on the cRFs, separately for each recording session. We then used this decoder to predict the luminance of the same square from the shadow board responses recorded in the same session (train paint/test shadow). We also did the opposite (train shadow/test paint). Train paint/test shadow decoders predicted significantly higher luminance than train shadow/test paint decoders for the majority of penetrations (permutation test $p<0.05$), consistent with the lightness constancy in the checker-shadow stimulus. In addition, train shuffled/test paint decoders predicted significantly higher luminance than train paint/test shuffled decoders, indicating that the dark surround in shadow regions contributes to the lightness constancy in the checkershadow stimulus, consistent with our psychophysics data in monkey and humans. We identified shadow-discounted lightness and simultaneous contrast signals in area V4 of the primate visual cortex. This suggests that V4 neurons may segment visual scenes into regions of illumination, in addition to their better understood role in figure-ground segmentation.

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Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

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Program #/Poster #: PSTR405.16/H4

Topic: D.06. Vision

Support: NIH Grant NS125843

Title: An opto-tagging approach to studying extrastriate projection neurons in macaque V1

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Abstract: As our understanding of cortical processing improves, the need for effective tools to target specific neuron types in the macaque brain grows. There are fewer tools available for this purpose in macaques than in rodents. Historically, functional inter-areal projections in macaque have been studied with antidromic electrical stimulation. This method does not define specific neuron types beyond the definition of their projection zone. Viral delivery of actuator proteins such as opsins to specific neuron populations can overcome this limitation. We injected *AAVretro-CAG-ReaChR-mCitrine* into V2 of 2 macaques. After a survival period of 7-9 mo, we recorded opto-tagged V1 to V2 projection neurons under anesthesia using multicontact linear recording arrays. We compared the visual responses of opto-tagged neurons to those described by El-Shamayleh et al. (2013, J Neurosci), in which V1 to V2 projecting neurons were identified using antidromic stimulation. We used the response to pulses of 620 nm light to identify 34 light

responsive neurons out of 92 total, across 4 recording arrays. We distinguished projection neurons from synaptically connected ones by measuring the onset latency and the trial to trial precision of the light-evoked spike trains, and took neurons to be projection neurons if they showed both short onset latency and high reliability. Eight of the 34 light responsive neurons (24%) met the criteria for a total yield of 9% (8/92), similar to the yield of antidromic stimulation experiments (10%). We studied the visual responses of a subset of neurons (70/92), including 4 projection neurons, using a battery of visual stimuli including drifting gratings varying in direction, drift rate (TF), and spatial frequency (SF). Compared to the antidromically identified neurons, the opto-tagged neurons displayed similar selectivity to stimulus orientation (mean orientation selectivity index: 0.4), and preferred similar SFs and TFs (mean preferred SF: 1.5 cpd, mean preferred TF: 5.1 hz), though they were less direction selective (mean direction selectivity index: 0.1). This may reflect a bias toward the superficial layers in our sample, which are less direction selective on average. These results suggest that opto-tagging is an effective way to characterize projection neurons in macaque visual cortex. Importantly, unlike electrical stimulation, this method can be used to target neuron subtypes with increasing precision as genetic and molecular tools are refined in the macaque.

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Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

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Topic: D.06. Vision

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LABEX CORTEX ANR 11 LABX 0042
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Title: Transcriptomic cell type composition and connectivity profiles define the retinotopic organization of macaque early visual cortex

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Abstract: Visual cortex is intensively studied as a model system for understanding the organizational principles of the neocortex, but previous studies of the early visual cortex were largely restricted to the representation of central visual field. Single nuclei RNA sequencing and single-cell spatial transcriptomics has recently been developed as a powerful tool for investigating cellular diversity across regions. Here, applied to the early visual cortex in areas V1, V2, V4 and MT this approach indicates significant variation of cell-type composition across retinotopic subdivisions, suggesting regional changes in the underlying in-put out-put relations, and marked difference according to eccentricity and dorsal ventral subdivisions. Particular attention was paid to primate specific cell types in layer 4 and layer 5 as defined by Chen et al. (2023). Tracer injections in these retinotopic subdivisions revealed dramatic variations of connectivity profiles. Injections in peripheral retinotopic subdivisions reveal projections that are not labeled by central injections. Connection strengths vary significantly with eccentricity in a systematic manner with respect to distance and origin, i.e., projections to central and upper visual fields are significantly stronger from ventral stream areas, projections to peripheral and lower fields are stronger from the dorsal stream areas. Non-invasive functional connectivity suggests a similar anatomical organization in the human brain. Comparison of transcriptomic based UMAPS and connectomic based multidimensional scaling show clear relationships. These results are related to ongoing efforts in the development of cell atlases of the human and NHP brain and are discussed with respect to the differing cognitive and perceptual roles of the retinotopic subdivisions of the early visual cortex.

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Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR405.18/H6

Topic: H.04. Executive Functions

Support: DFG SPP 2205
DFG SFB 1280

Title: The avian "visual cortex" at work - Basic properties and information flow in the pigeon's tectofugal visual system

Authors: *R. PUSCH[1], R. REICHERT[2], O. GÜNTÜRKÜN[3];
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Abstract: Profound visual abilities are fundamental to higher cognitive functions and enable visually guided adaptive behaviors in birds and mammals. Despite comparable 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 repetitive blueprint, the visual environment is centrally represented in a topographical manner. Additionally, sensory processing in the neocortex requires feedforward and feedback information flow between cortical areas. In contrast, the avian visual forebrain was thought to be organized in multiple clusters that at first glance resemble the histological pattern known from the basal ganglia. However, a recent anatomical study has shown that the cytoarchitectonic organization of the sensory avian forebrain shows striking similarities to its mammalian isocortical counterpart. This leads to the hypothesis, that neuronal computations and information flow, in the different aspects of the avian visual forebrain also correspond to this similarity. To test this hypothesis, we used a twofold strategy: In acute preparations, we performed electrophysiological recordings with multi-site silicon probes in the visual forebrain of pigeons to investigate basic coding properties and feedforward processing. We simultaneously recorded neuronal responses in the receiving visual forebrain center the entopallium, the visual associative mesopallium ventrolaterale, and the intermediate nidopallium - regions of a putative visual hierarchy. Visual stimuli such as gratings and random dot patterns were engaged. To additionally assess feedback information flow, pigeons were tested in an ABA

extinction learning paradigm, while we recorded from the above visual areas. During the acquisition phase, pigeons had to associate visual stimuli with a food reward in context A (i.e., white ambient light). In the subsequent extinction phase, the ambient light (context B) and the reward contingencies were changed so that responses to a stimulus were no longer followed by a food reward and thus extinguished. Once the response behavior ceased, the context conditions were changed back to the acquisition context to test for renewal. Critically, these experiments allow assessing both feedforward (stimulus properties) and feedback information (i.e. context and reward contingencies). Our research strategy thus permits a detailed analysis of the neural responses between each region at work, allowing for a direct comparison of avian and mammalian computations in the visual forebrain.

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Poster

PSTR405

Visual Cortical Circuits and Modulatory Mechanisms I

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Topic: D.06. Vision

Support: NIH Grant 1ZIAMH002967-07
BRAIN Initiative Grant U19NS107464

Title: Nonsensory "quiet" neurons in mouse V1 are highly excitable

Authors: *N. FRIEDMAN[1,2], C. M. PHILLIPS[3], P. K. LAFOSSE[3,2], Z. ZHOU[3], Y. DENG[3], M. H. HISTED[3];
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Abstract: In mouse primary visual cortex (V1), between 15-25% of neurons do not respond during visual stimulation, across a wide variety of natural and synthetic stimuli (Yoshida & Ohki, 2020; de Vries et al., 2020). These nonsensory ("quiet") neurons are seen throughout sensory cortices and species, for example in mouse primary somatosensory cortex (O'Connor et al., 2021; Pancholi et al., 2023), rat primary auditory cortex (Insanally et al., 2019), and both cat and rat visual cortex (Ohki et al., 2005). How do these neurons differ from sensory responsive neurons? Here we find, using single-cell optogenetics, that quiet neurons are highly responsive to input.
To measure responses to input we combine 2-photon calcium imaging and 2-photon optogenetic stimulation. We measure visual responses of excitatory neurons in layer 2/3 to a small stimulus (grating patch; raised cosine mask, FWHM=15 deg, spatial freq. 0.1 cyc/deg, pos. matched to V1 map at imaging location). In our data, 57% of neurons respond ($|dF/F| \geq 10\%$) to at least one visual stimulus. We photostimulate neurons chosen at random in small (N=1-11 neurons),

spatially dispersed groups. Neurons with small or no visual responses tend to have larger optogenetic responses (neurons with visual responses in lowest 25% of population, optogenetic dF/F mean: 20.5%±1.5, largest 25% of visual responses: 14.8%±1.9, N=100 cells, N=3 animals, power per cell held constant within experiment, Wilcoxon rank sum one-sided P<10-5). Deconvolution analyses also suggest higher spontaneous activity in quiet cells (Wilcoxon rank sum P<10-6; OASIS). These observations may suggest that quiet cells have different recurrent connectivity and input from the local network, compared to visually responsive cells. However, another possibility is that opsin expression differences might create these differences. To rule this out, we compared mRuby expression, the opsin's fluorescent tag, across populations and found no sig. difference between them (visual: 34.1±1.44 A.U. and quiet: 36.5±1.25 A.U., Wilcoxon rank sum one-sided P=0.13). In sum, we find quiet cells are highly excitable, showing both high spontaneous firing rates and high amplitude photoresponses. While there may be some sensory stimuli quiet cells respond to, it is nonetheless surprising that there is a subgroup of highly excitable cells with low amplitude responses to oriented stimuli. Neuronal activity can change over time to represent different sensory features (Deitch et al., 2021; Marks & Goard et al., 2021; Pancholi et al., 2022). These quiet cells may be permanently nonsensory, or cells may move between quiet and sensory states as animals undergo new sensory experiences.

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Poster

PSTR406

Motor Learning in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.01/H8

Topic: E.04. Voluntary Movements

Title: Fundamental processes in sensorimotor learning: Reasoning, Refinement, and Retrieval

Authors: *J. TSAY[1], H. E. KIM[2], S. D. MCDOUGLE[3], J. A. TAYLOR[4], A. M. HAITH[5], G. AVRAHAM[6], J. W. KRAKAUER[5], A. G. COLLINS[7], R. IVRY[8];
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Abstract: Motor learning is often viewed as a unitary process that operates outside of conscious awareness. This perspective has led to the development of sophisticated models designed to elucidate the mechanisms of implicit sensorimotor learning. In this poster we argue for a broader perspective, emphasizing the contribution of explicit strategies to sensorimotor learning tasks.

Furthermore, we propose a theoretical framework for motor learning that consists of three fundamental processes: Reasoning, the process of understanding action-outcome relationships; Refinement, the process of optimizing sensorimotor and cognitive parameters to achieve motor goals; and Retrieval, the process of inferring the context and recalling a control policy. We anticipate that this "3R" framework for understanding how complex movements are learned will open exciting avenues for future research at the intersection between cognition and action.

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Poster

PSTR406

Motor Learning in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.02/H9

Topic: E.04. Voluntary Movements

Support: NIH Grant R01

Title: Covert attention and implicit motor learning

Authors: *A. SHARMA, A. FORRENCE, S. D. MCDOUGLE;
Psychology, Yale Univ., New Haven, CT

Abstract: Motor adaptation is essential when people need to adjust to changes in the environment and body, ensuring accurate and efficient movements. While this form of motor learning is implicit - it proceeds fully without our awareness - it is thought to interact with cognitive systems involved with planning and decision-making. But how does motor adaptation interact with attention? Here we investigated two potential roles of attention, in particular covert spatial attention, in motor learning: a) whether it can serve as a context cue to partition motor memory, and b) whether it can modulate error sensitivity. In terms of covert attention acting as a context cue, it is generally assumed that mitigating interference requires associating competing learning contexts with distinct cues directly related to movement, like pre-planning and follow-through movements. In this study, we tested the role of nonmotor covert spatial attention signals in mitigating interference in a visuomotor adaptation paradigm. Second, while it is generally believed that the distance of a visual error from the fovea can influence the effect of those errors on visuomotor learning, it is not clear what role covert attention (rather than gaze location) may play in error sensitivity. Thus, we also asked if spatial alignment between covert attention and visuomotor errors influenced adaptation. Participants performed single-trial visuomotor rotation adaptation in a dual-adaptation paradigm, making wrist movements with a joystick. To ensure the covert nature of spatial attention in our task, eye-tracking was employed to monitor participants' fixation. Our current findings reflect a strikingly close relationship between covert

spatial attention and both action planning and error processing, though a role of covert attention in contextual cueing appears to be subtle or non-existent. These findings offer fresh perspectives on the interplay between covert attention and implicit motor learning, hinting that implicit motor learning may be directly influenced by covert attentional processes.

Disclosures: A. Sharma: None. **A. Forrence:** None. **S.D. McDougle:** None.

Poster

PSTR406

Motor Learning in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.03/H10

Topic: E.04. Voluntary Movements

Support: DST/CSRI/2021/164(C)(G)
SRG/2021/001125

Title: Interaction between contextualized motor memories during acquisition and expression

Authors: *N. KUMAR[1], A. D. KUMAR[2], A. KUMAR[3];
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Abstract: Learning, modification and expression of multiple motor skills is one of the most important motor behaviour that we possess. How do we build memories of all these skills without any interference among them and select and express the appropriate skill when required, is a fundamental question about our motor behaviour. One proposed hypothesis is that attaching distinct contexts to different skills helps in storing and retrieving them separately when presented with relevant attached context. How these contextual memories interact with each other is still not well understood. We investigated how presence of one context attached to one motor skill influence other memories during learning and expression. We employed a visuomotor rotation paradigm where participants learned Task A(30-degree clockwise rotation) and Task B(30-degree counterclockwise rotation), with distinct contexts. The tasks were learned for varying amounts of time and in different orders (block or interleaved). During expression, participants performed Task N with no task error but with either context A, context B, or no context. We examined how contextual cues drive the expression of motor memories across six experiments. The first two experiments revealed that although contextual cues lead to expression of distinct memories, the expression has bias towards more stable and more recent memories. The next two experiments where tasks were learned in block design but contexts were randomly switching during expression, also produced bias in expression based on stability and recency. However, the memories could not be distinctly expressed. The results suggest that different contextual memories interact with each other and multiple memories are updated even when one context is present based on the inference made about the present context. In the last set of experiments,

subjects learned the tasks in interleaved manner. Subjects were able to express the memories according to relevant contexts, irrespective of whether the contexts were presented in stable, block or random, interleaved manner. These results show the benefit of learning in a variable environment with high-transition probability across multiple contexts. Overall, the study presents behavioral evidence to support COIN model i.e we maintain a repertoire of multiple memories attached to different contexts and based on the inference about the current context, multiple relevant memories are updated or expressed. The findings also have significant implications for the development of more effective motor skill learning strategies and for understanding the neural mechanisms underlying motor skill learning.

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Poster

PSTR406

Motor Learning in Humans

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Program #/Poster #: PSTR406.04/H11

Topic: E.04. Voluntary Movements

Title: Difference in the retention of multiple motor memories following contextual learning

Authors: *A. OGAWA, N. ABEKAWA, H. GOMI;
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Abstract: People can acquire and retain multiple motor skills simultaneously. Previous studies have shown that two opposing visuomotor rotations (i.e., CW and CCW), which usually interfere with each other, can be learned when each is associated with valid contextual cues. If the learning rate and the final amount of learning were respectively equivalent for the two rotations, one might expect the memory retention to be similar for the two rotations. However, there is no experimental evidence for this yet. Here, we investigated the memory retention when multiple motor memories were acquired simultaneously in visuomotor rotation task. In the experiment, participants exhibited four types of trials which consisted of the spatial combination of visual targets (left and right of body center) and gaze positions (center, left and right of body center). They learned opposing visuomotor rotations (CW and CCW) whose angle gradually increased up to 20 deg. The direction of angle changed randomly across trials, but uniquely associated with reaching the target in the left and right visual fields. Participants were sitting idle for 12 minutes after learning, followed by a retention test session where they performed four trial types without visual feedback. The results showed that learning progress was similar across the four trial-types, but subsequent motor retention differed. More specifically, the adapted motor outputs were maintained (i.e., memory retention) in the two trial types in which the learned trajectory was away from the body center, whereas motor outputs returned to the baseline (i.e., memory forgetting) in the other two trial types in which the learned trajectory was towards the body

center. The results indicate that specific trial-type memories are strongly forgotten, contrary to the naïve prediction that memory forgetting is observed to the same extent across conditions. The forgetting trial type can be explained by any of the following three accounts for the reaching trajectory after learning: 1) being close to the front of body center, 2) changing toward the front of body center, and 3) approaching in two conditions. To disentangle these possibilities, we conducted Expt. 2 with target and gaze positions only in the left side of body. The results support the second account. These results highlight an important aspect of the learning mechanism in simultaneous learning: learning progress and memory retention are, at least in part, processed in different ways. The retention and forgetting we observed across conditions might be related to the reliability and/or systematic bias of hand proprioceptive position sense.

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Poster

PSTR406

Motor Learning in Humans

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Program #/Poster #: PSTR406.05/H12

Topic: E.04. Voluntary Movements

Support: NIH NINDS (NS116883)

Title: Computational mechanisms underlying strategy discovery in motor adaptation

Authors: *A. NIYOGI[1], E. CISNEROS[2], R. IVRY[3], J. S. TSAY[4];
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Abstract: Sensorimotor adaptation, the process of correcting motor errors through feedback and practice, keeps our movements well-calibrated in response to changes in the body and environment. Extensive research has been devoted to understanding the implicit processes underlying motor adaptation. However, less explored are the mechanisms underlying the discovery of a volitional explicit strategy, a learning process that can support rapid and flexible changes in behavior. To address this question, we developed a novel “Point and Click Task” designed to test strategy discovery in response to a wide range of visuomotor perturbations (rotation, reflection, translation, gain adjustments in the visual feedback), while minimizing the contribution of motor noise and implicit adaptation. Using computational modeling, we examined whether strategic discovery resembles a gradual process of error reduction, or an exploratory process of hypothesis testing involving a range of action-outcome relationships. Contrary to the predictions of the gradual error reduction model, we observed substantial individual differences in participant behavior, including sudden, punctuated jumps in performance (‘moments of insight’) at different time points across participants and varying

degrees of systematic exploratory behavior ('sign flips'). Both group and individual learning functions were well-captured by the hypothesis testing model, altogether furthering our understanding of the computational mechanisms underlying the discovery of an explicit sensorimotor strategy.

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Poster

PSTR406

Motor Learning in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.06/H13

Topic: E.04. Voluntary Movements

Title: A Bayesian decision-making model of implicit motor adaptation to small errors

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Abstract: Not all movement-related errors are created equal. The sensorimotor system is constantly tasked with deciding which errors to learn from and which to ignore. Recent work has shown that humans are able to accurately parse internal versus external sources of motor errors as small as 2 deg during reaching experiments involving external perturbations from either a force field or visuomotor rotation (Ranjan & Smith 2018). Participants implicitly adapted subsequent reaches in response to such externally-caused errors, moving in the direction opposite the perturbation. Remarkably, experiencing an error of the same size, but caused by an internal source (i.e., intrinsic motor noise), elicited no adaptation. The primary aim of the current study was to understand the computational principles underlying such finely-tuned adaptive responses. We started by successfully replicating the main results of Ranjan and Smith, testing 16 healthy, neurotypical young adults on a visuomotor rotation task in which rotations of 0, ±2, and ±4 deg were applied to the cursor feedback and pseudorandomized across the experimental block. All 16 participants demonstrated robust implicit adaptation to the small non-zero cursor rotations, while simultaneously showing little-to-no response to similar-sized errors due to motor noise. To formalize our understanding of this behavior, we developed a novel Bayesian ideal observer model referred to as the Parsing of Internal and External Causes of Error (PIECE) model. Similar to prior Bayesian models of motor adaptation (e.g., Wei & Kording 2009), causal inference regarding whether sensory feedback is perturbed or not is central to the PIECE model. Unlike prior models, though, the PIECE model assumes that, in addition to visual and proprioceptive cues, the observer also has access to an efference copy of the motor command which includes associated motor noise. The PIECE model conceptualizes adaptation as a process of utilizing all three cues to form a posterior belief regarding the presence (or absence) of a perturbation, with

motor output reflecting the weighting of the estimated perturbation size by this degree of belief. This framework contrasts with a class of computational models that frames adaptation as a process of aligning the perceived hand position with the movement goal (Wei & Kording 2009, Tsay et al 2022, Zhang et al 2024). The present study challenges such a view, as only the PIECE model could accurately capture the precise parsing of internal versus external errors observed. Our work instead supports a normative view of implicit adaptation in which prediction, perception, and error correction are unified.

Disclosures: H.E. Kim: None. **D. Hu:** None. **R. Chua:** None.

Poster

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Motor Learning in Humans

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Program #/Poster #: PSTR406.07/H14

Topic: E.04. Voluntary Movements

Support: NSF grant BCS 2216344

Title: Double dissociation between savings and long-term memory in motor learning in strong but not weak learners

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Abstract: A recent visuomotor adaptation study with a single target (Hadjiosif et al., 2023) showed that a persistent memory accounted for 24-hour retention, whereas a temporally volatile memory accounted for savings in relearning. Here, we ask whether a fast and slow adaptation model can account for these results and whether strong and weak learners, who show qualitatively different behaviors (Oh and Schweighofer 2019), differ in their 24-hour retention and savings. 37 subjects performed a two-day online visuomotor adaptation. On day 1, subjects adapted to a 30-degree perturbation to 8 targets in 3 blocks separated by short blocks of no feedback trials. On day 2, subjects performed a no-feedback retention block and a relearning block. A fast-slow model was individually fitted to all subjects' data on day 1. Slow and fast processes at the end of day 1 correlated with day 2 retention ($p < 0.0001$, $R = 0.56$) and savings ($p < 0.0001$, $R = 0.55$), respectively, but not with savings and retention ($p = 0.10$ and $p = 0.098$), showing a double dissociation. We then clustered the subjects into strong and weak learners based on overall learning level at the end of day 1. For strong learners ($N = 26$), despite the wide range of differences in learning rates (fast: 95% CI = [0.14, 0.27]; slow: 95% CI = [0.0030, 0.011]), a strong negative correlation between slow and fast processes ($p < 0.0001$, $R = 0.90$) showed that the two processes complement each other (also see Yamamoto et al. 2020). Weak learners exhibited retention (t-test, $p < 0.0001$) but no savings (t-test, $p = 0.43$), as well as a

positive correlation between slow and fast processes ($p = 0.032$, $R = 0.55$). This suggests a single memory process in this group, which was further supported by a bootstrapped BIC analysis comparing two- and single-process models. The single process correlated with retention ($p = 0.037$, $R = 0.40$) but not with savings ($p = 0.83$), and the single time constants of the weak learners and the slow process time constants of the strong learners showed no differences (t-test, $p = 0.42$). Our results suggest that, for strong learners, the temporally persistent and temporally volatile components of memory can be mapped to complementary fast and slow processes despite large interindividual variability in fast- and slow components in our multiple-target adaptation experiment. In contrast, the weak learner group did not develop a fast, volatile component. Our findings underscore the importance of considering both quantitative and qualitative individual differences in motor learning.

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Poster

PSTR406

Motor Learning in Humans

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Program #/Poster #: PSTR406.08/H15

Topic: E.04. Voluntary Movements

Title: Cross-sensory congruency facilitates motor adaptation

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Abstract: Motor adaptation is usually examined in a context of normal, upright posture with a general alignment of head axis and visual environment. We sought to identify how conditions where this default arrangement was violated, interfered with motor adaptation. In a visuomotor adaptation center-out reaching paradigm, participants (right-handed, 18-30 yrs, 17 female) used a table-mounted joystick to control a cursor on a monitor vertically positioned in front of them, moving the cursor from a starting position in the center of the monitor to a target at either 90º or 270º. Their head was supported by a laterally tiltable chin and headrest. The task followed a traditional baseline - exposure - post-exposure design. 39 participants were randomly assigned to one of three groups: Control group (G1) - head and monitor upright during exposure; Visual Incongruency group (G2) - head tilted 30º to the right, monitor upright; Visual Congruency group (G3) - head and monitor tilted 30º to the right. Three baselines (12 trials each) addressed the group-specific head/monitor orientations with veridical cursor feedback. During exposure (140 trials), the cursor was rotated 30º counterclockwise, requiring participants to direct their aim by the same amount clockwise to counter the perturbation. During early post-exposure (8 trials), each group's head/monitor orientation remained, but with veridical cursor feedback. In late post-exposure, participants' head was righted again to test for potential vestibular aftereffects. Results

showed that G3 adapted fastest to the perturbation, particularly for movement path linearity (RMSE), commonly associated with feedback control processes; conversely, G2 adapted slowest and least. Since for both G1 (controls) and G3 there was congruency between head and display axis, but G3 with tilted head and display axis outperformed G1, we suggest that the head and display monitor tilt condition benefitted from an additional kinesthetic congruency, established by hand movements towards the right side. We did not observe any aftereffects after the head was righted again, indicating a minor role of vestibular information in this adaptation.

Disclosures: A. Wolf: None. **V. Kalepu:** None. **F.A. Kagerer:** None.

Poster

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Motor Learning in Humans

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Topic: E.04. Voluntary Movements

Support: Australian Research Council DP200100234
Australian Research Council FT230100656
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Title: Investigating interactions between cardiovascular exercise, neuroplasticity, and implicit motor sequence learning

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Abstract: Developing and refining new motor skills are fundamental components of interacting with our environment. Acquiring novel skills commonly involves practicing sequences of motor actions (i.e., motor sequence learning; MSL), though this capacity varies substantially across individuals, and underlying mechanisms are an area of ongoing research. Recent work has demonstrated that the MSL occurs across multiple temporal and spatial scales in the brain (Bönstrup et al., 2019; Brooks et al., 2024). For instance, during early MSL, rapid improvements in skill occur across brief (e.g., 10s) 'micro-offline' rest periods, underpinned by parietal-hippocampal network activity. Comparatively, learning across longer timeframes is linked to gamma-aminobutyric acid (GABA) activity in motor areas (Stagg et al., 2011). However, this evidence remains preliminary and the mechanisms linking rapid 'micro-consolidation' to overall gains in skill following sustained practice are not clearly defined. Similarly, the degree to which interventions with known impact on learning, such as high-intensity interval exercise (HIIT), may influence these interactions remains unclear. As such, we investigated the effect of an acute bout of HIIT on the neural mechanisms underlying micro-consolidation of implicit MSL, using

resting-state fMRI and MR spectroscopy. Thirty-eight (39.5% female) right-handed, healthy adults (M_{age} = 22.55; range 19-28) were randomised into either 20-minutes of HIIT (n=19) or very low intensity (n=19) cycling, followed by an implicit serial reaction time task. MRI measures were acquired before and after exercise, and during the task. We report evidence of micro-consolidation of implicit MSL. However, HIIT did not impact micro-consolidation (p = .63, BF_{10} = 0.37), nor the overall degree of MSL following continued practice (p = .77, BF_{10} = 0.34), though in line with our previous work, we observed a modulation of sensorimotor GABA concentration following HIIT (p = .033, BF_{10} = 2.09). We also show that early MSL is associated with resting-state hippocampal network activity (p = .011, BF_{10} = 4.73), while overall gains in skill are linked to motor network connectivity and GABA concentration (R^2 = .27, p = .016). In summary, our findings indicate that implicit MSL is supported by networks implicated in both declarative and procedural learning. Further work is required to determine how interventions such as exercise may be applied to influence these dynamics.

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Poster

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Motor Learning in Humans

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Topic: E.05. Brain-Machine Interface

Support: NINDS Intramural Research Program (IRP)

Title: Optimized decoding of individual sequence actions and neural replay during human skill learning

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Abstract: Activities of daily living rely on our ability to learn new sequential motor skills. Hippocampo-neocortical neural replay, the temporally compressed reactivation of neural activity patterns representing behavior during rest, may contribute to early skill learning [1]. Decoding accuracy of individual sequence actions used to detect skill-related replay sequences has been limited to a range of 60.2-68.5% [1]. Improving decoding accuracy would substantially enhance our ability to investigate neural replay in humans with magnetoencephalography (MEG). Here, we explored various decoding strategies for individual finger movements embedded within action sequences. We analyzed MEG activity from 26 participants while they learned a novel

explicit keypress motor skill. We trained machine learning decoders to classify individual finger typing movements from MEG oscillatory activity measured in sensor or source space.We found that decoders trained on broadband consistently outperformed those trained on narrowband activity, and that whole-brain parcel-space (N = 148 brain regions or parcels) or voxel-space (N = 15684 voxels) decoders exhibited greater decoder performance (parcel: t = 1.89, $p = 0.035$; voxel: t = 7.18, $p < 0.001$) than individual voxel-space intra-parcel decoders. The highest performing intra-parcel decoders (superior frontal cortex) predicted individual finger movements with up to 68.77% accuracy, while accuracy for whole-brain inter-parcel decoders reached 74.51%. Next, we constructed hybrid space decoders (N = 1295 ± 20) combining all inter-parcel activity with main intra-parcel features (top 8 regions, optimally selected based on accuracy saturation) that improved the accuracy to 78.15%. Finally, manifold representation of these hybrid space features further improved decoding accuracy to 90.47%.We conclude that decoding of finger movement action sequences is optimized when neural activity recorded with MEG is represented in a hybrid spatial source space. This approach substantially enhances decoding accuracy compared with previous reports (90.47% vs ~65%) and furthers our ability to characterize neural replay and could contribute to non-invasive brain-computer-interface (BCI) applications in humans.References:1. Buch ER, Claudino L, Quentin R, Bonstrup M, Cohen LG: Consolidation of human skill linked to waking hippocampo-neocortical replay. *Cell Rep* 2021, 35(10):109193.

Disclosures: D. Dash: None. **F. Iwane:** None. **W. Hayward:** None. **M. Bönstrup:** None. **E.R. Buch:** None. **L.G. Cohen:** None.

Poster

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Motor Learning in Humans

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Program #/Poster #: PSTR406.11/H18

Topic: E.04. Voluntary Movements

Title: Deciphering single motor unit activities during motor skill learning

Authors: *L. HOU, S. BAO, Y. LEI;
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Abstract: To regulate motor behaviors, the motor cortex generates task-specific signals and transmits them to motoneurons. Consequently, the discharge pattern of the motor units reflects the control strategy selected by the nervous system. In this study, we examined the control of single motor units during motor skill acquisition, hypothesizing that recruitment and firing rates of motor units would be dynamically adjusted throughout the learning process. Participants were instructed to perform motor sequence learning with the first dorsal interosseous (FDI) muscle against a load cell to manipulate the position of a cursor on a display. To keep the cursor within

the designed pathway, participants generated muscle contraction at varying force levels in a specific sequence. Surface electromyography (sEMG) was captured using an 8 by 8 high-density EMG grid placed over the FDI belly and decomposed into waveforms of individual motor units via independent component analysis (ICA). The trajectory of the cursor was compared to the predesigned pathway, and the performance was assessed based on temporal and spatial (absolute) errors. Following the repetitive practice of the task, most of the participants demonstrated increased spatial accuracy, accompanied by changes in motor unit recruitment and discharge rates. Changes can also be observed by scrutinizing the inter-spike intervals between two units and coherence analysis in the frequency domain. These findings indicate that motor unit activities may serve as indicators of the neural strategies employed during motor skill learning.

Disclosures: L. Hou: None. **S. Bao:** None. **Y. Lei:** None.

Poster

PSTR406

Motor Learning in Humans

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Topic: E.04. Voluntary Movements

Support: CIHR MOP126158
NSERC PGS-D Graduate Award

Title: Prefrontal-hippocampal interactions support rapid behavioral adjustments during motor learning

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Abstract: Human motor adaptation is often treated as an iterative process, driven by the updating of an internal model by errors experienced during each trial. However, the parameter space for movements is generally extremely high dimensional, and iterative updating is inefficient until the brain has reduced the dimension of the problem space by identifying the goal-relevant features of the movement. This kind of dimension reduction has been well studied in the context of reinforcement learning, where prefrontal-hippocampal circuitry is believed to maintain a cognitive map of the task structure, allowing efficient learning in conditions of high complexity.
We trained subjects (N = 36) to generate curved movement trajectories in an fMRI scanner, subject to reward feedback. After learning to trace a visible target path, cursor feedback was

removed and score feedback reflected the accuracy in tracing the (invisible) mirror image path. In these and similar motor tasks, subjects often exhibit abrupt improvements in performance inconsistent with the gradual updating of internal models, and which may reflect (e.g.) the development of explicit knowledge of the task structure, or strategic changes in policy. We isolated these period of rapid learning, and studied changes functional connectivity between hippocampus and lateral/orbitofrontal cortex associated with individual differences in the rate of learning. In doing so, we replicated effects previously observed in Human one-shot learning, in which an increase in ventrolateral prefrontal and hippocampal functional connectivity signaled the onset of learning. We also observed a transient decrease in hippocampal-orbitofrontal functional connectivity during learning, which was restored upon a return to steady-state performance.
While these effects have not previously been observed in the context of Human motor learning, they have been well studied in animal models in the context of memory, reinforcement learning, and spatial navigation. In particular, they suggest a role for the episodic memory of action-outcome associations in the construction of a high-level model of the state and action spaces defining a motor learning task, and the subsequent use of this model to constrain the search space for efficient response selection.

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Poster

PSTR406

Motor Learning in Humans

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Topic: E.04. Voluntary Movements

Support: CIHR PJT 165987
CIHR PJT 183970

Title: The consolidation of newly learned movements depends upon somatosensory cortex in humans

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Abstract: Recent studies have indicated somatosensory cortex involvement in motor learning and retention. However, the nature of its contribution is unknown. One possibility is that the somatosensory cortex is transiently engaged during movement. Alternatively, there may be durable learning-related changes which would indicate sensory participation in the encoding of learned movements. In the present study we test the hypothesis that sensory system plasticity

underlies newly acquired movements, contrary to the prevailing belief that adaptation learning primarily involves motor controller adjustments. Participants were trained to make movements while receiving rotated visual feedback in a visuomotor adaptation task. The primary motor cortex (M1) and the primary somatosensory cortex (S1) were targeted for continuous theta-burst stimulation, while stimulation over the occipital cortex served as a control. Retention was assessed using active movement reproduction tests both immediately and 24h later. Suppression of the somatosensory cortex resulted in impaired motor memory in both tests. In contrast, suppression of the motor cortex had no impact on retention when tested immediately or 24h later, as indicated by comparable retention levels in control and motor cortex conditions. As an additional control, cTBS was applied to somatosensory cortex following training with unrotated feedback, yielding no impact on retention test movements, which indicated the specificity of cTBS effects on learning. These findings support the notion that S1 participates in the encoding of newly learned movements and in retaining human motor memory.

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Poster

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Motor Learning in Humans

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Topic: E.04. Voluntary Movements

Support: CIHR Grant PJT175012
NSERC Grant RGPIN-2017-04684

Title: Cortical and subcortical manifold structure during human motor learning and generalization

Authors: A. REZAEI[1], C. ARESHENKOFF[2], D. GALE[2], A. J. DE BROUWER[3], J. Y. NASHED[4], J. FLANAGAN[2], ***J. GALLIVAN**[2];
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Abstract: Being able to adjust our motor commands to cope with new and continuously changing environments is fundamental to daily life. Such adaptability is not just about mastering movements in a specific context, but also about how this learning transfers to different scenarios, a phenomenon termed ‘generalization’. Despite extensive research on the behavioral and computational processes that support motor generalization, its underlying neural mechanisms remain opaque. To bridge this knowledge gap, here we used functional MRI and advanced dimensionality techniques to investigate how brain regions reorganize their collective activity during sensorimotor adaptation and intermanual transfer. In our study, 38 human participants

learned to adapt their movements during a visuomotor rotation task performed with their right (dominant) hand. We then examined the transfer of that learning to the untrained (non-dominant) left hand. By projecting subjects' cortical, subcortical, and cerebellar functional connectivity patterns into a compact low-dimensional manifold space, we were able to disentangle functional connectivity changes related to the effector (right versus left hand) from those related to learning during both the adaptation and transfer phases of the task (independent of the effector). Our analyses revealed two main distinct patterns of neural reorganization. First, we found that the connectivity of several lower-order sensory-motor regions was selectively modulated based on the effector used, with manifold changes being topographically linked to contralateral hand control. Second, we found that several higher-order regions in transmodal cortex were selectively modulated based on the phase of the task (i.e., across baseline, early or late learning and transfer trials), with these manifold changes being largely bilateral in nature. In particular, we found that both the early learning and early transfer phases led to a significant expansion of transmodal regions along the whole-brain manifold, reflecting their increased covariance with other regions of association cortex. By contrast, during both the late learning and late transfer phases we found that these same transmodal regions now contracted along the manifold, which reflected their increased covariance with sensory-motor regions. Together, these findings provide a unique characterization of the whole-brain macroscale changes associated with sensorimotor adaptation, and offer a novel perspective on the role of transmodal cortex, and the DMN in particular, in motor generalization.

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Poster

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Motor Learning in Humans

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Topic: E.04. Voluntary Movements

Support: JST SPRING Grant JPMJSP2114

Title: Task complexity-related neurophysiological dynamics underlying visuomotor skill learning and interlimb transfer

Authors: J. ZHAO[1], Y. WANG[2], D. HOU[3], S. SUN[4], ***J. NEGYESI**[5,6], H. INADA[7], S. SHIOIRI[3], R. NAGATOMI[3];
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National Institute of Psychiatry, and Addictology, Budapest, Hungary; [7]Natl. Ctr. of Neurol. and Psychiatry, Kodaira, Tokyo, Japan

Abstract: Introduction: To understand how the human brain generalizes newly acquired motor skills to each hand is important for developing new rehabilitation methods. Previous research aimed to determine the neural correlates of either motor learning or interlimb transfer, however, its relation to task complexity is still unclear. Considering that laterality interacts with task complexity, we hypothesized that the level of task complexity determines the inter-hemispheric physiological dynamics of the brain.**Methods:** Healthy right-handed participants (n = 47) were randomly assigned to one of 4 conditions and performed 10 blocks of simple or complex visuomotor skill learning with their left or right hand with a 2-min rest between each block. The experiment was conducted in a dimly lit and electromagnetically shielded room, using a 19-in. (37.7 × 30.1 cm screen size) IBM LCD display (1280 × 1024 screen resolution). The stimuli were presented using a custom-made software. In addition to behavioural data (accuracy, task completion time), brain activity was monitored with a high-density electroencephalogram (EEG, BioSemi, Netherlands) at a sampling rate of 2048 Hz. The EEG data were processed using EEGLAB, an open-source toolbox running in the MATLAB2021b environment. Coherence between putative motor areas were also computed.**Results:** Task complexity affected motor skill performance and interlimb transfer, i.e., only participants who performed the complex visuomotor skill learning produced significant interlimb transfer, irrespective of the hand being trained (all $p < 0.05$). Regarding the neurophysiological data, participants who performed complex visuomotor skill learning with their right-dominant hand produced enhanced alpha coherence between the left primary motor cortex and supplementary motor area; and enhanced theta coherence between the left frontal and centroparietal cortex across training (alpha: $F_{(9, 99)} = 2.5$, $p = 0.023$, $\eta^2 = 0.185$; theta: $F_{(9,99)} = 2.9$, $p = 0.028$, $\eta^2 = 0.210$) and transfer sessions (alpha: $F_{(1,11)} = 9.6$, $p = 0.010$, $\eta^2 = 0.465$; theta: $F_{(1,11)} = 5.2$, $p = 0.043$, $\eta^2 = 0.321$).**Conclusions:** Overall, our study provides the first direct evidence of task complexity-related shared mechanisms between visuomotor skill acquisition and interlimb transfer as represented by the neural coherence within the motor, within the fronto-parietal, and across motor and parietal networks. Our ongoing research aims to comprehensively understand the influence of hemispheric asymmetries on disease development.

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Poster

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Topic: E.04. Voluntary Movements

Support: NIH Grant 1R15AG059095-01

Title: Using a reverse visually guided reaching task to distinguish between healthy aging and early alzheimer's disease

Authors: *B. WOOLMAN[1], A. WATRAL[2], K. TREWARTHA[1];
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Abstract: Changes in motor behavior may function as a proxy for cognitive decline. While Alzheimer's disease (AD) is associated with impairments in learning and memory, recent studies suggest that subtle changes in motor task performance may reflect early cognitive changes. For example, the visuomotor rotation task that manipulates visual feedback about hand position during reaching movements, can be used to examine cognitive changes in aging populations. The current study used the reverse visually guided reaching task (rVGR) which rotates visual feedback of participant's hand position 180º relative to the actual hand position. We sought to expand on previous literature by recruiting cognitively impaired individuals to characterize changes in rVGR performance in early AD. We also examined learning curves to assess the impact of cognitive impairment on learning in the rVGR task and probed the cognitive correlates of rVGR performance with a neuropsychological battery. We recruited young adults, and older adults (55 - 85 years old) with and without cognitive impairment to complete a VGR task with veridical mapping, and then the rVGR task. Overall, cognitively impaired adults exhibited longer reaction times and performed more corrective movements. Age differences were observed for nearly all overall measures of performance. The largest differences between healthy older adults and cognitively impaired adults were identified in the earliest stages of the learning curve. In the first few movements, the cognitively impaired group made more angular errors. Both overall- and early- measures of performance were correlated with measures of cognitive control. These findings add to the growing literature suggesting that sensorimotor adaptation tasks may be sensitive to early cognitive changes in AD.

Disclosures: B. Woolman: None. **A. Watral:** None. **K. Trewartha:** None.

Poster

PSTR406

Motor Learning in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.17/H24

Topic: E.04. Voluntary Movements

Support: NIH NINDS (NS116883)

Title: Impact of Parkinson's disease on explicit motor adaptation

Authors: *K. BOL[1], E. CISNEROS[1], S. J. ABRAM[1], L. SCHUCK[1], J. S. TSAY[3], R. IVRY[2]; [1]Psychology, [2]Psychology, Neurosci., Univ. of California, Berkeley, Berkeley, CA; [3]Psychology, Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Our ability to enact successful goal-directed actions involves multiple learning processes. Among these processes, the use of volitional, explicit strategies allow us to quickly and flexibly respond to changes in the environment. Whether Parkinson's Disease impacts explicit strategy use in response to a sensorimotor perturbation remains unknown. To address this question, we examined the performance of participants with Parkinson's disease (on medication) and age-matched controls (N = 16/group) on a visuomotor rotation task in which we delayed the feedback. The delay eliminates implicit recalibration and thus, successful learning requires discovering an appropriate strategy (i.e., to aim the movement in the opposite direction of the perturbation, see Brudner et al. 2016). Participants were exposed to a 60° visuomotor rotation in two learning blocks separated by a washout block with veridical feedback. This design allowed us to assess two components of this learning process: The initial exposure block assessed strategy discovery and the re-exposure block assessed strategy recall. Compared to age-matched controls, individuals with Parkinson's Disease exhibited intact motor adaptation performance during the discovery ($t(24.4) = 0.4$, $p = 0.7$) and recall ($t(23.2) = -0.9$, $p = 0.4$) blocks. These results demonstrate that neither strategy discovery nor strategy recall is impacted by Parkinson's Disease. Future experiments are required to examine whether the presence of dopaminergic medication would modulate performance in this population.

Disclosures: K. Bol: None. **E. Cisneros:** None. **S.J. Abram:** None. **L. Schuck:** None. **J.S. Tsay:** None. **R. Ivry:** Other; Co-founder with equity in Magnetic Tides, Inc..

Poster

PSTR406

Motor Learning in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.18/H25

Topic: E.04. Voluntary Movements

Support: NIH Grant R21HD111748
University of Minnesota Medical School Assistant Professor Award
National Center for Advancing Translational Sciences Grant UL1TR002494
Rehabilitation Science New Student Award
C-STAR Collaborative Mentorship Program Grant P2CHD101899

Title: Does cognitive load affect movement preparation and joint coordination differently based on lesion side?

Authors: *P. THAPA[1], L. ASGEDOM[1], M. FOLKERTSMA[2], S. LUNOS[3], D. M. CHAPPUIS[4], S. A. L. JAYASINGHE[1];
[1]Family Med. and Community Hlth., [2]Radiology, [3]Biostatistical Design and Analysis Ctr., Univ. of Minnesota, Minneapolis, MN; [4]Courage Kenny Rehabil. Inst., Eagan, MN

Abstract: Cognitive load can affect motor control, leading to decreased movement accuracy and changes in muscle coordination patterns. Previous studies have shown that with more cognitive demands, muscles involved in postural control on the right side of the body exhibit increased activity compared to those on the left side. Thus, there is likely a lateralized effect of cognitive load on muscle coordination patterns. In this study, we sought to examine whether each hemisphere contributes differently to the control of movement when presented with a cognitively challenging reaching task. We hypothesized that increased cognitive load results in motor deficits and muscle activation patterns that differ based on lesion side. We predicted increased reaction time and higher joint cocontraction levels with increased cognitive load in right hemisphere damaged (RHD) individuals compared to left hemisphere damaged (LHD) individuals with severe contralesional paresis. We developed a cognitively challenging reaching task (170 trials) on the Kinereach system where participants had to commit pictorial cues to memory in order to locate and reach for the target in a given period of time. In our ongoing study, we have recruited 14 chronic stroke survivors with severe hemiparesis (6 females; 8 males; age 57.07 years +/- 3.17 SEM; 8 RHD, 6 LHD). We attached 4 surface EMG sensors on the muscle bellies of the posterior deltoid, pectoralis, biceps, and triceps muscles of the ipsilesional arm to record muscle activity. We also collected position and orientation information of the ipsilesional arm using electromagnetic sensors. The level of cognitive load on the participant increased over the course of the task. Our results showed that reaction time increased with cognitive load level ($p < 0.0001$); however, no group differences were found. Joint cocontraction in the initial period after target appearance did not necessarily increase with cognitive load. We found no group differences in cocontraction at the shoulder joint ($p = 0.08$) or the elbow joint ($p = 0.20$). These preliminary results suggest that while movement preparation is impaired with increased cognitive load, this is not at the cost of differing muscle coordination patterns. More importantly, our results contradict our hypothesis by showing that these movement impairments are not lateralized. Perhaps this warrants further investigation of how future stroke rehabilitation paradigms are designed.

Disclosures: P. Thapa: None. **L. Asgedom:** None. **M. Folkertsma:** None. **S. Lunos:** None. **D.M. Chappuis:** None. **S.A.L. Jayasinghe:** None.

Poster

PSTR406

Motor Learning in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.19/H26

Topic: E.04. Voluntary Movements

Support: PVA 3188
Neilsen 890467

Title: Noninvasive Stimulation of the Sensory Thalamus Changes Sensorimotor Adaptation

Authors: *S. BAO[1], H. SOROUSHI[2], Y. LEI[2];
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Abstract: Noninvasive Stimulation of the Sensory Thalamus Changes Sensorimotor AdaptationShancheng Bao, Yuming LeiProgram of Motor Neuroscience, Department of Kinesiology & Sport ManagementTexas A&M University, College Station, TX, 77843.AbstractThe thalamus acts as a central hub for corticocortical communication in motor behaviors, integrating inputs from ascending pathways, the cerebellum, basal ganglia, and various cerebral lobes, and then relaying this information to the motor cortex. In this study, we focused on the sensory thalamus's role in dynamically controlling limb movements and adapting to perturbations. We utilized focal ultrasound stimulation (FUS) to induce the neuroplasticity of the sensory thalamus in 20 healthy subjects, ensuring precise targeting of the ventro-posterior lateral nucleus with personalized FUS parameters for each participant. Participants were tasked with controlling a cursor for reaching movements on a visual display following FUS. To assess the sensory thalamus's contribution to visuomotor adaptation, we introduced a 30-degree counterclockwise rotation to the cursor's position, requiring participants to adapt to the perturbation. After complete adaptation to this rotation, we switched the rotation direction to clockwise for 10 blocks and subsequently removed the cursor's visual feedback. Comparing performance in the FUS and sham conditions, we found that participants in the FUS group adapted significantly faster, supporting our hypothesis that the thalamus is pivotal in motor control and sensorimotor integration. These results also underscore the utility of FUS as an effective non-invasive method for influencing subcortical activity and altering motor behavior.

Disclosures: S. Bao: None. **H. Soroushi:** None. **Y. Lei:** None.

Poster

PSTR406

Motor Learning in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.20/H27

Topic: E.04. Voluntary Movements

Title: Enhancing motor learning with HD-tDCS: A pilot study on chopstick mastery and brain function in adults

Authors: *J. L. SCHOLL[1], L. A. BAUGH[1], T. J. BOSCH[2];
[1]BBS; CBBRe, Univ. of South Dakota, Vermillion, SD; [2]Psychology; CBBRe, Univ. of South Dakota, Vermillion, SD

Abstract: This pilot study extends our previous research on the neurological adaptations associated with learning to use chopsticks in right-handed adults. In this previous work, we observed increased functional activity and connectivity changes within the anterior supramarginal gyrus (aSMG), a brain region previously implicated in novel tool use. In the present pilot study, we investigated the effects of high-definition transcranial direct current stimulation (HD-tDCS) on motor learning by applying anodal stimulation to the aSMG in a double-blinded, sham-controlled session, 12 participants (7 active, 5 sham). Those in the active condition received 3mA of HD-tDCS focused over the aSMG while watching a 20-minute video of the task to be performed - picking up a marble with chopsticks and dropping it into a cylindrical container. In comparison, those within the sham condition watched the same video while being exposed to sham stimulation consisting of a 30s ramp-up and ramp-down at both the start and end of the 20-minute video. Immediately following the video task, participants performed 15 one-minute trials where they performed the task modeled in the video. Performance was assessed by the average number of successful marble drops per minute (MDPM) across trials. Results showed an overall significant increase in MDPM from the first to the last trial (11 vs. 19 MDPM; $p < .001$), with participants in the active stimulation group demonstrating an overall marked performance improvement across all trials compared to the sham group (18 vs. 13 MDPM; $p < .05$). These findings suggest that HD-tDCS enhances the rate of motor learning in novel tool use and underscore the potential of aSMG-targeted stimulation in facilitating complex motor tasks. Further studies are warranted to explore the broader applicability of HD-tDCS in skill acquisition and rehabilitation.

Disclosures: J.L. Scholl: None. **L.A. Baugh:** None. **T.J. Bosch:** None.

Poster

PSTR406

Motor Learning in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.21/H28

Topic: E.04. Voluntary Movements

Title: Investigating the lateralized role of the posterior parietal cortex for fine motor control during a tablet-based tracing task using HD-tDCS

Authors: S. SHARP[1], ***N. YAGHOUBI**[1], J. MANNING[1], C. SELB[1], P. E. PIDCOE[1], B. DEXHEIMER[2];
[1]Virginia Commonwealth Univ., Richmond, VA; [2]Virginia Commonwealth Univ., Henrico, VA

Abstract: Dominant hand (DH) motor control deficits can arise from various conditions, necessitating compensatory use of the non-dominant hand (NDH). Previous research has shown that NDH compensation (the left hand in right-handed individuals) during precision drawing coincides with increased functional connectivity in a left-lateralized parietal-prefrontal network. Applying non-invasive stimulation to a specific region in this network, the left posterior parietal cortex (PPC), facilitates gross motor learning transfer from the DH to NDH. Thus, we hypothesized that NDH compensation during precision drawing involves the left PPC, and we predicted that applying non-invasive stimulation to this region might facilitate NDH compensation. In our pilot sample, 22 right-handed young adults received 15 minutes of High-Definition Transcranial Direct Current Stimulation (HD-tDCS) at 2 mA, over left PPC (n = 7), right PPC (n = 6), or under sham conditions (n = 9). During HD-tDCS, participants completed a shape-tracing task with their DH using a stylus on a touch screen. After 60 trials, they completed the same task with their NDH (no HD-tDCS applied). To quantify task performance, we computed tracing error (Euclidean distance between the stylus tip and shape outline at every sampling timepoint), mean velocity, and speed-accuracy relationships (tracing error normalized to mean velocity). We used mixed-model ANOVAs to assess the effect of repetition (averaged in 10-trial cycles), group (left PPC, right PPC, sham), and hand (DH, NDH) on task performance. There was a significant increase in tracing error ($p < .0001$), a decrease in velocity ($p = .0003$), and an increase in speed-accuracy relationships ($p <.0001$) when participants switched from their DH to NDH. There was a trend towards differential effects of repetition on speed-accuracy relationships per hand ($p = .07$). While the DH improved speed-accuracy relationships across cycles, the NDH remained consistent. To further quantify performance, we used Fast Fourier Transformations (FFTs) within each trial to decompose continuous tracing into distinct frequency components. This preliminary analysis revealed a significant difference in very low-frequency spectral power (0.5 Hz) between the right PPC and sham groups ($p = .001$), regardless of hand. In summary, our preliminary data shows that participants exhibited slower speeds and significantly higher tracing errors when switching to their NDH. The right PPC group had lower proportions of low-frequency oscillatory movement despite similar task performance. Contrary to our hypothesis, left PPC HD-tDCS did not facilitate motor learning transfer from the DH to NDH.

Disclosures: S. Sharp: None. **N. Yaghoubi:** None. **J. Manning:** None. **C. Selb:** None. **P.E. Pidcoe:** None. **B. Dexheimer:** None.

Poster

PSTR406

Motor Learning in Humans

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Program #/Poster #: PSTR406.22/H29

Topic: E.04. Voluntary Movements

Support: NIH Grant R01HD059783
AHA Grant 16GRNT31010001
Dorothy Foehr Huck and J.Lloyd Huck Distinguished Chair of Kinesiology and Neurology awarded to R.L. Sainburg

Title: The effect of stimulation timing on anodal HD-tDCS to the left and right posterior-parietal cortices (PPC) in a skill task with the dominant right arm.

Authors: *P. R. RUELOS[1], J. YUK[2], N. KITCHEN[3,4], M. YAROSSI[5], E. TUNIK[6], R. L. SAINBURG[7];
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Abstract: Evidence from studies in patients with focal brain lesions and studies using non-invasive brain stimulation indicate that the left posterior parietal cortex (PPC) is a critical hub for motor learning, at least for visuomotor rotation adaptation. In a previous study, we extended such findings to a non-perturbation-based skill-task, which required striking a virtual puck at a precise angle into the goal area, in order to score points. Using excitatory (anodal) high-definition (HD) transcranial direct current stimulation (tDCS) applied to the left or right PPC, we found that only left PPC stimulation enhanced learning of a skill task. In that study, stimulation was coincident with the start of practice of the task. In order to examine the effect of stimulation timing, we now delay stimulation until participants performed the task for 15 trials and expect that specific group effects of stimulation will arise after the onset of stimulation. Subjects controlled a virtual paddle presented in a 2D virtual reality environment to strike a virtual puck towards a target region of the workspace. Participants were directed to begin their movements from starting positions situated at 45° and 135° angles relative to the initial puck location in order to hit the puck straight forward (90°). This required participants to learn how to glance the puck toward a specific target direction, regardless of puck distance. Healthy, right-handed participants between the ages of 18-35, were recruited and randomly assigned to one of three groups according to the type of HD-tDCS they received: Anodal Left PPC stimulation (LPPCS), Anodal Right PPC stimulation (RPPCS), and sham stimulation group (Sham; applied to either right or left PPC). Participants first performed an initial 15 trials of the task with their dominant, right arm in the absence of stimulation. They then continued with a further 150 trials concurrent with 20 minutes of stimulation at an intensity of 2 mA. At the end of this block, participants performed an additional 150 trials but without stimulation and using their non-dominant, left arm to assess inter-manual transfer of learning. Preliminary results suggest that only LPPCS resulted in improved rate of learning, relative to sham and RPPCS.

Disclosures: P.R. Ruelos: None. **J. Yuk:** None. **N. Kitchen:** None. **M. Yarossi:** None. **E. Tunik:** None. **R.L. Sainburg:** None.

Poster

PSTR406

Motor Learning in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.23/H30

Topic: E.04. Voluntary Movements

Support: NIH Grant R01NS085122

Title: Double-pulse TMS delivered to the left inferior parietal lobe before the movement onset enhanced interlimb transfer of adaptation.

Authors: *H. HIBINO[1], A. AKBAS[2], B. UITZ[1], T. MURPHY[3], R. L. SAINBURG[4], M. YAROSSI[5], E. TUNIK[6];
[1]Northeastern Univ., Boston, MA; [2]Inst. of Sport Sci., Dept. of Human Motor Behavior, Acad. of Physical Educ., Katowice, Poland; [3]Pennsylvania State Univ., State College, PA; [4]Kinesiology & Neurol., Penn State Univ., University Park, PA; [5]Physical Therapy Movement and Rehabil. Sci. / Electrical and Computer Engin., Northeastern Univ., Boston, MA; [6]Dept. of Physical Therapy, Movement, and Rehabil. Sci., Northeastern Univ., Boston, MA

Abstract: Motor learning involves a refinement of movement preparation for an upcoming movement based on
within-trial errors. Evidence suggests that motor planning and error correction processes are separate
aspects of the learning processes. There is compelling evidence for posterior parietal cortex (PPC)
involvement in preparation/planning as well as in error adjustment; however, its role in adaptation has
been sparsely investigated. We investigated whether the PPC involvement in visuomotor adaptation is
during the planning or the error correction stage of the movement by delivering MRI-guided double-
pulse TMS to the left inferior parietal lobe (IPL) at different timing. A total of 30 healthy right-handed
participants were randomly assigned to one of the three TMS groups; (1) No TMS (Control), (2) Prior-
To-Movement TMS (Early TMS), and (3) Later-In-Movement TMS (Late TMS). All participants reached
to each of eight targets over four sessions: (1) left-arm baseline, (2) right-arm baseline, (3) right-arm
adaptation, and (4) left-arm transfer. The visually displayed and actual hand motion were matched
during the baseline sessions, whereas the visual feedback of the hand deviated from the actual movement
direction of the hand by 30◦ during the adaptation and transfer sessions. Participants who were assigned

to Early TMS and Late TMS groups received double-pulse TMS during the right-arm baseline and right-
arm adaptation sessions. While Early TMS group received the TMS at 50ms after a target visualization,
Late TMS group received the TMS when the hand reached a spatial location 8cm away from the starting
position. The absolute angular difference between the cursor path and the vector defined from the start
circle to target at the peak velocity was calculated. The average of directional error from the 16 trials
(epoch) was quantified. The average of the first and last three epochs (Initial and Late, respectively)
during the right-arm adaptation and the left-arm transfer sessions was further quantified as measures
of adaptation and transfer, respectively. Despite the comparable directional error between groups in
both Initial and Late adaptations, we observed lower direction error in Early TMS at the Initial transfer
compared to two other groups. Such group difference was not observed at the Late transfer. Our
observations suggest a role of left IPL in trial-to-trial modification of motor planning in the facilitation
of right-to-left interlimb transfer, which requires further investigation as to the hemispheric lateralization
on adaptation (e.g. right PPC), and the interlimb transfer direction (e.g. left-to-right interlimb transfer).

Disclosures: H. Hibino: None. **A. Akbas:** None. **B. Uitz:** None. **T. Murphy:** None. **R.L. Sainburg:** None. **M. Yarossi:** None. **E. Tunik:** None.

Poster

PSTR406

Motor Learning in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.24/H31

Topic: E.04. Voluntary Movements

Support: JSPS KAKENHI, JP22K17628

Title: Transcutaneous Spinal Random Noise Stimulation Enhances Motor Memory Consolidation in Healthy Individuals

Authors: *M. NITO[1], D. KUDO[2], T. KOSEKI[3], S. TANABE[4], T. YAMAGUCHI[5];
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Abstract: The functional oscillatory coupling between the primary motor cortex and the spinal motoneurons, which can be measured as corticomuscular coherence, is known to increase after motor skill training, and this change may contribute to motor memory consolidation. Transcutaneous spinal random noise stimulation (tsRNS) over the cervical level can enhance corticospinal drive to spinal motoneurons. Therefore, we hypothesized that tsRNS can improve motor skill acquisition and corticomuscular coherence and facilitate memory consolidation. Here, we investigated the effects of tsRNS over the cervical level on motor performance and corticomuscular coherence in healthy individuals. Forty healthy volunteers were randomly allocated to the real or sham tsRNS group. tsRNS was delivered through a pair of rubber electrodes placed on the anterior part of the neck and the spinous process of C6-T1. Ranges of variation in current and frequency of tsRNS were -3 mA to +3 mA and 0.1 Hz to 640 Hz. Participants performed a 20-min visuomotor accuracy tracking task, which requires rapid shifts in pinch force levels and is measured as the average percentage of time on target. The real group received tsRNS for 20 min and the sham group for 0.5 min during the motor training task. The motor performance was assessed for 2 min. The motor performance assessment consisted of the same task used in the motor training task but without tsRNS. To quantify corticomuscular coherence, electroencephalography (C3 and its 2cm anterior) and electromyography from the first dorsal interosseous muscle were measured during tonic isometric contraction of the thumb and index finger for 2 min. The motor performance and corticomuscular coherence were tested before and after (Day 1), a day (Day 2), and 7 days (Day 8) after the motor training task. Motor performance was significantly improved in the real and sham tsRNS groups on Day 1, but there was no significant difference between the groups. However, the tsRNS group performed better than the sham group on Day 2 and 8. There was a significant increase in beta-band corticomuscular coherence after training in both groups. A significant correlation was only observed between changes in corticomuscular coherence on Day 1 and motor performance on Day 8 in the tsRNS group. These findings suggest that tsRNS combined with motor training facilitates the consolidation of motor skills. An increase in the beta-band corticomuscular coherence after motor training with tsRNS may contribute to a more efficient consolidation process of motor skills.

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Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.01/H32

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant 1R43MH135814-01

Title: Lightweight wireless headstages for small and large animal electrophysiology research

Authors: C. L. HOWARD[1], *R. J. GERTH[1], V. GO[2], N. ARMSTRONG[2], N. RAMOS[2], P. HARDISON[2], **J. C. MORIZIO**[2];
[1]Spike Neuro, Ann Arbor, MI; [2]Duke Univ., Durham, NC

Abstract: Wireless electrophysiology has a power consumption problem. The simultaneous transmission of high-fidelity single-unit signals and injection of electrical stimulation pulses creates a tradeoff concern with battery weight and battery life. The typical solution is to increase battery capacity, but the added weight is often prohibitive for small animal models. Novel lightweight, low-power wireless solutions would allow researchers to conduct more complex awake behaving experiments in a wide variety of animal models, better informing our understanding of the nervous system.

Spike Neuro, LLC and the Wireless Electrophysiology laboratory at Duke University, Department of Electrical and Computer Engineering, are developing two new wireless solutions for real-time neural recording and electrical stimulation. With small animal models in mind, we developed a lightweight Bluetooth solution providing 4 channels of single-unit recording weighing < 2.5 g and requiring only 6 mA. We have demonstrated transmission of simulated signals over 12 ft while meeting power consumption goals and maintaining a low bit error rate. Adding 4 channels of electrical stimulation would only require an additional 0.5g and 2 mA of battery current. This provides an excellent lightweight solution for small animal researchers; however, these features do not scale to higher channel counts while maintaining an appropriate weight.

For higher channel counts in larger animal models (including rats), we have developed a novel low power hybrid wireless radio system. Our hybrid radio leverages backscatter modulation technology, utilizing an incident radio frequency (RF) signal to transmit high data rates, reducing the need for larger batteries. This technique uses passive reflection and digital modulation of the incoming RF signal that is digitally encoded for data communications. The active components are contained in a base-station (receiver) with only a passive chip antenna in the headstage, further reducing weight and current consumption. This system provides up to 16 channels of single unit recording and 2 channel of +/-1mA constant current biphasic electrical stimulation (up to +/-5V) while weighing < 8 g and requiring only 9 mA. We have demonstrated binary backscatter modulation and demodulation using a constant RF transmitter and demodulation receiver components.

This novel wireless technology provides useful solutions for studying the neural basis of natural behaviors across a wide range of animal models. In our upcoming work we will add integrated electrical stimulation and test these devices in vivo demonstrating performance previously limited to wired research setups.

Disclosures: C.L. Howard: A. Employment/Salary (full or part-time):; Spike Neuro LLC. **R.J. Gerth:** A. Employment/Salary (full or part-time):; Spike Neuro LLC. **V. Go:** None. **N.**

Armstrong: None. **N. Ramos:** None. **P. Hardison:** None. **J.C. Morizio:** F. Consulting Fees (e.g., advisory boards); Spike Neuro LLC.

Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.02/H33

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF GRFP DGE-2139754
NIH UG3 NS120172
NIH R01 DC019498

Title: Concurrent recording and stimulation system with artifact mitigation for investigating immediate stimulation-evoked neural activity

Authors: *C. SCHMITZ[1], K. WINGEL[3], C. WANG[1], C.-H. CHIANG[1], B. PESARAN[3], J. VIVENTI[1,2,4,5];
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Abstract: Recording neural activity while delivering electrical stimulation can be useful for investigating the biophysical mechanisms of neuromodulation. Neural recordings can also be used in closed-loop neuromodulation devices for controlling the delivery of stimulation, which enables targeted treatment and extended battery life. However, stimulation produces large voltage transients (stimulation artifacts) that can saturate recording amplifiers, preventing the system from capturing the immediate stimulation-evoked neural activity and potentially limiting therapeutic outcomes. We developed a neuromodulation system with low-gain, high-input-range recording amplifiers and programmable stimulation current sources to investigate the mechanisms of neuromodulation typically masked by artifacts. We designed a custom headstage that connects with National Instruments M Series multifunction I/O data acquisition devices (NI DAQ) to support concurrent recording and stimulation. The headstage generates current-controlled stimulation waveforms using four Howland current sources and records neural signals with a gain of 4V/V, enabling an input dynamic range of up to +/- 2.5V. The headstage also contains analog switches that can be connected to ground to correct for charge imbalance between stimulation pulse trains and four 16:1 multiplexers to programmatically select stimulating and recording channels. The NI DAQ uses an 18-bit analog-to-digital converter, enabling low-noise, high-resolution neural recordings. We designed a MATLAB graphical user interface for configuring the hardware, setting stimulation waveform parameters, plotting a live trace of the recording, and logging data. We performed in vitro testing of the system with both platinum iridium microprobes and cortical surface microelectrodes. We delivered symmetric,

biphasic stimulation pulses and recorded externally generated sine waves through the same electrode contact in phosphate buffered saline. We found that the system successfully prevents saturation of the recording amplifier during stimulation with current amplitudes commonly used in the literature and preserves the integrity of recorded sine waves between stimulation pulses. Future work will involve testing the system in non-human primates, which will enhance understanding of the biophysical mechanisms of neuromodulation and support the development of more consistent and effective neuromodulation devices, such as prosthetics, for humans with neurological disorders.

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Poster

PSTR407

Neuroprosthetic Implantable Devices

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Topic: E.05. Brain-Machine Interface

Support: NIH Grant 1R01DC019498
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DOD EP200077

Title: Liquid crystal polymer as an encapsulating layer for implantable electronics

Authors: *R. VERRINDER, C. PAZDAN, I. RACHINSKIY, J. E. SMITH, C. WANG, J. VIVENTI;
Biomed. Engin., Duke Univ., Durham, NC

Abstract: Background: Long-term implantable devices for neural recording are essential for understanding brain function and developing effective therapies for neurological disorders. Existing implantable devices, however, often include bulky packages for electronics. There is a need for more compact encapsulation strategies that can accommodate high-density feed-throughs to high-channel-count electrode arrays. Liquid crystal polymer (LCP) is a flexible, biocompatible polymer that has shown promise as a long-lasting material for micro-electrocorticographic (µECoG) and stereotactic-electroencephalographic (sEEG) electrodes. However, there has been limited longevity testing of actively-powered circuits using LCP encapsulation. Here we show accelerated aging of encapsulated active circuits using a wirelessly powered light-emitting diode (LED) circuit. Methods: We developed a heat press procedure for laminating LCP sheets into a monolithic material. We optimized temperature ramping and pressure parameters to achieve thermal lamination of layers of LCP sheets. We developed inductively-powered LED circuits on an LCP substrate and encapsulated the circuit in LCP. We assessed lamination using micro-CT to detect air bubbles and material heterogeneities. To assess

device longevity, we submerged the LCP-encapsulated LED circuit in a jar of phosphate-buffered saline (PBS) and delivered power to the sample wirelessly. We used a canning procedure to seal the jars. We maintained samples at 60°C in PBS to obtain an accelerated aging factor of ~5x. Using continuous video monitoring, we assessed the resistance of the encapsulated device to PBS ingress by measuring the time until device failure caused the LED to turn off. Results: Micro-CT and visual inspection of the LCP lamination samples indicated a monolithic structure. We confirmed repeatability of the heat press procedure and successful wireless power delivery to the LED. We confirmed an effective vacuum seal by noting no change in jar weight after a 1-month period. Evaluation of device performance in PBS is ongoing. Discussion: We developed a procedure for encapsulating active circuits in LCP and assessing longevity in an accelerated aging environment using soak testing. Preliminary results are promising for the use of LCP as an encapsulating layer for implantable electronics.

Disclosures: R. Verrinder: None. **C. Pazdan:** None. **I. Rachinskiy:** None. **J.E. Smith:** None. **C. Wang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Blackrock Neurotech. **J. Viventi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Duke University.

Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.04/H35

Topic: E.05. Brain-Machine Interface

Support: NIH 1U01NS099697

Title: Measuring the long-term reliability of a wireless, fully implantable neural interface

Authors: *J. E. SMITH, I. RACHINSKIY, R. VERRINDER, T. JOCHUM, J. VIVENTI; Duke Univ., Durham, NC

Abstract: Electrocorticography has been used to decode speech production from the sensorimotor cortex, a development with promising implications for people with neuromuscular disorders that prevent natural speech. To restore communicative ability in everyday life, the electrodes and requisite circuitry must be fully implanted. Conventional packaging for implanted electronics is bulky and relies on ceramic feedthroughs that limit the number of electrodes that can be implemented. Yet, the accuracy of speech decoding increases with spatial resolution. We propose a method of encapsulation wherein both an electrode array and the electronics for signal acquisition and wireless communication are enveloped by liquid crystal polymer (LCP). This approach achieves a near-hermetic seal and, since the array and electronics share a single substrate without need for feedthroughs, allows for high channel counts. While prior research has

shown that LCP outlasts competing materials like parylene and polyimide in soak testing, it is yet unclear how long an LCP implant might function and whether or not the inclusion of active electronics diminishes its long-term reliability. This work presents a wireless test device for soak testing that emulates our design for a high-channel-count fully implantable speech prosthetic. To sense possible moisture ingress, the test device measures the conductance of an array of interdigitated electrodes. To gauge the quality of electrographic recordings after prolonged exposure to implanted conditions, the test device measures electrode impedance over frequency (at 1, 2, 5, 10, 20, 50, 100, 200, 500, and 1000 Hz). Measurements are requested of an encapsulated microcontroller wirelessly using near field communication and are logged automatically. Firmware can be flashed to the embedded controller over-the-air. Power is provided through an inductive link. The accuracy of electrochemical impedance spectroscopy is comparable to other commonly used wired solutions. In future work, we will use this device to evaluate the stability of LCP encapsulation through accelerated aging to quantify the viability of chronic applications and predict expected lifetimes.

Disclosures: J.E. Smith: None. **I. Rachinskiy:** None. **R. Verrinder:** None. **T. Jochum:** None. **J. Viventi:** None.

Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.05/H36

Topic: I.08. Methods to Modulate Neural Activity

Support: 2022M3C1A3081294
IITP-2023-2020-0-01822

Title: Fully flexible neural interface with temporary mechanical reinforcement

Authors: *S. HONG, M. CHO, M. CHOO, S.-M. PARK;
POSTECH, Pohang, Korea, Republic of

Abstract: Fully flexible neural interface with temporary mechanical reinforcement for spinal cord stimulationAuthors ***S. HONG**, M. CHO, M. CHOO, S.M. PARK; POSTECH, Pohang, Korea, Republic of **Disclosures S. HONG**: None. **M. CHO**: None. **M. CHOO**: None. **S.M. PARK**: None. **Abstract** Neural interfaces serve as crucial mechanical components for neuromodulation. They are categorized into commercialized stiff interfaces (Young's modulus > 1 GPa) and research-oriented flexible interfaces (Young's modulus < 2 MPa), depending on the properties of the base materials. Recently, there has been a transition to flexible interfaces to enhance compatibility and biomechanics of the spinal cord. However, this transition has reintroduced limitations such as unintended damage during handling processes, low electrical stability, and short lifespan, thereby restricting their application in real-world. In this study, we

propose a fully flexible neural interface that integrates temporary stiffness reinforcement technology and liquid metal electrode technology to harness the advantages of both stiff and flexible implants. Temporary stiffness reinforcement technology can provide high stiffness similar to stiff interfaces in vitro and low stiffness similar to flexible interfaces in vivo, thereby minimizing unintended damage during handling processes. The liquid metal electrode enables the proposed neural interfaces to maintain high electrical stability even in the presence of flexible spinal cord movements, thereby increasing the lifespan of proposed neural interfaces. The device and implantation procedure have been validated in vitro and on rat models. This offers significant advantages in long-term biometric signal acquisition and electrical signal stimulation for real-world application. The device design and material development reported here allow for low-cost neural interface fabrication using inexpensive equipment in small research labs, suggesting a wide range of applications in system development for neuromodulation.

Disclosures: S. Hong: None. **M. Cho:** None. **M. Choo:** None. **S. Park:** None.

Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.06/H37

Topic: I.08. Methods to Modulate Neural Activity

Support: 2022R1A2C2092821
RS-2023-00220534

Title: Effects of electrode arrangement on wound healing: insights of in vivo and in silico

Authors: *S. KIM, E. PARK;
Soonchunhyang Univ., Asan-si, Korea, Republic of

Abstract: A chronic wound presents a major clinical problem. Chronic wound is defined as wound that has not healed within 3 months and the risk of chronic wound are low quality of life and high death rates by sepsis, complications, and necrosis. Electrical neurostimulation treatment that mimics endogenous electrical field (EF) can heals and prevents chronic wounds. Previous reports described the wound healing effect of electrical neurostimulation. Although the exogenous EF that affect to wound healing is changed by electrode arrangement, research into this area remains unexplored. In this study, we aim to compare the healing effects as electrode arrangement and determine the relationship between electrode arrangements and wound healing. We have used rats (Sprague Dawley, 8 weeks, male, 250g) to compare the healing effects. Full-thickness wounds (~4mm in depth, ~10mm in diameter) were created using a biopsy punch on the dorsal of rat. To investigate efficacy of the treatment in vivo, control group and three treatment groups (n=7~8) were treated with the electrical neurostimulation (V=50V, F=100Hz, Twin-peak pulse, duty 1%, monophasic). We have used image from rat to show the wound

healing rates, which were approximately 26% (mean 25.59% SD 9.833) in control group and 16% (16.128% ± 6.671), 17% (17.474% ± 8.034), and 9% (8.925% ± 2.007) in each treatment group. The results demonstrated that electrical neurostimulation was effective after day 10. On the day 17, we have sampled the wound section of rat and stained with H&E to investigate the degree of wound healing in a histological analysis. We have arranged the wound area clearly, confirmed the healed epidermal layer, and found the collagen area, indicative of evidence of healing. The collagen area was found in treatment groups only, and there were differences in the thickness of the epidermis (each group 0μm, 2μm, 2μm, 4μm), indicative of effect of electrode arrangement to wound healing. To determine relationship between electrode arrangements and wound healing, we have simulated the EF and heat distribution using COMSOL Multiphysics. We have confirmed different penetration depth and intensity of EF in the epidermis and dermis as each group. Furthermore, the simulations have shown different heat distribution as distance of electrode between anode and cathode. Our findings provide histological and functional evidence that electrode arrangement affects to the chronic wound healing by electrical neurostimulation. We propose the most effective electrode arrangement considering the EF formation and heat. Together, these findings can optimize electrical neurostimulation to accelerate speed of chronic wound healing.

Disclosures: S. Kim: None. **E. Park:** None.

Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.07/H38

Topic: I.08. Methods to Modulate Neural Activity

Title: Innovative sEEG based device for neural recordings, ablation and drug delivery into the brain

Authors: *A. KULLMANN[1], S.-Y. CHANG[2], M. PORTO CRUZ[3], M. VOMERO[4], M. MCNEIL[5], G. ZEPEDA[5], F. MIVALT[6], I. KIM[7], J. KIM[6], A. CHAVEZ[5], G. A. WORRELL[7], C. DIAZ-BOTIA[5];
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Abstract: Stereoelectroencephalography (sEEG) electrodes are routinely used to identify the epileptogenic zone (EZ) in patients with drug resistant epilepsy. The sEEG electrodes are FDA-cleared for temporary (<30 days) neural activity monitoring, recording and stimulation. Here we present bench and in vivo studies for two new functions added to the sEEG electrodes:

temperature-controlled radiofrequency ablation (RFA) and convection enhanced drug delivery (CED). **Methods.** RFA was tested in ex vivo chicken breast and in vivo swine brain, using a proprietary RF generator. Lesions obtained for different ablation parameters (time and temperature) were evaluated with MRI and histology. CED was tested in 0.6% agarose gel. Concomitant neural recordings and CED were evaluated in vivo in rodent and swine. CED-sEEG electrodes were stereotactically implanted into the hippocampus (HC) and putamen (PUT), and penicillin (5000 units/µl) was used to modulate neural activity. MRI with gadolinium (Gd; swine) and histology (rodent) were used to evaluate diffusion volume (Vd). **Results.** sEEG-guided RFA created reproducible lesions with sizes proportional to temperature and time. In vivo and ex vivo lesions were comparable and varied from ~4 to 10 mm diameter, depending on the RFA parameters. Bench studies demonstrated CED for infusion rates of 0.5-15 µl/min and infused volumes (Vi) of 60-300 µl, with Vd/Vi ratio of 2.7-3.4. Penicillin elicited seizure-like episodes consisting of large amplitude bursts of coordinated activity interspaced by quiet periods, in both rodent and swine HC. MRI visualization of Gd demonstrated localized infusions in the swine PUT and HC. Histological examination of the rodent brain tissue showed confined Vd. **Conclusions.** These data demonstrate the ability of one sEEG-based device platform to perform multiple functions: record brain activity, stimulate, ablate and deliver drugs. This has the potential to increase the accuracy of diagnosis and offer treatment within one surgical procedure. Furthermore, real time monitoring of neural activity during infusion of therapeutic compounds can be used to probe the function of various brain structures, and/or evaluate onset and mechanism of action, dosing, efficacy and safety of therapeutic compounds.

Disclosures: A. Kullmann: A. Employment/Salary (full or part-time):; NeuroOne. **S. Chang:** None. **M. Porto Cruz:** A. Employment/Salary (full or part-time):; NeuroOne. **M. Vomero:** A. Employment/Salary (full or part-time):; NeuroOne. **M. McNeil:** A. Employment/Salary (full or part-time):; NeuroOne. **G. Zepeda:** A. Employment/Salary (full or part-time):; NeuroOne. **F. Mivalt:** None. **I. Kim:** None. **J. Kim:** None. **A. Chavez:** A. Employment/Salary (full or part-time):; NeuroOne. **G.A. Worrell:** None. **C. Diaz-Botia:** A. Employment/Salary (full or part-time):; NeuroOne.

Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.08/H39

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH SPARC HORNET Initiative RFA-RM-21-024

Title: Can a lab build their own packaged, implantable sensing device for clinical trials? Update on the open source COSMIIC/HORNET HIVE module

Authors: *O. G. LEE[1], D. M. WALLACE[4], M. KELBERMAN[5], J. L. GBUR[6], D. B. SHIRE[8], J. LAMBRECHT[7], A. K. VASKOV[2], S. R. NASON-TOMASZEWSKI[9], C. A. CHESTEK[3]; [2]Plastic Surgery, [3]Biomed. Engin., [1]Univ. of Michigan, Ann Arbor, MI; [4]Biomed. Engin., [5]Univ. of Michigan, Ann Arbor, Ann Arbor, MI; [6]Case Western Reserve Univ., Cleveland, OH; [7]Case Western Reserve Univ., Cleavland, OH; [8]Cornell Univ., Ithaca, NY; [9]Biomed. Engin., Emory Univ., Atlanta, GA

Abstract: Currently, there are limited options for implantable, voltage sensing devices for early feasibility clinical trials. One limitation of current systems is that they require wired connections to external equipment, hindering participant mobility during experiments (Ajiboye et al., 2017). Regulatory approval is also necessary for clinical trials, hindering customization of these systems for specific applications (Vaskov et al., 2022). To address these challenges, our group was recently funded through the SPARC HORNET mechanism to develop a high-channel count, implantable sensing device adapted from the Networked Neural Prosthesis (NNP) under an open-source framework (Makowski et al., 2021). This 64-channel module includes existing and new circuit elements to the NNP, as well as a novel titanium package.

A 35x28x44mm titanium package was designed with 64 hard-wired sensing channels. The sensing channels are composed of 1x7x0.25mm 35N LT 28% silver-cored drawn filled tube wires housed in perfluoroalkoxy alkane insulation and jacketed in a silicon tube. These leads are hard-wired to internal electronics and exit the package via eight commercial 8-pin feedthroughs. Additionally, two Bal Seal-style connectors support power and communication with the existing NNP power module. Two 30x24mm printed circuit board (PCB) panels connected via flexible circuitry were designed to fold and fit into the titanium package. One panel houses an Intan amplifier connecting to the 64 sensing channels. Diodes were added on each channel from the Intan to protect against electrostatic discharge (ESD) during implant. The second panel houses a microcontroller unit (MCU) and power management circuitry. This design isolates analog and digital signals to reduce signal contamination. A similar device developed by our group consumed <40mW of power (Bullard et al., 2019). The target power budget for this new device is <25mW by consolidating electronic components and using a more efficient MCU operating in direct memory access mode with frequent sleep periods.

The titanium package and the PCB were fabricated and assembled at third-party manufactures. All design files, part numbers, vendors, contractors, and manufacturers are available at COSMIIC.org and the Chestek Lab website. Communications with the FDA have been initiated with investigational device exemption pre-submissions. Mechanical testing on the sensing leads and electrical current leakage testing from the Intan has been performed. Required future testing includes functionality, hermiticity, ESD, sterility, and biocompatibility, which will all be done under open-source principles.

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Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.09/H40

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Stimulating Peripheral Activity to Relieve Conditions (SPARC) Program, U41-NS129436

Title: Cosmiic open source implantable neuromodulation platform

Authors: *C. G. REXROTH[1], C. A. CHESTEK[2], K. L. KILGORE[1];
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Abstract: Background: Active implantable devices are an important means of treating disease and disability, in large part by achieving direct and targeted control of portions of the nervous system through electrical stimulation. However, limited access to this technology and related resources is a barrier to research progress and adoption. The goal of COSMIIC is to establish a community with an open source neuromodulation platform for researchers to build upon the world's only modular active implantable device. COSMIIC System: The open source COSMIIC System is based on the modular implant system developed at Case Western Reserve University known as the networked neuroprosthesis. The COSMIIC System consists of pulse generator and biopotential recording units as well as specialized components in-development by collaborators. New components will add function related to health monitoring, movement tracking, high-density recording and stimulating, and high-frequency nerve conduction block. The modular design allows daisy-chaining of components in limitless orientations to provide both specific and body-wide function. This system has already achieved use in human studies under an Early Feasibility Investigational Device Exemption (IDE). Open source materials to be released include all: circuit designs and layouts; mechanical drawings for enclosures, connectors, cabling; annotated code for software, firmware, bootloaders; instructions of fabrication techniques; regulatory documents and test data, including the approved IDE document as an example. Open Source Impact: The COSMIIC System will provide an expansive tech platform enabling researchers to develop novel therapies that require focused device specifications not currently provided in the market, without compromising their budgets nor acquiring expertise in development of implantable devices. In addition to open technology, COSMIIC seeks to build a collaborative ecosystem across neuromodulation indications and support users with educational and regulatory resources from the benchtop to animal studies culminating to human use. Above all, the COSMIIC System will advance new therapies leading to better patient outcomes. We are seeking input from the research community to determine areas of need related to technical features, support for regulatory submissions, and customer support for system use and implementation. We are open to discussing how adopting the open source COSMIIC System can fit needs of individual studies. Open source materials can be found at cosmiic.org.

Disclosures: C.G. Rexroth: None. **C.A. Chestek:** None. **K.L. Kilgore:** None.

Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.10/I1

Topic: I.08. Methods to Modulate Neural Activity

Support: AMED JP23gm1510010
JSPS 23K19217

Title: In vitro experimental platform with bulk platinum electrodes for high-intensity electrical stimulation and wide-field imaging.

Authors: *Y. TERASAWA[1,2,3], H. TASHIRO[3], J. OHTA[2];
[1]NIDEK CO., LTD., Gamagori, Japan; [2]Nara Inst. of Sci. and Technol., Ikoma, Japan; [3]Kyushu Univ., Fukuoka, Japan

Abstract: Multielectrode arrays (MEAs) are a popular tool in neuroscience, used to study the physiological characteristics of neural tissues or cells, including hippocampus slices, stem cell-derived neurons, and retina. In our study, we are examining the neural response of the retina to temporally-interferential electrical stimulation using a stereoscopic microscope and calcium fluorescent dye. The application of interferential stimulation requires a relatively large electric current amplitude. However, the use of large currents on commercially available MEAs may cause irreversible damage to the electrodes due to the vulnerability of the metal thin film used in the MEAs. In this study, we developed an experimental platform with a custom-made microelectrode array capable of large electric charge injection without damaging the electrodes. The electrode material employed was 100-um-diameter platinum wires. Sixteen wires were inserted into holes formed in the acrylic plate in a 4 x 4 arrangement and fixed in place by an adhesive. The plate was positioned on the bottom of the 18-mm-diameter and 5-mm-deep acrylic chamber filled with saline. A cover glass was placed on the top of the chamber to avoid noise due to the shaking of saline. The 4 x 4 multielectrode array with 200 um interelectrode distance was successfully fabricated. Two 1-mm-diameter silver wires were embedded in the sidewall of the chamber, with their cross sections serving as the return electrodes for electrical stimulation.The use of bulk platinum allows high-intensity electrical stimulation without causing electrode damage. In this study we fabricated 100-um-diameter 16 electrodes, but the electrode diameters and their arrangement are flexible enough to be selected arbitrarily depending on the expriments. Moreover, the proposed platform allows microscopic observation using a stereoscopic microscope, which allows a relatively large field of view compared with an inverted microscope conventionally used in MEA applications.

Disclosures: Y. Terasawa: A. Employment/Salary (full or part-time):; NIDEK CO., LTD. **H. Tashiro:** 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.; NIDEK CO., LTD. **J.**

Ohta: 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.; NIDEK CO., LTD..

Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.11/I2

Topic: I.08. Methods to Modulate Neural Activity

Support: R01NS120850

Title: Bioelectric Router for Adaptive Isochronous Neuro Stimulation (BRAINS) Board - A Programmable Device for Multipolar Stimulation

Authors: *E. SAHAI[1], D. J. DENMAN[2], J. L. HICKMAN[3];
[1]Univ. of Colorado, Anschutz Med. Campus, Aurora, CO; [2]Univ. of Colorado Anschutz, Aurora, CO; [3]Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: Multipolar intracranial electrical brain stimulation is a promising method with the potential to improve Brain Computer Interfaces in clinical settings. To enable research on multipolar stimulation using common research electrode arrays we developed an integrated circuit, which we call the Bioelectric Router for Adaptive Isochronous Neuro Stimulation (BRAINS) Board—a cost-effective, customizable device enabling scientists to both observe and manipulate multipolar stimulation across a 16-channel electrode array. Designed to interface with an Arduino microcontroller, the board allows users to configure each channel to receive cathodal or anodal input, establish a grounded connection, or remain floating. The stimulation delivered from a conventional isolated stimulator via the BRAINS Board shows no significant change in RMSE noise or signal/noise ratio to those produced by the isolator. The BRAINS board can switch configuration of each channel within 100us, enabling high-frequency switching for temporally multiplexed multipolar stimulation. The BRAINS Board has the potential to streamline brain stimulation research by offering an intuitive tool for conducting sophisticated, reproducible, and finely controlled stimulation experiments.

Disclosures: E. Sahai: None. **D.J. Denman:** None. **J.L. Hickman:** None.

Poster

PSTR407

Neuroprosthetic Implantable Devices

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Program #/Poster #: PSTR407.12/I3

Topic: I.08. Methods to Modulate Neural Activity

Support: This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education 2022R1A6A3-A01087318

Title: Cross-acquisition of tonic dopamine and serotonin levels in vivo with interleave scanning of MCSWV and N-MCSWV waveforms

Authors: *H. CHO[1], S. HWANG[2], Y. KWAK[3], H. KWON[4], J. JANG[5], D. JANG[6]; [1]Hanyang Univ., Seoul, Korea, Republic of; [2]Biomed. Engin., Hanyang Univ., Seongdong-gu, Seoul, Korea, Republic of; [3]Biomed. Engin., Hanyang Univ., Seoul, Korea, Republic of; [4]Dept. of Electronic Engin., Hanyang Univ., Seoul-City, Korea, Republic of; [5]Electrical Engin., Hanyang Univ., Seoul, Korea, Republic of; [6]Biomed. Engin., Hanyang Univ., Seoul-City, Korea, Republic of

Abstract: Dopamine (DA) and serotonin (5-HT) are key neurotransmitters regulating various brain activities, such as behavior, emotions, and motivation through diverse neural circuits. Various studies have aimed to measure the changes in DA and 5-HT concentrations in the brain, by traditional methods like microdialysis and fast scan cyclic voltammetry (FSCV). However, these tools have limitations in analyzing neurotransmitter concentrations, including low temporal resolution and difficulty distinguishing between DA and 5-HT. Our group previously developed Multi-Cyclic Square Wave Voltammetry (MCSWV) to measure basal neurotransmitter concentrations with high spatial and temporal resolution, distinguishing them by their oxidation-reduction patterns. However, the oxidation signals of DA and 5-HT are still similar, making it challenging to differentiate each neurotransmitter's concentration. Therefore, an additional analytical method is needed to refine the data from MCSWV. In this work, we implemented a combined waveform technique with the N-Shaped Multiple Cyclic Square Wave Voltammetry (N-MCSWV), which selectively measures 5-HT, enabling effective separation and basal concentration measurement of DA and 5-HT. MCSWV and N-MCSWV are applied every 10 seconds periodically, ensuring the sensitivity and selectivity of each waveform for neurotransmitters. By the unique property of N-MCSWV, we could complement the information obtained from MCSWV for DA and 5-HT, ultimately allowing the extraction of only DA basal concentration. The developed waveform was validated for their ability to separately measure basal concentrations of DA and 5-HT mixture solution. Additionally, the waveform successfully differentiated and measured the basal concentration changes of DA and 5-HT in the striatum area of the rat brain. Future studies aim to investigate changes in neurotransmitter concentrations related to specific drugs and brain diseases such as depression.

Disclosures: H. Cho: None. **S. Hwang:** None. **Y. Kwak:** None. **H. Kwon:** None. **J. Jang:** None. **D. Jang:** None.

Poster

PSTR407

Neuroprosthetic Implantable Devices

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.13/I4

Topic: I.08. Methods to Modulate Neural Activity

Support: R01-NS104923
NSF IOS-123213
R01-EY035826

Title: Modular implantable microdrive (MIM) for simultaneous neural recording and stimulation

Authors: *K. WINGEL[1], J. CHOI[2], M. F. KHAZALI[3], C. WANG[4], A. DUBEY[1], C.-H. CHIANG[4], C. SCHMITZ[4], J. VIVENTI[4], B. PESARAN[1];
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Abstract: Neural prostheses to replace lost sensory and motor functions depend on implanting neural interface devices to record and modulate neural activity in the brain. Trade-offs govern the functionality of devices that interface with neural activity at the cortical surface or penetrate the cortical surface. However, due to technical limitations, the effects of recording and modulating neural activity at the surface and within cortex have often been compared in different experiments. Without performance comparisons in the same population of neurons measured at the same time, the tradeoffs remain unclear. To directly compare the relative properties of surface and penetrating neural interfaces we designed a modular implantable microdrive (MIM) array. The MIM houses customized micro-electrocorticography (uECoG) electrode array for cortical surface microstimulation and recording with customized hole patterns to give access to independently-actuated neuropixel penetrating electrode arrays for intracortical recording, traditional microelectrodes for intracortical recording and microstimulation, and optrodes for intracortical optical stimulation and recording. The uECoG surface electrode was manufactured from a liquid crystal polymer substrate with 244 contacts (200 um dimensions and 900 um intercontact spacing; Dyconnex) and a hole pattern. Each contact underwent electrodeposited platinum-iridium coating (Platinum Group Coatings) to reduce the electrode impedance for surface microstimulation and recording. The uECoG array was molded into an artificial dura and the hole pattern aligned to a microdrive assembly. The microdrive holds 3 Neuropixel-1.0 NHP electrodes each with a customized dovetail shuttle as well as a combination of up to 10 electrically stimulating microelectrodes or optically stimulating optrodes. Bench testing and in vivo testing in non-human primates (NHP, macaca mulatta) ensured electrical and mechanical integrity of the design and associated software. We then implanted the MIM in two NHPs to obtain a population of neural recordings from the prefrontal cortex (PFC), posterior parietal cortex (PPC), and motor cortical areas (M1/ PMd) during task performance. The MIM has the potential to provide valuable new insights into the development of next-generation sensory and motor protheses.

Disclosures: K. Wingel: None. **J. Choi:** None. **M.F. Khazali:** None. **C. Wang:** None. **A. Dubey:** None. **C. Chiang:** None. **C. Schmitz:** None. **J. Viventi:** None. **B. Pesaran:** None.

Poster

PSTR407

Neuroprosthetic Implantable Devices

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Program #/Poster #: PSTR407.14/I5

Topic: I.08. Methods to Modulate Neural Activity

Title: Athena - A flexible platform for closed-loop neuromodulation and bioelectronic medicine studies

Authors: S. HIATT, S. BARRUS, A. THIESSEN, ***K. LOIZOS**, A. M. WILDER;
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Abstract: Neurostimulation has had a tremendous impact on human health and wellbeing over the past few decades and currently is maturing into a substantial global market. In 2023, the lives of over 200,000 individuals were improved through the implantation of a neurostimulator and global revenue for active implants and related services totaled over $10B (with an annual growth rate of 10-15%). Today, systems for treating a variety of major health conditions have received regulatory approval and are commonly prescribed. Examples include spinal cord stimulation for chronic pain, hypoglossal nerve stimulation for obstructive sleep apnea, and cochlear implants for hearing loss. The future holds tremendous promise for the development of many new neurostimulation applications including for major health concerns such as obesity and depression. Unfortunately, despite the large number of groups demonstrating successes in early-stage research, very few new neuromodulation approaches end up making it to human-subject studies. Two major hurdles currently limiting the translation of promising initial research are the high cost and long timeline required to to develop human-use systems from scratch. The average implantable stimulator can cost upwards of $10M and require 5-7 years to develop and qualify, often far beyond the means of many early-stage teams.In response to this need, IRIS Biomedical has developed and qualified a highly-capable neurostimulation platform, the ATHENA, for use in early-stage human clinical trials. The ATHENA platform offers highly-flexible stimulation and broadband sensing capabilities and is compatible with existing neurostimulation leads, allowing it to support a wide range of neuromodulation paradigms out of the box. The platform also provides a programmable processor for development of novel closed-loop stimulation algorithms and comes with a dossier of functional and safety testing data that can be leveraged to greatly accelerate time to achieve regulatory approval for an IDE study. With a greatly reduced timeline and up to 10x reduction in cost, the ATHENA platform makes it possible to execute clinical studies with a wide range of existing funding sources (including federal research grants and seed-stage venture capital). We believe the ATHENA platform will greatly facilitate the successful introduction of many new neuromodulation therapies over the coming decade.

Disclosures: S. Hiatt: A. Employment/Salary (full or part-time):; Ripple LLC. **S. Barrus:** A. Employment/Salary (full or part-time):; Ripple LLC. **A. Thiessen:** A. Employment/Salary (full or part-time):; Ripple LLC. **K. Loizos:** A. Employment/Salary (full or part-time):; Ripple LLC. **A.M. Wilder:** A. Employment/Salary (full or part-time):; Ripple LLC.

Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.15/I6

Topic: I.08. Methods to Modulate Neural Activity

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Title: An ultra-flexible microelectrode based auditory brainstem implants for stable and spectrally selective activation of the auditory pathway

Authors: H. WU[1], ***J. PAN**[2,3], B. LIU[1], Z. WANG[4], G. CHEN[4], C. WANG[4], H. JIA[1], C. REN[4], Z. ZHAO[4];
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Abstract: Electronic central auditory prostheses hold promise for restoring hearing in deaf individuals ineligible for cochlear implants. However, current rigid surface auditory brainstem implants (ABIs) face challenges due to the complex anatomy of the cochlear nucleus (CN) and the imprecise macro electrode stimulation. Soft surface electrodes, though explored, struggle to access CN's tonotopic organization effectively. To address this, we propose a soft penetrating

ABI (~1 μm thickness) using ultra-flexible microelectrodes (UFμEs) for improved neural-electrode interface with excellent biocompatibility, minimal damage, broad tonotopic coverage, and high spatial resolution of neural recording and stimulation. IrOx coating on UFμE sites (~50 μm diameter) significantly increased safe stimulation current limits and allowed 100 million micro-electrical stimulation (μES) cycles with effective charge injection (~1.2 $mC \cdot cm^{-2}$) for CN stimulation. To test the effectiveness of our soft penetrating ABIs, we implanted UFμEs in both CN and its downstream region inferior colliculus (IC) along the auditory pathway in normal hearing rats. The IC neural responses provided a straightforward and timely readout of CN stimulation. The UFμE covered most of CN's tonotopic organization revealed by pure tone stimulus. Selective μES of CN with specific characteristic frequency sites induces site-specific spatiotemporal distribution of IC neural responses akin to pure tone stimuli of the same frequency. Furthermore, the CN μES-evoked IC response patterns remained stable over 150 days. We also explored an innovative circuit-level stimulation strategy by temporally coordinated selective stimulation of CN and IC at the microampere level. This aligns electrical stimuli-evoked IC responses more closely with pure-tone-evoked responses with lasting effects, suggesting that circuit-level coordinated stimulation could induce clinically beneficial plasticity along the natural auditory pathway. To our knowledge, this is the first clinically relevant application of UFμEs. These results demonstrate the great potential of our soft penetrating ABI to achieve long-term, stable hearing restoration with both refined frequency specificity and broad tonotopic coverage. Dual-nucleus synergistic stimulation may be a promising strategy to optimize electrical stimulation effects for more natural artificial auditory experiences. These advancements hold significant translational implications for next-generation clinical ABIs and improved therapeutic outcomes in individuals with hearing impairments.

Disclosures: H. Wu: None. **J. Pan:** None. **B. Liu:** None. **Z. Wang:** None. **G. Chen:** None. **C. Wang:** None. **H. Jia:** None. **C. Ren:** None. **Z. Zhao:** None.

Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

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Program #/Poster #: PSTR407.16/I7

Topic: I.08. Methods to Modulate Neural Activity

Title: A Detection Circuit for High-Pixel-Count Electrochemical Measurements

Authors: *M. JAMALZADEH, P. MARKOWSKI, D. SHAHRJERDI;
New York Univ., Brooklyn, NY

Abstract: Recording neuromodulatory signals with sub-cellular spatial resolution from a large network of neurons can significantly contribute to advancing our understanding of the nervous system. Among various methods, electrochemical sensing has great prospects due to its potential

applicability in human subjects. However, creating an electrochemical probe with a high sensor pixel count, akin to a camera, remains a challenge. The main requirements of such an electrochemical camera probe are conceptually similar to those of optical imagers, such as two-photon electron microscopy. Specifically, to provide detailed spatial and temporal information about neurochemical activities in a large network of neurons, they must offer a high pixel count, high spatial resolution, and high temporal resolution. Achieving high-quality imaging necessitates numerous small electrochemical sensors with dedicated CMOS detection circuits. Additionally, these electrochemical sensors must exhibit selectivity to measure different neurotransmitters simultaneously and provide good sensitivity to detect low concentrations of neurotransmitters. In recent years, significant progress has been made in developing novel carbon nanomaterials to simultaneously fulfill the demand for high sensitivity while achieving a compact sensor size. In addition to enhancing the characteristics of electrochemical sensors, another critical requirement for reducing the pixel size of an electrochemical camera probe is to develop compact readout circuits for interfacing with these sensors. In this work, we introduce a compact electrochemical readout circuit unit. Rather than using conventional op-amp-based transimpedance amplifiers for the readout, we propose a common-gate structure that utilizes much smaller space and allows the individual pixels to be read independently with this new design. We describe how our proposed detection circuit utilizes the advantageous characteristics of emerging electrochemical sensors made of carbon nanomaterials, as well as the principles of voltammetry measurements, to create a new readout concept. We demonstrate the feasibility of the proposed detection circuit through proof-of-concept in-vitro sensing experiments with different concentrations of dopamine. The proposed compact electrochemical readout circuitry promises significant advancements in scalable, selective neurochemical imaging with unprecedented spatial and temporal resolutions. This study is an important step forward for enriching the existing capabilities for recording neuromodulatory signals from a large neuron population.

Disclosures: M. Jamalzadeh: None. **P. Markowski:** None. **D. Shahrjerdi:** None.

Poster

PSTR407

Neuroprosthetic Implantable Devices

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Program #/Poster #: PSTR407.17/I8

Topic: C.03. Parkinson’s Disease

Title: Miniscope-based neural circuit profiling in freely behaving animals for preclinical therapeutic assessment

Authors: S. HUANG[1], D. CHENG[2], A. SIMONNET[3], É. NOÉ[3], ***G. PORRAS**[4], E. BEZARD[5], J. ZAPATA[6], J. J. NASSI[7];
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Abstract: Conventional preclinical tests for CNS disorders, which depend on behavioral or histological outcomes, frequently struggle to distinguish between various mechanisms. The lack of mechanistic insights is often causative of translational failure at the clinical stage. More sensitive and predictive assays shall speed up the progression of effective new therapies into clinical settings. The miniscope imaging platform allows cellular resolution activity measurements from hundreds of genetically defined neurons simultaneously. These large-scale neural activity recordings, paired with simultaneous behavioral measurements in freely moving animals, present an opportunity to advance translational research by revealing detailed relationships between neural circuit activity and behavioral symptoms and allow for the construction of predictive preclinical assays based on the treatment-induced response of large populations of neurons. We have established a robust pharmacological dataset with FDA-approved medications for Parkinson's Disease (PD) to compare this approach with traditional behaviour-based assays. In 6-OHDA lesioned mice, we have gathered a multidimensional dataset which includes cell-type specific activity of striatal D1 receptor-expressing medium spiny neurons (D1-MSNs) with synchronised locomotor metrics during free exploration under a breadth of conditions: pre-lesion, post-lesion, therapeutic and dyskinesia-inducing doses of L-DOPA, and dyskinesia-alleviating doses of amantadine. We have observed distinct neural activity patterns across these disease and treatment conditions. Additionally, and as a proof of concept, we've assessed how progressive alpha synuclein aggregate spread and inclusion impact striatal activity and motor behaviors by injecting preformed fibrils (PFFs) into either the dorsal striatum or substantia nigra. Our investigation has yielded a comprehensive dataset along with synchronized locomotor measurements during unrestricted exploration over multiple weeks following introduction of PFFs. Utilising these neurobehavioral profiles to inform preclinical assessments of target engagement and drug efficacy holds the potential for greater predictability in clinical outcomes compared to conventional assays. This approach will accelerate the advancement of next-generation therapeutics for a wide range of CNS disorders, beginning with Parkinson Disease.

Disclosures: S. Huang: A. Employment/Salary (full or part-time):; Inscopix. **D. Cheng:** A. Employment/Salary (full or part-time):; Inscopix. **A. Simonnet:** A. Employment/Salary (full or part-time):; Motac. **É. Noé:** A. Employment/Salary (full or part-time):; Motac. **G. Porras:** A. Employment/Salary (full or part-time):; Motac. **E. Bezard:** A. Employment/Salary (full or part-time):; Motac. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac, TREEFROG Therapeutics, SE Therapeutics. **J. Zapata:** A. Employment/Salary (full or part-time):; Inscopix. **J.J. Nassi:** A. Employment/Salary (full or part-time):; Inscopix.

Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

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Program #/Poster #: PSTR407.18/I9

Topic: E.05. Brain-Machine Interface

Support: NIH R01NS094396
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Title: Finite element model predicts tissue strain distributions surrounding Utah arrays that correlate with device performance

Authors: *A. M. FORREST, J. P. VANDE GEEST, T. D. Y. KOZAI;
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Abstract: Utah arrays are the predominant recording device for intracortical brain-computer interfaces (BCIs) in both humans and non-human primates (NHPs). A predominant hurdle to advancing BCI technologies is the decline in recording capabilities of these arrays over time. A potential cause for the decline in performance is the glial and fibrotic scarring resulting from relative motion that persists between the array and the cortical tissue leading to mechanical injury. Here, we employed a finite element model (FEM) to determine how the tissue strain surrounding Utah arrays correlates to recording performance metrics. While previous work has produced FEMs of tissue strain for linear arrays, this has yet to be demonstrated in Utah arrays. Due to the planar geometry of the array, we hypothesized that different peri-electrode tissues would experience different micromotion-derived mechanical strains based upon the electrode's location within the array. We further predicted that electrodes with the best recording capabilities would exert less strain on the surrounding tissue. Using our FEM, we found that the mean von Mises strain in the tissue near the edge electrodes was on average 27% greater than the strains in tissue near interior electrodes (ANOVA with post-hoc Tukey's HSD: $p<0.0001$). We next looked to see if the modelled strains correlated with array performance metrics including impedance, mean peak-to-peak waveform voltage (PTPV), and signal-to-noise ratio (SNR). Electrode-averaged impedance, PTPV, and SNR were computed across arrays of the same geometry. For motor cortex arrays in human participants (N = 2 participants, n = 4 arrays), we found modelled tissue strain to be negatively correlated with impedance, PTPV, and SNR at 1 year post implantation (simple linear regression: $p<0.0001$, $p<0.01$, $p<0.05$). The correlation with impedance was the strongest with $R^2 = 0.3$. For NHP arrays in area V4 (N = 2 animals, n = 4 arrays), we observed similar trends in impedance, but found a positive correlation between SNR and modelled strain (simple linear regression: $p<0.01$). These correlations may be influenced by multiple factors. Electrodes that experience higher strains from micromotions may have increased glial scarring, contributing to reduced relative performance. Alternatively, curvature of the cortical surface may result in edge electrodes to be in different cortical layers compared to interior shanks. Differences in vascular supply and metabolic support may also contribute to the

observed differences. In all, the relationship between FEM strains and recording metrics suggests a spatial dependence on electrode performance in the Utah array.

Disclosures: A.M. Forrest: None. **J.P. Vande Geest:** None. **T.D.Y. Kozai:** None.

Poster

PSTR407

Neuroprosthetic Implantable Devices

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Topic: E.05. Brain-Machine Interface

Support: NIH Grant UH3NS107714

Title: The relationship between sensorimotor cortical activity and perceptual detection of intracortical microstimulation in somatosensory cortex.

Authors: *S. SELIG[1], A. ALAMRI[2], C. EGGLESTON[1], C. M. GREENSPON[3], N. G. HATSOPOULOS[4];
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Abstract: We previously investigated motor cortex responses evoked by intracortical microstimulation (ICMS) of primary somatosensory cortex (S1). However, the relationship between sensorimotor cortex activity and perceptual detection of stimulation using peri-threshold current amplitudes remains largely unexplored. Here, we analyzed sensorimotor cortex activity in a participant of an ongoing human clinical trial with intracortical arrays implanted in both somatosensory and motor cortices. We stimulated electrodes in S1 with peri-threshold current amplitudes and compared somatosensory and motor cortex activity between successful and failed detection of stimulation based on the subject's reports. Our findings revealed significantly different patterns of somatosensory and motor cortex activity between successful and failed detection trials. Before onset of ICMS of S1, motor cortex exhibited significantly higher activity on successful detection trials. After onset of ICMS of S1, both somatosensory and motor cortices exhibited significantly higher activity in failed detection trials. Our findings suggest a potential correlation between sensorimotor cortex activity and conscious sensory perception.

Disclosures: S. Selig: None. **A. Alamri:** None. **C. Eggleston:** None. **C.M. Greenspon:** None. **N.G. Hatsopoulos:** F. Consulting Fees (e.g., advisory boards); N.G.H. serves as a consultant for BlackRock Microsystems, the company that sells the multi-electrode arrays and acquisition system used in this study..

Poster

PSTR407

Neuroprosthetic Implantable Devices

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Program #/Poster #: PSTR407.20/I11

Topic: E.05. Brain-Machine Interface

Support: NIH NINDS 1R01NS124222 (MRO)

Title: Histological Evaluation of Chronic Implantation of a Regenerative Ultramicro Multielectrode Interface

Authors: *K. HUSSEIN[1], S. F. COGAN[2], M. I. ROMERO-ORTEGA[1];
[1]Biomed. Engin., Univ. of Arizona, Tucson, AZ; [2]Bioengineering, The Univ. of Texas at Dallas, Dallas, TX

Abstract: Regenerative Multielectrode Interfaces (REMIs) have been used to record from and stimulate regrowing rat axons. However, these arrays have relatively large shaft electrodes (200 µm OD) with 2000 μm^2 geometric surface area (GSA) of individual electrodes. We have reported that these electrodes evoked a foreign body response (FBR) but remained anchored to the nerve due to the fibrotic capsule. In order to reduce the electrode-induced FBR, we fabricated a 32-electrode Regenerative Ultramicro-electrode Array (RUMA), with a 25-fold reduction in thickness (8 µm) and a 10-fold reduction in electrode GSA (200 μm^2). One 12- and two 9-electrode arrays were placed, one in the common conduit, and the other two in the left and right arms of a 1-mm Y-shape RUMA. The peroneal and tibial nerves were implanted onto the left-right arms of the RUMA and connected distally in an end-to-end repair to allow re-innervation of their natural gastrocnemius and tibialis anterior muscles in the lower limb. We investigated the FBR to the RUMA after 134 days of implantation and compared to the REMI arrays. Gross evaluation of the explanted RUMA interfaced nerve showed reduced fibrotic tissue compared to that with the REMI implant. The regenerated Y-nerve showed normal Y-shaped regeneration and no signs of edema, trauma or injury was noted in the RUMA regenerated nerve. Histological evaluation of the number of regenerated unmyelinated and myelinated (MBP) axons in the REMI and RUMA was compared using Neurofilament and P0 double immunofluorescence histology. The amount of inflammation was evaluated by visualization of the ED1 immunological biomarker. This work will provide a detailed understanding of the tissue response to smaller and thinner electrode arrays in the RUMA and compare reliability of the electrode array (i.e., number of recorded neural signals). Acknowledgments: We thank NIH NINDS for funding this work 1R01NS124222 (MRO).

Disclosures: K. Hussein: None. **S.F. Cogan:** None. **M.I. Romero-Ortega:** None.

Poster

PSTR407

Neuroprosthetic Implantable Devices

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Topic: E.05. Brain-Machine Interface

Support: This work was supported by Electronics and Telecommunications Research Institute (ETRI) grant funded by the Korean government. [24YB1210, Collective Brain-Behavioral Modelling in Socially Interacting Group]

Title: A fully Implantable multi-site monitoring system for deep brain neural signals in unrestrained primate

Authors: *C. JE[1], J.-Y. KIM[1], Y. KANG[1], Y. LEE[2], J. WON[2], M. KIM[2], C.-Y. JEON[2], K. LIM[3], S. LEE[4];
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Abstract: In the deep part of the brain, there are many areas that are related to the emotion and behavior. In small animals, the deep brain is easy to access at a depth of several millimeters, but in large animals such as non-human primates, it is tens of millimeters below the skull, making it difficult to access. Consequently, signal acquisition in primate deep brain has been mainly done under behavioral constraints. In this study, in order to continuously monitor the emotional and behavioral status together in the deep brain region of primates for a long period of time without restraint, the wireless brain signal acquisition system connected to the several tens of mm long electrodes was integrated and packaged, so that it can be completely inserted into the body. The deep brain electrodes are made of a needle-type tungsten metal electrodes (WPI inc., 0.356 mm diameter, 0.5 MΩ impedance) that are tailored to the depth of the target area (several tens of mm) and soldered to the electrode PCB. According to the pattern of the electrode PCB, electrodes for different target areas can be placed at the same time. The electrode PCB is connected to the wireless signal acquisition substrate through FPCB connector so that the device could be flexibly bent to fit the shape of the skull, making it easier to implant into the body. The device was multi-coated using epoxy/film/biocompatible-silicones for stable operation in the body. The size of the packaged device body is 32(L)x17(W)x4(H) mm^3, which can be attached and fully implanted to the skull of a small primate. The fabricated deep brain signal recording electrode device was inserted into the right STN and amygdala through drilled holes in the skull of an adult Cynomolgus monkey, and then the flexible device body was brought into close contact with the skull and fixed using dental cement. The procedure was performed on the custom-built CT/MRI-compatible stereotaxic frame under general anesthesia. With the implanted battery, it was possible to record the nerve signals of STN and Amygdala in freely moving primates for 8 weeks. Even after 8 weeks, the electrodes were still in good condition with no significant drop in electrical impedance, and nerve signals were also acquired in good condition. Our system is very small and flexible, allowing it to fit into the skull of a small primate and monitor multiple areas of the deep brain simultaneously through implanted multi-electrodes. In

addition, it is expected that it will be able to continuously monitor for a long time without behavioral restraint through our system, which will contribute to studying the correlation of the brain signals with social behaviors such as interaction of multiple individuals.

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Poster

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Neuroprosthetic Implantable Devices

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Topic: E.05. Brain-Machine Interface

Support: Science Foundation Ireland (SFI) Grant SFI/12/RC/2289_2
Neural Signals Inc

Title: Simulation of extracellular action potentials detected by an intracortical NeuroNexus neurotrophic electrode (NXNE)

Authors: *P. SAIKIA[1,2], P. R. KENNEDY[3], D. S. ANDREASEN[4], K. SRIDHAR[5], M. LOWERY[1,2];
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Abstract: Speech brain-computer interfaces (BCIs) offer the possibility of restoring communication in individuals with locked-in syndrome, brainstem stroke, ALS, or other neurodegenerative conditions. Intracortical microelectrode-based BCIs that capture neuronal electrical activity are among the most promising solutions for enabling communication using speech BCIs. However, their effectiveness decreases over time due to factors including electrode-tissue interface changes. Furthermore, the relationship between the recorded signal and relative neuron location, along with the influence of electrode properties, is not well-understood. Data on the original neurotrophic electrode indicates longevity up to 13 years. Understanding and addressing these challenges is crucial for improving the long-term stability and performance of BCIs. The aim of this study was to develop a computational model to simulate extracellular action potentials (EAPs) detected by the NXNE in the rodent brain. The electrode consists of 16 recording contacts of diameter 70 µm embedded within an insulating polyimide and glass conical structure 1,956 µm long, with a diameter of 126 µm at the narrow lower and 494 µm at the wide upper end. Nerve growth factor inside the electrode tip facilitates the growth of neurites from neighbouring neurons towards and into the electrode. A finite element (FE) model of the electrode and surrounding tissue in the rat cortex was developed. The FE model was coupled to a

line source model of propagating intracellular APs to simulate EAPs detected from 1,500 axons lying within the electrode. The model was used to examine how amplitude and shape of EAP waveforms are influenced by variations in electrode parameters, including monopolar and bipolar recording configurations. Bipolar EAPs were lower in amplitude compared to those detected using monopolar configurations. For axons within the electrode, the attenuation of APs with increasing distance from the recording contacts was faster for bipolar than monopolar electrodes, with EAPs from fibers located at 165 µm from the electrode contacts having a root mean square amplitude of 86 % and 93 % of that of the closest fibers (67µm), for bipolar configurations. The insulating structure of the electrode had a substantial effect on the electric field distribution, amplifying the signal from fibers lying within the electrode and attenuating the signal from those lying outside it. The model presented can be used to provide insight into detection volume of intracortical electrodes and the relationship between electrode properties and the signals recorded, facilitating optimization of electrode designs for intracortical BCIs.

Disclosures: P. Saikia: None. **P.R. Kennedy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Kennedy has patents associated with the research.. **D.S. Andreasen:** None. **K. Sridhar:** None. **M. Lowery:** None.

Poster

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Topic: E.05. Brain-Machine Interface

Support: HORIZON-EIC-2021-PATHFINDERCHALLENGES-01-02, Grant Agreement n. 101070908

Title: Tailoring brain disease treatments: evaluating wireless implantable microbots using high-resolution 3D imaging.

Authors: *T. GIANNATTASIO[1], M. FRATINI[2,3], F. BRUN[4,5], L. BROMBAL[4,5], S. NIZAMI[1], V. PALUMBO[1], J. F. RIBEIRO[6], F. FRANCESCHINI[1], M. MICALI[1], E. GUIDA[1], S. DOLCI[1], L. BERDONDINI[7], M. SCIMECA[1], A. MAURIELLO[1], A. CONTI[1], N. TOSCHI[1,8];
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Abstract: Brain pathologies exhibit considerable variability, impacting specific regions and altering neural electrical activity uniquely across individuals. This variability necessitates personalized treatments tailored to individual differences. The CROSSBRAIN EU project[1] aims to enhance neuromodulation through the development of wireless, implantable microbots (μBots – 100x100x100μm^3size), designed for precise modulation of brain activity with minimal invasiveness and targeted resolution of specific spatiotemporal events. We evaluated various imaging techniques to identify the most accurate method for locating μBots in brain tissue, a critical step for their optimal placement and functionality.The experiments involved non-functional *μBot silicon (Si) dummies* (Fig. a) - replicating the μBots' shape and size - implanted in ex-vivo adult wild-type mouse brains. The imaging modalities used included light microscopy, transmission electron microscopy (TEM), computed tomography (CT), and synchrotron radiation-based X-ray phase-contrast 3D virtual histology (XPCT) [2] .Our findings indicated challenges with the high density of μBot Si dummies, which compromised tissue integrity during histological sections (Fig. a), thereby diminishing the efficacy of 2D imaging methods (standard microscopy and TEM). However, CT imaging effectively localized the dummies at the implantation sites (Fig. b). XPCT 3D virtual histology provided detailed visualization of both the dummies and adjacent brain tissue (Fig. c-e), revealing differences in absorption indices (Fig.c) and offering insights into the cellular and vascular structures of the brain.This study confirms that XPCT is a promising ex-vivo technique for precisely investigating the location and interactions of small brain implants within brain tissue. This method holds promise for the development of tailored brain treatment technologies, especially for new generation devices incompatible with MRI, representing a significant advance in personalized neuromodulation therapies.

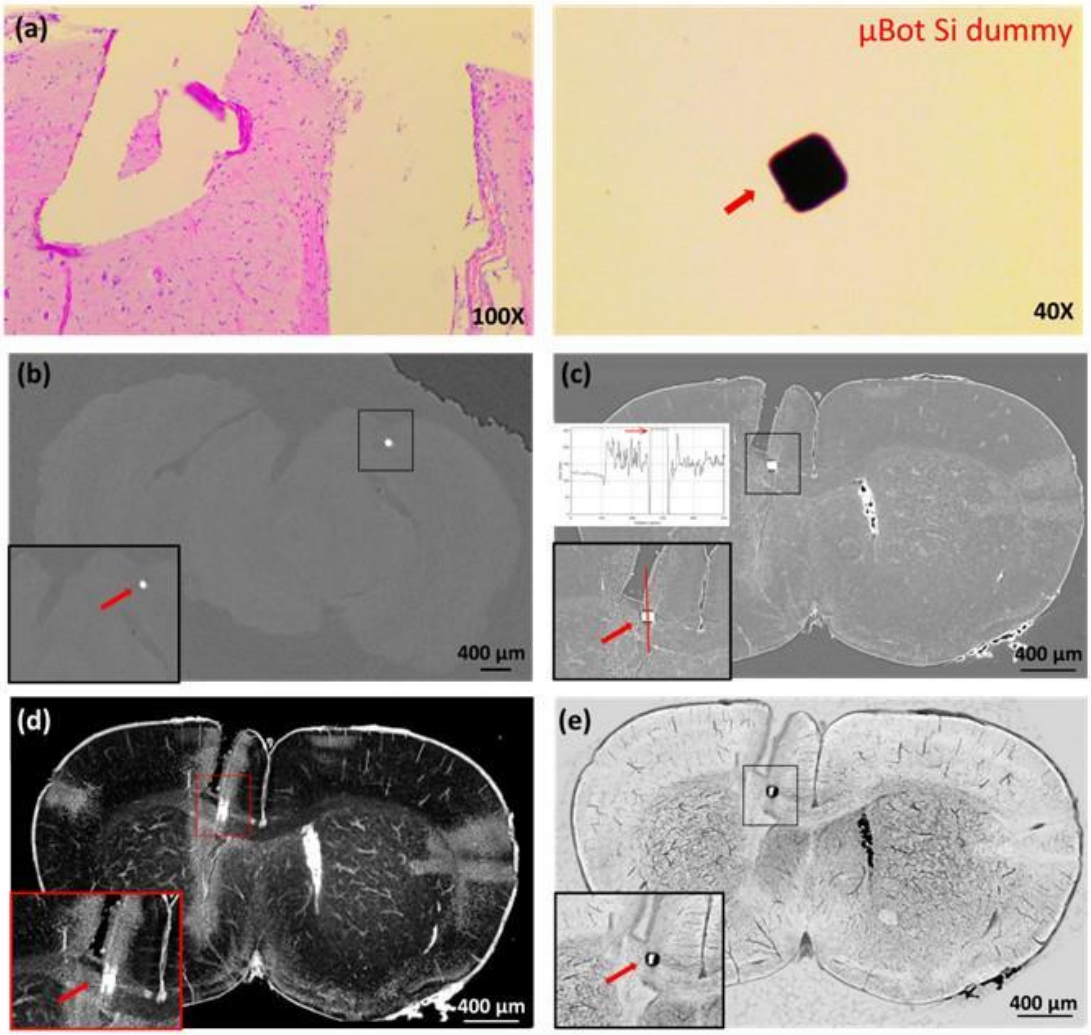

Figure 1. Ex-vivo imaging techniques used to assess the µBots placement and interaction with the brain tissue. (a) hematoxylin/eosin staining and µBot Si dummy visualized with standard microscope; (b) CT – voxel size: 20x20x20 µm3; (c-e) segmented XPCT 3D brain virtual slices – voxel size: 3x3x30 µm3; scale bar: 400 µm. Graph reported in (c) is representative for intensity profile of structures observed; red arrows point µBot Si dummy into the brain tissue and its intensity peak value.

1 www.crossbrain.eu
2 Palermo et al. Front. Neurosci. 202014:584161

Disclosures: T. Giannattasio: None. **M. fratini:** None. **F. Brun:** None. **L. Brombal:** None. **S. Nizami:** None. **V. Palumbo:** None. **J.F. Ribeiro:** None. **F. franceschini:** None. **M. Micali:**

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Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.24/I15

Topic: E.05. Brain-Machine Interface

Support: NINDS (NIH) Grant UF1NS107659
NINDS (NIH) Grant RF1NS128667
NINDS (NIH) Grant R01NS118606
NIMH (NIH) Grant 1RF1MH120005-01
NSF Grant 1707316
NSF Grant 2129817

Title: Uncovering the recording capacity of subcellular-scale carbon fiber electrodes across laminae in rat motor cortex

Authors: *J. G. LETNER[1], P. R. PATEL[1], M. G. COPENHAVER[1], J. L. W. LAM[2], J. RICHIE[1], E. J. WELLE[1], D. CAI[3], C. A. CHESTEK[1];
[1]Biomed. Engin., [2]Neurosurg., [3]Cell and Developmental Biol., Univ. of Michigan, Ann Arbor, MI

Abstract: Conventional practice when using motor brain machine interfaces (BMIs) is to aim for sampling the spiking activity of large pyramidal neurons in layer V due to their robust signals (Moran 2010), which directly project to the spinal column (Losanno 2023). While previous studies have considered the potential utility of recording from other cortical layers (e.g. Parikh 2009), the relationship between recording depth and BMI performance is largely unknown. Highly-biocompatible (Patel 2016) carbon fiber electrodes (CFEs) have the capacity to reliably record nearby neural activity as large spike waveforms (>100 $\mu V_{peak\text{-}peak}$) when implanted chronically in rat cortex, a trait attributed, in part, to the miniscule recording tip (6.8 µm diameter, 10.3 µm height) (Welle 2020). Therefore, CFEs could be sufficiently sensitive to capture neural activity at an expanded range of depths. To measure their recording yield across layers, we implanted CFE arrays into N=9 anesthetized rats (male, Long Evans) during non-survival procedures targeting M1. Each array had six CFEs supported by permanent silicon shuttles (Huan 2021) with small recording sites (257 μm^2 surface area) (Welle 2020) and was manually driven to multiple depths. Electrophysiology recordings were collected at estimated depths ranging 0-2470 µm. Viable recordings (120s each, N=227) were spike-sorted using a slightly modified Kilosort3 (Pachitariu 2024) with parameters updated to yield comparable results to manual sorting. The output was minimally curated to remove clusters that were

putative noise, duplicates, or greatly offset in timing, and recordings that would require manual sorting entirely were excluded. Recordings were also categorized by cortical layer, which was derived from the estimated recording depth and layer boundaries defined in literature (Skoglund 1997). We then visually inspected waveforms that had peak-peak amplitudes >100 µV in the clusters to quantify recording capacity and classified whether implanted fibers had recorded clear spike waveforms at each depth. As expected, we found that these large waveforms were captured on at least one fiber in 48/54 (89%) recordings in layer V and 64/64 (100%) in layer VI. Interestingly, layers III and IV also demonstrated high yield of large waveforms, with 22/28 (79%) and 29/35 (83%) recorded in layers III and IV, respectively. Overall, these results suggest that CFEs are suited for sampling layers III-VI in rat motor cortex. Future work will include improving the spike-sorting with Kilosort4, improving spike modelling with CFEs, and repeating the experiment with CFEs that have even smaller recording sites (105 μm^2 surface area) (Richie 2024).

Disclosures: J.G. Letner: None. **P.R. Patel:** None. **M.G. Copenhaver:** None. **J.L.W. Lam:** None. **J. Richie:** None. **E.J. Welle:** None. **D. Cai:** None. **C.A. Chestek:** None.

Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.25/I16

Topic: E.05. Brain-Machine Interface

Support: NIH Grant 1R44NS103714-01

Title: Ultra-low impedance, platinum-iridium arrays with liquid crystal polymer substrates for bidirectional deep brain interfacing

Authors: *A. PETROSSIANS;
EPIC Med. Inc., Pasadena, CA

Abstract: *Objective:* High-density deep brain electrode arrays have the potential to significantly increase the feature space for clinician-programmed and adaptive deep brain stimulation (DBS) therapies. Such arrays have been fabricated with polyimide substrates mechanically wrapped around and epoxied to a carrier lead. In this study, we report on development and evaluation of a high-density DBS array with ultra-low impedance electrodes and with a liquid crystal polymer (LCP) substrate that was thermally reshaped around carrier lead. *Methods:* Deep brain LCP arrays were fabricated with 100 µm (n=24) and 50 µm (n=24) diameter electrodes (12 rows x 4 columns), each coated with a rough platinum-iridium layer to increase charge-storage capacity, and then implanted in the globus pallidus of two parkinsonian non-human primates. Evaluation of the arrays included analysis of electrochemical impedance spectroscopy, resting-state local field potentials (LFPs), and electrically-evoked LFPs. *Results:* Both chronically implanted LCP

arrays retained electrode viability after one year of implantation and impedance magnitudes did not significantly change between pre-implant and post-explant measurements. The higher density electrode configurations also revealed spatially heterogeneous LFP features in the globus pallidus that were lost when recordings were analyzed in a 'ring'-mode configuration that would be consistent with standard-of-care DBS leads for human use. Conclusion: LCP arrays can maintain long-term bidirectional interfaces. Significance: LCP substrate technology paired with innovative site coatings allow higher amplitude stimulation, recording of heterogeneous neural signals, and good resiliency during chronic implantation.

Disclosures: A. Petrossians: None.

Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.27/I17

Topic: E.05. Brain-Machine Interface

Support: NIH Grant 4UH3NS095557 - 03

Title: Stability of AIROF intracortical electrodes implanted in a human for 17 months

Authors: *P. TROYK[1], M. P. BARRY[1], J. SZLYK[2], S. COGAN[3], G. DAGNELIE[4], V. L. TOWLE[5], F. LANE[6], G. DEMICHELE[7], B. BAK[8], M. J. BAK[9], S. SUH[10];
[1]Illinois Inst. of Technol., Chicago, IL; [2]Low Vision, The Chicago Lighthouse, Chicago, IL; [3]Bioengineering, The Univ. of Texas at Dallas, Dallas, TX; [4]Ophthalmology, Johns Hopkins Univ., Baltimore, MD; [5]Univ. of Chicago, Chicago, IL; [6]Psychology, Illinois Inst. of Technol., Chicago, IL; [7]Engin., Sigenics Inc., Chicago, IL; [8]MicroProbes for Life Sci., Gaithersburg, MD; [9]Microprobes for Life Sci., Inc., Gaithersburg, MD; [10]Biomed. Engin., Illinois Inst. of Technol., Chicago, IL

Abstract: Microelectrodes implanted into brain tissue are often used for both recording and stimulation. For recording the electrode surface area, typically assessed by impedance measurement, is often desired to be small since this maximizes the likelihood of recording single units. For stimulation, the surface area of the electrode must be sufficient to support the desired charge injection, with the recommended injectable charge density related to the electrode material type. Assessment of the artificial neural interface using voltage transients measured during constant-current stimulation is one method for assessing the stability of the interface. As part of an on-going clinical trial (NCT04634383) to investigate the safety and efficacy of an intracortical visual prosthesis (ICVP), 400 activated-iridium-oxide (AIROF), (length 1.0-1.5 mm), intracortical electrodes were implanted into the dorso-lateral surface of the right occipital lobe in a human volunteer who has bare light perception in February 2022. The 3000-sq-micron electrodes were grouped within 25 Wireless-Floating-Microelectrode-Arrays (WFMA), 16

electrodes in each WFMA, which allowed for wireless telemetry of constant-current stimulation voltage transients (VT). The VTs, as well as perceptual thresholds, were periodically measured over a period of two years (on-going). 30uA/200usec/100Hz were used for VT measurement. Two critical parameters extracted from the VTs are Access Resistance (RA) and Polarization (VP) with those data presented here for 17 months of implantation. For all 400 electrodes, the average RA was 54.1kohms; SD=12.4kohms, and the average polarization voltage was -0.27V; SD=0.31V. The variation of these parameters for each WFMA (16 electrodes) was low, suggesting high electrical stability of the interfaces. There is an interesting behavior of the electrodes for which VT shapes and magnitudes seem to be WFMA-specific. It is unknown whether this behavior is associated with the local biological environment or WFMA-specific subtleties in the AIROF film, perhaps caused by variations in the activation process. The high electrical stability of this large group of intracortical electrodes, implanted in a human, is encouraging relative to chronically-implanted brain-machine-interface type devices. Preclinical histopathology results in our work suggests that the floating nature of the WFMA which has no tethering wires may be a significant factor facilitating the high electrical stability.

Disclosures: P. Troyk: A. Employment/Salary (full or part-time):; Sigenics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sigenics, Inc. **M.P. Barry:** None. **J. Szlyk:** None. **S. Cogan:** F. Consulting Fees (e.g., advisory boards); Qualia Oto.. **G. Dagnelie:** None. **V.L. Towle:** None. **F. Lane:** None. **G. DeMichele:** A. Employment/Salary (full or part-time):; Sigenics, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sigenics, Inc. **B. Bak:** A. Employment/Salary (full or part-time):; Microprobes for Life Science. **M.J. Bak:** A. Employment/Salary (full or part-time):; Microprobes for Life Science. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Microprobes for Life Science. **S. Suh:** None.

Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.28/I18

Topic: I.08. Methods to Modulate Neural Activity

Support: private gifts
NIH Award 1S10OD025181

Title: Implantable Intracortical Microchips for Fully Wireless Patterned Focal Stimulation Across Wide Areas of Cortex

Authors: *J. LEE[1], A.-H. LEE[2], V. LEUNG[3], A. V. NURMIKKO[2];
[1]Brown Univ., providence, RI; [2]Sch. of Engin., Brown Univ., Providence, RI; [3]Baylor Univ., Waco, TX

Abstract: A major unresolved question for brain-computer interfaces (BCI) is how to use electronic means to ‘write in’ meaningful sensory information over large areas of the cortex. One suggested alternative to monolithic current injecting multielectrode arrays is based on the idea of autonomous wireless microchip stimulators toward BCI-related stimulatory interventions. Exploratory microstimulator designs for such microscale devices reported to date support only a single channel/device and lack the ability to deliver focal current at multiple locations across wide areas of the cortex. In the realm of neural recording, our group has previously introduced 'neurograins' — a network of spatially distributed, autonomous silicon microchips, as a scalable solution for large-scale wireless neural sensing. This work reports on the development of wireless microchips of 500 μm in size for intracortical stimulation. Importantly, the microelectronic circuitry is designed so that current stimulus across large populations of chips can be wirelessly programmed from a remote radio-frequency source. The telecommunication scheme is based on a novel, collision-free, low duty-cycle communication protocol by which an external RF transmitter issues prescheduled commands across the entire chip population via a high-speed downlink (1 Mbps) whereby a population even up to 1000 chips can be programmed, each for specific current amplitude (up to 60 μA), pulse duration, and repetition rate. Proof-of-concept demonstration of the multipoint patterned stimulation capability of the spatially distributed microchip system has been assessed in several phases. Following benchtop validation in saline, we applied patterns of intracortical microstimulation in vivo on anesthetized rats and recorded the evoked neural response over a range of stimulus conditions. We then implanted 30 microchips for chronic experiments across the cortex of a rat and applied spatially targeted stimulation in randomly sampling the vast stimulus parameter space for a freely moving animal over three months. Clear neuromodulation effects were observed as the animal engaged in a trained lever-pressing task. While the size of the rat brain limits the number of implants, extrapolation of the animal data suggests that a wireless system composed of up to 1000 microstimulators can be accessed in less than 3 milliseconds to deliver complex dynamical patterns of cortical excitation at RF powers which should remain below specific absorption SAR limits in a primate model.

Disclosures: J. Lee: None. **A. Lee:** None. **V. Leung:** None. **A.V. Nurmikko:** None.

Poster

PSTR407

Neuroprosthetic Implantable Devices

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.29/I19

Topic: I.08. Methods to Modulate Neural Activity

Support: Private gifts
NIH Award 1S10OD025181

Title: Minimally Invasive Injectable Wireless Subdermal EEG Microimplant for Long Term Chronic Use

Authors: *A.-H. LEE[1], J. LEE[1], S. S. CASH[2], V. LEUNG[3], A. V. NURMIKKO[1,4];
[1]Sch. of Engin., Brown Univ., Providence, RI; [2]Dept. of Neurol., Harvard Med. Sch., Boston, MA; [3]Electrical and Computer Engin., Baylor Univ., Waco, TX; [4]Carney Institute for Brain Science, Brown University, Providence, RI

Abstract: Long term continuous 24/7 monitoring of brain activity over weeks and months is becoming increasingly recognized as crucial for many patients with epilepsy. Currently available electroencephalogram (EEG) systems are designed for long-term monitoring during relatively short-term hospital stays. Inspired by recent developments as well as our expertise in microelectronic neural sensors, we have developed a minimally invasive unobtrusive wireless subdermal neural microsensor concept that should allow patients to go about their normal daily activities without interference for weeks, months, or even longer. The sensor is based on a 0.2-millimeter-thick, 1-millimeter wide, and up to 10 centimeters long polymer strip which houses four channel electrodes. Detected brain signals from the electrodes are collected at a microchip located at one end of the sensor strip. The silicon chip, measuring 800 μm × 500 μm, harvests around 1 GHz radio-frequency (RF) energy to amplify, process, and securely transmit multichannel brain signal data via RF backscattering through the scalp. The miniaturized form factor of the device enables subdermal insertion through a simple incision in a doctor's office (behind the ear) using a thin, flat custom insertion tool. Importantly, the device uses time-domain multiple access technology for wireless communication whereby multiple subdermal strips can in principle be inserted through the same millimeter-size small incision to fan out and cover larger brain areas, all recorded signals collected by a coin size scalp-mounted radio receiver. We have conducted initial tests with a wireless EEG four-channel prototype strip where this prototype captured proxy EEG signals injected into a saline bath using a function generator. We also tested the insertion technique on a head-proxy phantom, using a flat 15-gauge hypodermic needle as the guide and integrating a bio-resorbable anchor at the end of the strip for secure placement. Additionally, in-vivo experiments have demonstrated the efficacy of the streamlined insertion process and subdermal neural recording capabilities of our system.

Disclosures: A. Lee: None. **J. Lee:** None. **S.S. Cash:** None. **V. Leung:** None. **A.V. Nurmikko:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.01/I20

Topic: E.06. Posture and Gait

Title: Novel approach to induce internal perturbation during walking on a treadmill using functional electrical stimulation

Authors: *T. MIYATA[1,2,3], N. IZUMI[4,2,3], T. YAMAGUCHI[4], S.-I. YAMAMOTO[5], K. MASANI[6,2];
[1]Inst. of Syst. Engin., Shibaura Inst. of Technol., Saitama, Japan; [2]University of Toronto, Toronto, ON, Canada; [3]University Health Network, Toronto, ON, Canada; [4]Tohoku Univ., Miyagi, Japan; [5]Shibaura Inst. of Technol., Saitama, Japan; [6]KITE Res. Inst., Univ. Hlth. Network, Toronto, ON, Canada

Abstract: Falling while walking is a life-threating issue in various populations, including individuals with neurological diseases and the elderly. Extensive research has been conducted to understand the mechanism behind falls. Typically, researchers introduce various perturbations to study reactive balance in these groups and to investigate the deterioration of impaired neural systems. Here we propose a novel approach to induce internal perturbations within the control system of walking using Functional Electrical Stimulation (FES). In the current study we aimed to develop an optimal strategy for inducing instability while walking on a treadmill. Eleven healthy participants walked on a treadmill at three different speeds: fast (1.5 m/s), medium (1.3 m/s), and slow (1.1 m/s). We applied FES to the tibialis anterior (TA), soleus (SOL), rectus femoris (RF) and biceps femoris (BF) muscles at 25, 50, 75 and 100% of gait cycle for 0.2 sec, with 0% set at the moment of heel contact. The Margin of Stability (MoS) in the anterior-posterior direction decreased significantly under all conditions compared with normal waking, particularly at 75% of gait cycle with FES on SOL, 50% with RF and 75% with BF, where the decrease in MoS was significantly greater than at other stimulation timings. In these three conditions, knee joint flexion increased at heel contact on the first step following FES, and the stride interval was significantly shorter than in normal walking. The knee joint plays a critical role in maintaining stability during walking, particularly by absorbing the impact of heel contact. Applying FES to BF at 75% of gait cycle, which occurs just before heel contact, significantly disrupted the knee's role at this critical moment resulting in the decrease of MoS and shortened stride interval. Applying RF at 50% of gait cycle likely indirectly increased the knee flexion as well, resulting in a decrease in MoS. Applying FES to SOL at 75% of gait cycle directly increased the plantarflexion and indirectly increased the knee flexion resulting in a decrease of MoS and shortened stride interval. In conclusion, we have successfully developed an effective strategy to induce mechanical postural instability during walking, without the need for mechanical perturbations, but with inducing artificial muscle contraction. This novel approach can be usable to internally and mechanically perturb dynamic balance and facilitate deeper investigation into the dynamics of human gait.

Disclosures: T. Miyata: None. **N. Izumi:** None. **T. Yamaguchi:** None. **S. Yamamoto:** None. **K. Masani:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.02/I21

Topic: E.06. Posture and Gait

Support: NIDILRR Grant 90SFGE0040-01-00
NIH Grant R01NS115487

Title: Baseline trunk variability predicts motor learning of trunk postural control in children with cerebral palsy

Authors: *S. YAN[1], I. HAMEEDUDDIN[2], H. LIM[3], W. Z. RYMER[3], M. WU[4];
[1]Shirley Ryan AbilityLab, Northwestern Univ., Chicago, IL; [2]Univ. of Illinois Chicago, Bensenville, IL; [3]Shirley Ryan AbilityLab, Chicago, IL; [4]Legs and Walking Lab., Shirley Ryan Abilitylab- Chicago, CHICAGO, IL

Abstract: Excessive trunk movement in the frontal plane during walking is commonly observed in children with cerebral palsy (CP), occurring at a rate of 72% in this population. It is characterized by an increased trunk angle in the frontal plane toward the stance leg. This excessive trunk movement attributes to a compensatory strategy for lower limb motor deficits and/or trunk control deficits, which can result in increased energy cost and less efficient use of extremities during walking. This study aimed to determine the effects of repeated trunk perturbation force during walking on reducing excessive trunk movement in the frontal plane (obliquity) in children with CP. We hypothesized that increasing the error size by applying lateral trunk perturbation force during walking would reduce excessive trunk movement and facilitate motor learning of trunk postural control during walking in children with CP. Fourteen children with CP completed two experimental conditions consisting of 10 min treadmill walking 1) with trunk perturbation force (PERTURB) or 2) without force (CONTROL). A session of overground walking was conducted before, immediately after, and 10 min after treadmill walking. During the treadmill walking of PERTURB condition, a controlled lateral perturbation force was applied to pull the trunk toward the stance leg. The perturbation force, approximately 12% of body weight, was applied during early-mid stance phase for a duration of 400ms. Variables included trunk obliquity and its variability (standard deviation) during treadmill walking and spatiotemporal parameters during overground walking. Participants showed reduced trunk obliquity toward the stronger side immediately after the removal of perturbation for PERTURB condition ($P < 0.05$). Improved trunk obliquity was correlated with higher baseline variability of trunk obliquity ($r = 0.738$, $P < 0.05$). The baseline variability of trunk obliquity can predict the improvement in trunk obliquity following a treadmill walking session with perturbation ($P < 0.05$). Moreover, trunk obliquity variability at early time of perturbation and post-perturbation periods decayed over time, suggesting a motor adaptation to motor variability of trunk movement. Additionally, reduced trunk obliquity during treadmill walking may promote overground walking speed and step length, as well as single-limb and double-limb support time of the stronger leg. Repeated motor adaptation to trunk perturbation force during walking may

induce error-based motor learning of improving trunk control in children with CP. High initial variability of trunk movement can predict motor learning ability of trunk postural control.

Disclosures: S. Yan: None. **I. Hameeduddin:** None. **H. Lim:** None. **W.Z. Rymer:** None. **M. Wu:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.03/I22

Topic: E.06. Posture and Gait

Title: Temporal gait symmetry during treadmill walking in response to electrical stimulation

Authors: *S.-J. KIM[1], S. JAFARI[2], O. J. DYKE[3], J. CHO[2];
[1]Biomed. Engin., California Baptist Univ., Riverside, CA; [2]California Baptist Univ., Riverside, CA; [3]California Baptist Univ., New York, NY

Abstract: Gait asymmetry is observed following neurological conditions. Contemporary rehabilitation methods employ a split-belt treadmill to alter symmetrical gait patterns. Additionally, functional electrical stimulation (FES) presents an alternative intervention to modulate gait symmetry. However, there is limited research exploring FES as a perturbation method to induce gait symmetry adaptation. The objective of this study was to examine the effects of FES perturbation applied bilaterally to subjects' legs, to induce asymmetric gait adaptation during treadmill walking. 20 healthy subjects walked on a treadmill while connected to an electrical stimulator (MCS_STG_4002) via two electrodes (one on the mid-calf, and the other on the lower ankle) on both legs. Subjects' stride duration, heel strike, and toe-off were measured using an Optotrak system. The trial included a 3-min baseline period, a 7-min perturbation period, and a 5-min post-perturbation period (a total of 15 minutes). During the perturbation period, electrical pulses (14~16 mA with 600 μs biphasic pulses for a total of 25.50 ms) were applied to both legs, eliciting a slight plantar flexion. The two pulses were applied at the same period of the subject's stride one. A temporal gap existed between them, equivalent to the interval time between the right and left toes' off. Subjects were instructed to align their right and left toe-off phases with the stimulation. The temporal gap between the two pulses gradually decreased, ranging from -20 ms to -100 ms. By modifying the timing of the pulses applied to legs, we hypothesized that the subject's gait could be adjusted asymmetrically. We measured step length, stance time (ST), and double limb support time (DST) for each leg and analyzed the symmetry changes between the right and left leg for these parameters. We observed bidirectional changes in ST symmetry, with the right ST becoming longer than the left (*positive direction, n=14*) and vice versa (*negative direction, n=6*). The induced ST asymmetry resulted in slight

aftereffects during the post-perturbation period. In addition, we observed unidirectional symmetry changes in DST, with right DST being shorter than the left. No consistent pattern in changes in step length symmetry was noted. These results demonstrate that the FES perturbation method can induce adaptive changes in temporal gait asymmetries (ST and DST). Future experiments would offer deeper insights into the long-term effects of FES perturbation and the reasons behind the bidirectional changes in ST asymmetry observed among different subjects.

Disclosures: S. Kim: None. **S. Jafari:** None. **O.J. Dyke:** None. **J. Cho:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.04/I23

Topic: E.06. Posture and Gait

Support: NIH Grant R01 HD107145-01

Title: Whole body angular momentum differentiates successful versus unsuccessful responses during reactive balance gait training in people with multiple sclerosis

Authors: *M. ADAM[1], A. S. HYNGSTROM[1], T. G. HORNBY[2], B. D. SCHMIT[3]; [1]Physical Therapy, Marquette Univ., Milwaukee, WI; [2]Physical Med. and Rehabil., Indiana Univ., Indianapolis, IN; [3]Dept. of Biomed. Engin., Marquette Univ. Dept. of Biomed. Engin., Milwaukee, WI

Abstract: This study aimed to determine whether whole body angular momentum (H) can differentiate “successful” versus “unsuccessful” responses during reactive balance gait training in people with multiple sclerosis (PwMS). Because falls in PwMS are common,[1] can be extremely debilitating,[2] and may be due to a delayed or ineffective corrective stepping response,[3] reactive balance gait training is becoming frequently employed to reduce fall risk.[4-5] During this training, “falls” are often deemed as unsuccessful, but are determined based on body weight bearing through a fall arrest harness, making this measure heavily dependent on harness placement. H may be well suited to capture more nuanced changes in the effectiveness of these responses due to its ability to reflect a variety of direct and indirect balance control strategies.[6] To improve objective quantification of the reactive response of PwMS, we aimed to determine whether measures of H differed between common definitions of “non fall” and “fall” events. Discrete unexpected treadmill-based perturbations were provided to 5 PwMS (4F/1M) while ambulating on a treadmill at their overground walking speed. Perturbations occurred during single limb stance mediolaterally through surface translation, and anteroposteriorly through rapid slips and stops of the treadmill tread. For a given stance limb and perturbation direction, perturbations increased in amplitude and velocity until a fall was elicited or the maximum

difficulty was reached. Falls were defined as greater than 20% of body weight bearing through a fall arrest harness. Participants were assessed with this protocol 3 to 6 times on separate days, and the differences between all non fall and fall events were compiled and compared. H significantly differed between non fall and fall events after mediolateral and slip type perturbations. Following mediolateral perturbations, fall events demonstrated shorted step widths (0.055m vs 0.096m, $p = 0.03$) and lengths (0.48m vs 0.54m, $p = 0.04$), greater peak to peak differences in sagittal plane H (0.061 vs 0.048, $p = 0.005$), and decreased normal H trajectories (48% vs 65%, $p < 0.001$). Following slip perturbations, fall events demonstrated shortened step lengths (0.55 vs 0.67, $p = 0.02$), greater peak to peak differences in sagittal plane H (0.096 vs 0.048, $p = 0.02$), and decreased normal H trajectories (42% vs 51%, $p = 0.01$). Following stop perturbations, fall events demonstrated shortened step lengths (0.29m vs 0.45m, $p = 0.004$), but no differences in H-based variables. The use of H may define reactive balance response quality more objectively than current standards, especially following mediolateral and slip type perturbations.

Disclosures: M. Adam: A. Employment/Salary (full or part-time):; Marquette University. **A.S. Hyngstrom:** A. Employment/Salary (full or part-time):; Marquette University. **T.G. Hornby:** A. Employment/Salary (full or part-time):; Indiana University. **B.D. Schmit:** A. Employment/Salary (full or part-time):; Marquette University.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.05/I24

Topic: E.06. Posture and Gait

Support: Fondecyt Iniciacion. ANID.11230645

Title: Effect of a perturbation-based balance training combined with targeted Neuromuscular Electrical Stimulation on reactive balance control and early fall risk predictors in persons with stroke

Authors: *G. VARAS;
Physical Therapy, Pontificia Univ. Católica de Chile, Santiago, Chile

Abstract: ***Background.*** In this study, we aimed to investigate whether a 4-weeks Perturbation-based balance training (PBT) can improve kinematic and spatiotemporal parameters of reactive balance control, and kinematic and neuromuscular gait parameters, described as early fall risk predictors, in persons with stroke. Additionally, we aimed to determine whether an impairment-oriented intervention aimed to correct the gait patterns during the proposed PBT, using a targeted neuromuscular electrical stimulation (NMES) applied to the rectus femoris and tibialis anterior

muscles during PBT, could enhance the potential benefits of the proposed training protocol among stroke population. ***Methods.*** The study employs a primary two-arm randomized, controlled design. Twenty participants were randomly assigned to the NMES (n=10) or No-NMES group (N=10). Participants were asked to walk over a six by two meters computer-controlled movable platform at a self-selected speed. Unexpected slip-like perturbations were induced by the device software that moved the platform 12 inches forward at 0.7 m/s with an acceleration of 9.4 m/s2. In total, all the participants experienced 24 perturbation trials per training session. Participants were asked to come to the Laboratory two times per week, so each participant completed 8 PBT sessions, 1 baseline gait assessment and 1 post training gait assessment. The following outcome measures were assessed: Perturbation outcomes (fall or recovery), and Center of mass (CoM) stability. All these outcome measures were assessed after an externally-induced balance perturbation before and after the 4-weeks PBT. On the other hand, step-to-step transition (time of occurrence of the minimal CoM vertical velocity during gait) and altered neuromusuclar patterns (AMAP)(deviation of lower limb EMG activity during gait with respect of age-matched healthy participants), were assessed before and after the training during an instrumented gait assessment in The analysis of Movement Laboratory at Pontificia Universidad Católica de Chile. Two by two ANOVA was conducted to test the time and group effect of this protocol. ***Result.*** Both groups demonstrated improved CoM stability and reduced laboratory fall post-training. Additionally, participants showed improved step-to-step transition and less deviations from normal values of lower limb EMG activity ($p<0.05$). While no difference was observed in step-to-step transition between NMES and No-NMES groups, the NMES group exhibited improved AMAP values post-training.

Disclosures: G. Varas: None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.06/I25

Topic: E.06. Posture and Gait

Support: Tateishi Science and Technology Foundation, Research grant (A)

Title: Effect of walking speed on dynamic stability during non-linear walking

Authors: *A. HIRANO[1], K. HATA[2], M. SHINYA[3];
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Abstract: Effects of Walking Speed Changes on Dynamic Stability During Non-Linear Walking TasksAuthors*A. Hirano, K. Hata, M. Shinya; Hiroshima Univ.DisclosuresA. Hirano: None. K. Hata: None. M. Shinya: None.Non-linear walking, such as turning and avoiding obstacles, inherently poses risks related to balance and stability. The central nervous system controls the walking trajectory and foot placement to concurrently maintain dynamic balance and ensure efficient body movement. In this study, we aimed to quantify the dynamic stability during non-linear walking. Seven young adults (4 males, 3 females, 22 ± 2 years) performed a 180 degree turning after a 5m straight walk, rotating clockwise or counterclockwise back to the start. The participants walked at three speeds: preferred, slow (0.7 times preferred), and fast (1.5 times preferred). Full-body motion capture was performed using reflective markers and 9-camera system (Qualisys, Miqus M3 cameras, 240 fps). Walking path was obtained by fitting a 6th-order polynomial to the center of mass (CoM) trajectory (Dingwell et al. 2024). At each foot contact, a local reference frame was defined by the estimated walking path. Step length, step width, and the turn angle of the steps were calculated based on the local reference frame. Dynamic stability was assessed using the margin of stability (MoS, Hof et al., 2005) which was calculated along with the anteroposterior (AP) and mediolateral (ML) axes of the local reference frame. Recorded walking speeds were 0.75 ± 0.15 m/s (slow), 1.04 ± 0.27 m/s (preferred), and 1.22 ± 3.21 m/s (fast), with step lengths increasing accordingly. For all the speed conditions tested, maximum turn angles occurred during the outside foot's step toward the turning direction (58.6 ± 6.6, 63.8 ± 6.9, and 71.7 ± 6.4 degrees for slow, preferred, and fast conditions; mean ± standard deviation), coinciding with the smallest ML MoS value (-0.07 ± 0.08, -0.10 ± 0.08, and -0.16 ± 0.06 m for slow, preferred, and fast conditions, respectively). The negative ML MoS values indicate that the step was performed. This might be regarded as a strategy of the able-bodied young participants that emphasizes mobility rather than instantaneous balance. Future research could compare these dynamics among older adults and children to further elucidate the impacts of aging and development on dynamic balance control and fall risk.

Disclosures: A. Hirano: None. **K. Hata:** None. **M. Shinya:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.07/I26

Topic: E.06. Posture and Gait

Support: R01NS124814
R01NS088679
R01NS113746

Title: Stepping response to forward and backward postural perturbations in Parkinson's disease with vs. without REM sleep without atonia: 3-year follow up

Authors: *Y. CHOI[1], S. L. AMUNDSEN HUFFMASTER[1], J. CHUNG[1], C. LU[1], A. VIDENOVIC[2], P. TUITE[1], M. HOWELL[1], C. D. MACKINNON[1], S. E. COOPER[1]; [1]Neurol., Univ. of Minnesota, Minneapolis, MN; [2]Massachusetts Gen. Hosp., Brookline, MA

Abstract: A high percentage (~40%) of people with Parkinson's disease (PD) exhibit increased muscle activity during rapid eye movement (REM) sleep (REM sleep without atonia; RSWA). RSWA serves as an electromyographic marker of REM sleep behavior disorder (RBD). Individuals diagnosed with both PD and RBD are more likely to develop postural instability than individuals with PD alone. Using a reactive stepping task, we examined the impact of RSWA on reactive postural stability by comparing PD patients with RSWA to PD patients without RSWA, and healthy controls (HC). Furthermore, we reassessed the same individuals after 3 years, to investigate how reactive postural instability had progressed over time. We tested the hypothesis that people with PD and RSWA have greater reactive postural instability, and this instability progresses differently over three years, compared to those without RSWA and HC.Ten people with PD and RSWA (65.0 ± 6.3 yrs, F=4), 17 people with PD with normal REM sleep muscle tone (63.1 ± 8.2 yrs, F=5), and 17 HC (61.1 ± 8.0 yrs, F=7) participated in the study. Participants stood on a force plate which measured center of pressure (COP) while the support surface underwent a sudden anterior or posterior displacement sufficient to require at least one step to recover balance (Cmill, Amsterdam). Each participant completed 5 trials in both the forward-stepping and backward-stepping directions. This was repeated 3 years later. Testing in the PD participants was conducted off-medications. A principal components analysis was used to extract most of the COP variance into the first principal component (PC1). We compared the PC1 projection values among three groups and across two time points (baseline and 3 years later) using a linear mixed model with SUBJECT as random-intercept factor.We found a significant group effect ($p=0.04$), but no significant time effect ($p=0.2$) for forward stepping responses. A significant group-by-time interaction was also noted ($p=0.001$). In general, the PD with RSWA group exhibited the lowest PC1 projection value compared to other groups, with a significant difference from HC ($p=0.03$). After 3 years, all groups showed an increase in PC1 projection value, but only the RSWA group demonstrated a statistically significant increase ($p<0.05$). For backward stepping responses, while there was an overall increase in PC1 value across all groups over time ($p<0.001$), no significant differences were found between the groups either at baseline or after 3 years ($p>0.1$).In conclusion, the presence of muscle tone during REM sleep (RSWA) significantly alters postural response to perturbations when compared to individuals with PD who have normal muscle tone and HC.

Disclosures: Y. Choi: None. **S.L. Amundsen Huffmaster:** None. **J. Chung:** None. **C. Lu:** None. **A. Videnovic:** None. **P. Tuite:** None. **M. Howell:** None. **C.D. MacKinnon:** None. **S.E. Cooper:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.08/I27

Topic: E.06. Posture and Gait

Support: Cerebral Palsy Alliance Research Foundation Doctoral Grant (PHD00421)
Kimel Family Graduate Student Scholarship in Rehabilitation (Imaging)
SPARK Lab Research Support Fund
NSERC Grant (RGPIN-2019-06033)

Title: Functional and neurological outcomes following the application of a motor learning treatment paradigm during exoskeleton-assisted physiotherapy in two children with cerebral palsy

Authors: *S. S. BRADLEY[1,2], V. WRIGHT[3,4], T. CHAU[3,5];
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Abstract: Cerebral palsy (CP) encompasses a group of neurological disorders that affect posture, balance, and coordination. Use of motor learning principles (i.e., intensity, repetition, variability, task-specificity) have been associated with structural changes in the brain following blocks of physiotherapy (PT). Newly developed robotic gait technologies offer upright mobility opportunities for children with CP, while supporting use of motor learning principles. The Trexo Plus is an overground pediatric lower limb exoskeleton that guides and powers leg movements for minimally ambulatory children. Our study explores the functional and neuroplastic outcomes of a PT-based motor learning treatment paradigm with the Trexo. **Methods**: Pre/post analysis of two children with CP from a larger pre-/post-test feasibility study. The Trexo-based intervention was given twice weekly for 6 weeks in our outpatient center by a physiotherapist team trained in motor learning principles. Child A (male, 5 y.o., spastic unilateral CP [left hemiplegia]) and Child B (male, 6 y.o., mixed bilateral CP [right-handed]) were categorized as Gross Motor Function Classification System level IV, with no independent ambulation. Pre/post functional assessment measures (Gross Motor Function Measure [GMFM-88 subset] and Goal Attainment Scaling [GAS]) were completed by physiotherapists. Pre/post MRI head scans were obtained via a 3T Siemens scanner and subjected to whole brain volumetric analysis (Freesurfer Version 7.4.1). Motor learning principles applied during PT sessions were documented using the Motor Learning Strategies Rating Instrument. **Results (Child A, Child B)**: The functional categories of GAS goals were determined to be walking endurance, stepping, upper extremity function, head control, and trunk control. Pre/post GAS T-scores (56.20, 59.10); pre/post GMFM change scores (+28.48% points, +10.91% points). MRI volumetric analysis showed pre/post cortical gray matter (GM) changes (% of original volume) in the left hemisphere (LH) (+1.48, +0.52) and right hemisphere (RH) (+0.77, -1.89). There were also pre/post changes in cerebral white matter (WM) in the LH (+1.89, +2.12) and RH (+1.64, -1.21). **Conclusions**: Following exoskeleton-mediated PT, functional changes (goal-based and gross motor) were present in both children.

The increases in WM and GM are consistent with results of other exercise interventions, and more notable LH results may be due to both children's right-side dominance. Characterization of therapy-dependent neuroplasticity in children with CP may help clinicians better understand relationships among PT, neurological changes, and clinical outcomes.

Disclosures: S.S. Bradley: None. **V. Wright:** None. **T. Chau:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.09/I28

Topic: E.06. Posture and Gait

Support: NSF Career Award 1847891
NSF-GRFP 2139321

Title: Human perception of leg-speed differences in walking adheres to Weber's Law

Authors: *M. GONZALEZ-RUBIO[1], P. A. ITURRALDE[2], G. TORRES-OVIEDO[3]; [1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Engin., Univ. Católica del Uruguay, Montevideo, Uruguay; [3]Dept. of Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Sensation plays a central role in the planning, execution, and adaptation of our movements; however, research has concentrated on the characterization of motor adaptability due to its observable consequences. Understanding sensation during walking is crucial for effectively responding to environmental changes, such as varying terrains. Thus, we aim to quantify sensation non-invasively using perceptual tasks on a split-belt treadmill (i.e., one leg moves faster) and test if perception of leg-speed differences adheres to Weber's Law which has been identified across multiple sensory modalities. Weber's law posits that sensitivity to a change in sensory stimulus scales proportionally to the intensity of the background sensory information. For example, one can detect a small change in lumens in the dark, but this sensory change needs to be large for detecting it under the bright sunlight. We hypothesized that sensitivity to leg-speed differences scales accordingly. To test this hypothesis, we assessed the sensitivity of leg-speed differences in healthy, young adults who walked at different mean walking speeds: slow (0.7 m/s), medium (1.05 m/s) and fast (1.75 m/s). According to Weber's Law, we expected people would be more sensitive to leg-speed differences when walking at slower (low sensory background) than when walking at faster (high sensory background) speeds. We estimated each participant's sensitivity to leg-speed differences with a series of 2-alternative-forced choice tasks (2AFC) presented in a pseudorandom order while walking at each one of the walking speeds. In each 2AFC task, participants experienced a series of leg-speed differences and had to indicate within 8 seconds which leg moved slower. We fitted a logistic regression on

participants' responses as a function of the perturbation size and analyzed the slope of the curve. We found that participants' responses had a significantly higher slope for the slow compared to medium walking speed logistic regression ($p=0.0048$; $n=13$) and a significantly lower slope for the fast than medium walking speed logistic regression ($p=0.0005$; $n=6$). Our results align with what would be predicted by Weber's Law, where sensitivity to sensory stimuli (leg-speed differences) is higher (i.e., higher slopes) upon low sensory backgrounds (slow walking) than high sensory background (fast walking). Future studies should address how to incorporate these findings into the design of split-belt adaptation protocols and investigate interplay between sensory and motor adaptation.

Disclosures: M. Gonzalez-Rubio: None. **P.A. Iturralde:** None. **G. Torres-Oviedo:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.10/I29

Topic: E.06. Posture and Gait

Support: University of Colorado Anschutz-Boulder Nexus
NIH National Center of Neuromodulation for Rehabilitation (P2CHD086844)

Title: Effects of acute intermittent hypoxia on lower limb voluntary activation and torque output during repeated maximal contractions

Authors: *A. BOGARD[1], A. POLLET[1], J. BORDEWICK[1], L. PELLEGRINO[1], A. Q. TAN[2]; [1]Univ. of Colorado Boulder, Boulder, CO; [2]Integrative Physiol., Univ. of Colorado Boulder, Boulder, CO

Abstract: Promising investigations underscore the therapeutic benefits of acute intermittent hypoxia (AIH) to enhance motor performance after motor incomplete spinal cord injury and to promote motor learning in able-bodied individuals. Our recent work has identified AIH-induced enhancements in corticospinal excitability as a marker of improvements in motor learning. However, questions persist regarding how AIH affects voluntary activation during repetitive lower limb muscle contractions. Voluntary activation refers to the ability of the nervous system to fully activate muscle fibers during voluntary contractions. Voluntary activation reflects the composite neural drive from descending and spinal networks. In able-bodied individuals, repeated maximal voluntary contractions (MVCs) lead to a reduction in both lower limb voluntary activation and maximum torque production, alongside an increase in corticospinal excitability. Therefore, augmenting descending neural drive could serve as a control strategy to maintain the requisite voluntary activation needed to preserve high torque output. Accordingly,

we are conducting a pre-post study to assess the effects of AIH on voluntary activation and torque during 20 consecutive, isometric plantarflexion MVCs. Supramaximal electrical stimuli are applied to the posterior tibial nerve every 5 contractions. We are utilizing the central activation ratio (CAR) as an index of voluntary activation, with greater values indicating heightened voluntary activation. CAR is calculated as the ratio of the average voluntary torque 50 ms preceding the electrical stimuli to the superimposed torque 10 ms after the electromechanical delay. Participants undergo AIH for four consecutive days at the same time each day. During each AIH treatment, participants receive 15, 90-second intervals of low-oxygen air (9% O_2) alternated with 60-second intervals of room air (21% O_2). Our preliminary findings show higher CAR values during repeated MVCs post-AIH, indicating that AIH may attenuate voluntary activation deficits. Notably, our results also demonstrate that AIH may mitigate the decline in torque during repetitive contractions. These early observations support the use of AIH in enhancing voluntary activation and ankle torque production during tasks that challenge the neuromuscular system. Optimizing voluntary activation is critical for facilitating the recovery of functional walking skills after neurological injury.

Disclosures: A. Bogard: None. **A. Pollet:** None. **J. Bordewick:** None. **L. Pellegrino:** None. **A.Q. Tan:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.11/I30

Topic: E.06. Posture and Gait

Title: Combining a multilevel trunk-hip-knee orthotic, focused gait diagnostics with neurobiomechanical biofeedback unveil gait and cortical adaptations in a CP patient - a case report

Authors: K. GÖTZ-NEUMANN[1], J. HERNANDEZ GLORIA[2,3], N. MRACHACZ-KERSTING[3], *U. G. KERSTING[4];
[1]Observational Gait Instructor Group, Los Angeles, CA; [3]Sport and Sport Sci., [2]Albert-Ludwigs Univ. Freiburg, Freiburg, Germany; [4]Biomechanics and Orthopaedics, German Sport Univ. Cologne, Cologne, Germany

Abstract: Children with Cerebral Palsy (CP) often face challenges in motor function and gait mechanics that persist into adolescence and adulthood despite conventional therapies and orthotic interventions. This case report focuses on one child with CP who exhibited stagnant motor development and (worsening) gait abnormalities despite extensive therapeutic interventions, including ankle-foot orthotics and orthopedic surgeries. We aimed to explore the

efficacy of a novel approach combining multilevel trunk-hip-knee orthotics, focused gait diagnostics, and neurobiomechanical biofeedback therapy in promoting cortical plasticity and enhancing functional outcomes.The infant underwents comprehensive assessments of gait kinetics and kinematics, electromyography and electroencephalography (EMG, EEG), and neurobiomechanical evaluations in a single session of about 2 h duration. Based on initial assessments, a tailored intervention plan was implemented directing the kids attention to recruitment and activation of hip external rotators, combined with a soft and multijoint orthotic mainly addressing hip external rotation. EEG activities were compared before and after the intervention in the alpha-band, EMG data of hip external rotators and gait kinematics.Following the intervention, the participant demonstrated immediate improvements in gait patterns by altering the foot angle at touch-down, showing less Knee flexion and a trend for more externally rotated thighs during stance bilaterally. Muscle activation of the adductors were reduced while external rotators increased. EEG analysis identified pattern changes in the alpha band implying a clear cortical response due to the intervention. This case report underscores the potential of a concerted approach integrating advanced orthotic interventions, neurobiomechanical diagnostics, and targeted therapy to induce cortical plasticity and promote functional improvements in children with CP. We rate such improvements superior to conventional therapies, e.g., with rigid orthoses which promote a less active motor pattern. The data also demonstrates that afferent input and cortical activity increase. If such alterations would be strengthened due to coninuous practice a wide opportunity for preventing musculoskeletal deterioration will be possible.

Disclosures: K. Götz-Neumann: None. **J. Hernandez Gloria:** A. Employment/Salary (full or part-time):; Albert-Ludwigs University Freiburg. **N. Mrachacz-Kersting:** A. Employment/Salary (full or part-time):; Albert-Ludwigs University Freiburg. **U.G. Kersting:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.12/I31

Topic: E.06. Posture and Gait

Support: University if Colorado Anschutz - Boulder Nexus
NIH Grant P2CHD086844

Title: The relationship between acute intermittent hypoxia-induced changes in serum brain-derived neurotrophic factor and ankle torque steadiness

Authors: *A. POLLET, A. BOGARD, A. Q. TAN, L. DEMINSKI, M. CEDRO;
Univ. of Colorado, Boulder, Boulder, CO

Abstract: Neuroplasticity following incomplete spinal cord injury (iSCI) is key in restoring functional mobility and quality of life. One emerging adjuvant used to induce neuroplasticity is acute intermittent hypoxia (AIH), which involves breathing mild bouts of low-oxygen air. Evidence from spinally injured rodent models demonstrate that AIH induces increased synthesis of brain-derived neurotrophic factor (BDNF). Importantly, increased BDNF concentration has been linked to greater excitability of motor neurons and improved motor performance in animal studies. While a similar BDNF dependent mechanism may be responsible for the AIH-induced motor improvements seen in humans, this has yet to be investigated. Quantifying the AIH-induced changes in systemic BDNF concentration and associated changes in motor control is important for understanding the therapeutic potential of AIH. It is well established that ankle torque steadiness is predictive of walking speed and endurance in individuals with multiple sclerosis. Thus, we investigated the effects of repetitive AIH on serum BDNF as well as ankle torque steadiness. Prior to AIH, study participants completed a visually-guided, isometric, torque steadiness assessment. Participants gradually increased plantarflexion torque to reach and maintain a target torque of both 20% and 40% of their maximum voluntary contraction. The coefficient of variation (CV) of torque was used as a measure of torque steadiness. CV is calculated as the ratio of the standard deviation of torque and the mean torque, with lower values indicating greater torque steadiness. Participants then received AIH treatments for four consecutive days. A single AIH treatment consisted of breathing 90-second bouts of hypoxic air (9% O_2) alternated with 60 seconds of normoxic air (21% O_2) for 15 episodes. On the fourth day, participants completed the same torque steadiness assessment. Venous blood samples were taken on day one, prior to AIH and following AIH on days 1, 3, and 4. We quantified changes in the serum concentration of BDNF following the 1st, 3rd, and 4th AIH exposure. Preliminary data demonstrates a decrease in the CV of torque during plantarflexion from pre- to post-AIH, suggesting improvements in lower limb motor control. Additionally, results indicate a possible influence of AIH on BDNF concentration which may validate one mechanisms behind AIH induced neuroplasticity in humans.

Disclosures: A. Pollet: None. **A. Bogard:** None. **A.Q. Tan:** None. **L. Deminski:** None. **M. Cedro:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.13/I32

Topic: E.06. Posture and Gait

Support: Natural Sciences and Engineering Research Council of Canada Discovery Grant [2017-06632]

Title: The effect of normobaric hypoxia and postural demand on cortical and spinal excitability

Authors: E. C. BENNETT[1], A. D. PAISH[2], Q. MALONE[3], J. M. J. R. CARR[4], C. J. MCNEIL[5], *B. H. DALTON[1];
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Abstract: Balance control is essential for performing activities of daily living and, ultimately, independence throughout the lifespan. Standing balance relies on the integration of multiple sensory signals (e.g., proprioceptive, vestibular, visual, and auditory) with motor commands to produce appropriate postural adjustments to keep the body upright. One known factor that may alter balance is an inadequate availability of inspired oxygen (i.e., hypoxia), which is often encountered when traveling to high altitudes. Alternatively, high altitude can be simulated within an environmental chamber capable of reducing the fraction of inspired oxygen (F_IO_2) while maintaining ambient atmospheric pressure (i.e., normobaric hypoxia). Because sensory signals are integrated at different levels of the corticospinal pathway (e.g., cortical, motoneuronal and peripheral), it is important to understand how distinct components of the corticospinal tract function during hypoxia. The purpose was to determine if two hours of normobaric hypoxia ($F_IO_2 \approx 0.11$; Everest Base Camp, 5364m) altered cortical, motoneuronal or peripheral excitability during sitting and standing. Six (4 females) participants completed three plantar flexor maximal voluntary contractions (MVCs) to assess torque and voluntary activation in a sitting posture. While seated, electric stimulation of the tibial nerve was used to evoke a maximal compound muscle action potential (M_{max}) from the soleus. Further, transcranial magnetic stimulation of the motor cortex and electric stimulation over the thoracic spine were used to elicit soleus motor evoked potentials (MEPs) and thoracic motor evoked potentials (TMEPs), respectively of ~5-10% M_{max}. To evaluate excitability at the same relative muscle activity for sitting and standing, participants performed five trials for each posture in which they maintained an integrated electromyography (iEMG) amplitude of the soleus that was equivalent to that obtained at 20% MVC torque while sitting. During each trial, a fixed series of stimuli were delivered to elicit a TMEP, MEP, and M_{max}. Ratios of the area of the evoked potentials were used to evaluate cortical (MEP/TMEP) and motoneuronal (TMEP/M_{max}) excitability, and M_{max} area was used to evaluate peripheral excitability. Mean voluntary activation and plantar flexion MVC torque were 95.4±2.3% and 108.6±38.7Nm, respectively. There were no main effects of posture ($p \geq 0.098$) or hypoxia ($p \geq 0.102$), nor interactions ($p \geq 0.051$) for cortical, motoneuronal, and peripheral excitability. Therefore, our findings indicate that excitability of the corticospinal tract may not be influenced by 2 hours of normobaric hypoxia.

Disclosures: E.C. Bennett: None. **A.D. Paish:** None. **Q. Malone:** None. **J.M.J.R. Carr:** None. **C.J. McNeil:** None. **B.H. Dalton:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.14/I33

Topic: E.06. Posture and Gait

Support: NIH/NIA R01-AG071585

Title: Does pain context effect retention of a locomotor learning paradigm?

Authors: *S. JACKSON[1], S. M. MORTON[2];
[1]Univ. of Delaware, Newark, DE; [2]Physical Therapy, Univ. of Delaware, Newark, DE

Abstract: The effect of pain on motor learning and retention is not well understood, which could have important consequences for patients undergoing rehabilitation. Our group and others have shown that pain could interfere with retention of motor learning. However, some evidence suggests this effect may not be truly due to pain, but due to the effect of a context change from learning (in a painful state) to retention testing (in a nonpainful state). Yet retention with and without a context change has not been directly compared. Therefore, we examined the effect of pain on locomotor learning and retention, specifically the effect of a context change between learning and retention conditions, on the magnitude of retention. Three groups of young healthy adults performed a locomotor learning task: a 'no pain' group received no intervention; a 'pain during learning' group received a painful stimulus during Day 1 learning; and a 'pain during learning and retention' group received the same painful stimulus during both Day 1 learning and Day 2 retention testing. Pain was induced via topical capsaicin (0.1%) and heat applied to the anterior lower leg. On Day 1, all participants learned a new asymmetric stepping pattern using visual feedback. A monitor displayed real-time feedback of step lengths, represented as bars growing vertically on the screen. During learning, the feedback was distorted, making one leg appear to take longer steps and the other to take shorter steps. To hit the visual step length targets, participants had to acquire a novel step asymmetry of 9%. Twenty-four hours later, retention was tested with the 9% abrupt distorted visual feedback presented. Force plates and a 3D motion capture system recorded gait events and measured step lengths, respectively. Results indicate there are no differences in learning between groups, consistent with our prior work. Retention, quantified by calculating a forgetting index across days (mean±SD; no pain: 3.69±2.4, pain during learning: 5.14±2.6, pain during learning and retention: 4.16±3.8) suggest acute pain impairs retention of locomotor learning and that a context change during retention testing may affect retention magnitude. Taken together, these findings provide further evidence that acquisition of a new motor skill is possible for individuals experiencing acute pain and suggest that acute pain impairs retention.

Disclosures: S. Jackson: None. **S.M. Morton:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.15/I34

Topic: E.06. Posture and Gait

Title: Targeted downward pelvic force induces improvements in crouch gait in children with cerebral palsy

Authors: *I. HAMEEDUDDIN[1], S. YAN[2], H. LIM[3], M. WU[4];
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Abstract: Cerebral palsy (CP) is a common motor disorder in children caused by brain damage around the time of birth. Crouch gait, characterized by the hyperflexion of the hips and knees during stance, is a common and debilitating gait disorder in children with CP. A crouched posture is significantly less efficient when walking and can lead to overworked muscle. Current treatments for crouch gait have not been optimized to effectively address key gait deficits in many children with CP. Thus, there is a clear need to develop new interventions for improving crouch gait, requiring an examination of motor learning mechanisms of the interventions. The purpose of this study is to determine whether application of targeted downward pelvic pull will be more effective compared to constant downward pull via increased extension and decreased flexion of the hip and knee joints. We hypothesized that applying downward pulling force at mid-stance of gait to increase error will facilitate motor learning of improved crouch gait. Eleven participants (age 9 to 14, 3 girls, GMFCS I - III) with crouch gait participated in a one-visit study consisting of treadmill walking for 10 minutes with three conditions of downward pulling force: Early-Stance, Mid-Stance, and Constant. The walk consisted of 1 minute of no force, 7 minutes of force (adaptation), and 2 minutes of no force. For the two targeted conditions, a downward force was applied for 250ms at either the early-stance phase or the mid-stance phase. For the Constant condition, the downward force was applied throughout the gait cycle. The downward force was applied via a pelvis belt attached to two motors to provide more flexion for the participant voluntarily counteract against during stance phase of gait. The participants adapted to the targeted downward force and showed an aftereffect consisting of increased hip extension. Specifically, participants showed significant increase in hip extension in both the weaker ($\Delta 1.66 \pm 0.0159$ °) and stronger legs ($\Delta 3.02 \pm 0.7848$ °), and decreased activity of the tibialis anterior during the post-adaptation period for the Mid-Stance condition (2-way ANOVA $p<0.05$). Conversely, the participants showed significant increase in knee flexion in the weaker leg ($\Delta 4.1975 \pm 1.6331$ °) for the Early-Stance condition (2-way ANOVA $p<0.05$) and did not show significant changes for the Constant condition. These results suggest that applying targeted force at the mid-stance phase may be more effective in facilitating motor learning of improved crouch gait, as opposed to constant or at early-stance. Results from this study may be used for the development of new interventions to improve crouch gait in children with CP.

Disclosures: I. Hameeduddin: None. **S. Yan:** None. **H. Lim:** None. **M. Wu:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.16/I35

Topic: E.06. Posture and Gait

Support: Brinson Foundation
NIH R01HD082216

Title: Spatiotemporally controlled transcutaneous spinal cord stimulation enhances paretic leg motor control during walking in persons with stroke

Authors: *H. LIM[1,2], S. YAN[1,2], I. HAMEEDUDDIN[3,1], W. Z. RYMER[1,2], M. WU[1,3,2]; [1]Shirley Ryan AbilityLab, Chicago, IL; [2]Northwestern Univ., Chicago, IL; [3]Univ. of Illinois at Chicago, Chicago, IL

Abstract: *Background:* After stroke, individuals demonstrate impaired motor control and muscle weakness in the paretic leg, which interferes with mobility. Transcutaneous spinal cord stimulation (tSCS) has shown promising results in enhancing volitional leg motor control and walking function in people with spinal cord injury. Recent evidence suggests that multi-site stimulation of the lumbosacral spinal cord, tailored to specific gait phases, induces superior effects compared to single-site, continuous stimulation. Despites its potential effectiveness, the impact of spatiotemporally controlled tSCS targeting the paretic leg to improve walking in individuals with stroke remains unclear.*Objective:* To determine whether the spatiotemporally controlled tSCS during treadmill walking with constraint force applied to the non-paretic leg, facilitates paretic leg motor control during walking in individuals post-stroke. *Methods:* Thirteen individuals with chronic stroke were tested under two conditions: active vs sham tSCS. Each condition involved either active or sham tSCS during treadmill walking, with backward constraint force (~3% of body weight) applied to the non-paretic leg during the swing phase of gait. tSCS was delivered through two cathodes, which were placed 1.5 cm laterally from the L4 and S1 spinous processes toward the paretic side, and two anodes were placed on the anterior superior iliac spine on each side. Spatiotemporally controlled stimulation was applied at the L4 during swing phase of the paretic leg and the S1 during stance phase. The stimulation parameters were monophasic rectangular pulses with a pulse width of 100 μs at a frequency of 80 Hz for L4 and 30 Hz for S1, filled with a carrier frequency of 9.5 kHz. Spatiotemporal gait parameters were measured during treadmill walking before and after the application of tSCS and constraint force. Overground gait parameters and propulsive force of the paretic leg were assessed before, immediately after, and 10-minute after treadmill walking.

Results: Participants showed greater increase in paretic step length and paretic step height for the active tSCS condition compared to the sham tSCS condition. Active tSCS improved paretic stance time, while the sham tSCS did not. These changes partially transferred to overground walking, indicated by increased paretic step length and walking speed 10-minute after treadmill walking. *Conclusion:* Our findings suggest that the spatiotemporally controlled tSCS during treadmill walking with constraint force applied to the non-paretic leg may improve motor control of the paretic leg, which may partially transfer to overground walking in individuals post-stroke.

Disclosures: H. Lim: None. **S. Yan:** None. **I. Hameeduddin:** None. **W.Z. Rymer:** None. **M. Wu:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.17/I36

Topic: E.06. Posture and Gait

Title: Gait factors related to walk ratio in Parkinson’s disease

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Abstract: Gait impairment in Parkinson's disease (PD) patients is primarily assessed by gait velocity, which is the product of stride length and cadence. Both stride length and cadence are critical factors to consider. Therefore, this study focused on the walk ratio, defined as the ratio of cadence to stride length, aiming to identify motor symptoms and gait indices that influence the walk ratio in PD. In this study, gait analysis was conducted on 25 PD patients using accelerometry. The assessment revealed various parameters including gait speed, walk ratio, stride length, cadence, and time components such as stance, swing, and stride phases, along with their coefficients of variation (CV), arm swing amplitude, asymmetry, and trunk motions. For data analysis, gait indices—excluding stride length and cadence—were reduced dimensionally through principal component analysis (PCA). Multiple regression analysis was then performed using the derived principal components (PCs), basic demographic characteristics, and the MDS-UPDRS motor score sub-score as explanatory variables, with walk ratio as the dependent variable. Among the five PCs identified, PC2—which included more severe side's stance and swing times (β = 3.617e-04, p-value = 0.004)—emerged as a significant variable, achieving an R^2 of 0.744. Increased movement time was observed throughout the gait cycle in PD, possibly reflecting compensatory strategies for slower gait and reduced stride length. Given that stride length in PD is indicative of disease progression, whereas cadence does not change as the disease

progresses, it is possible that the walk ratio may also change with progression. Therefore, it is necessary to expand the study population and further investigate whether similar gait control strategies are adopted based on disease severity in future studies.

Disclosures: Y. Terasawa: None. **S. Morioka:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.18/Web Only

Topic: E.06. Posture and Gait

Support: JSPS KAKENHI Number 24K02840

Title: Anticipatory postural adjustments to compensate for horizontal moments associated with pulling a handle toward different directions

Authors: *M. SHINYA[1], T. AKAMATSU[2];
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Abstract: Anticipatory postural adjustments (APAs) are executed to compensate for the predictable perturbations associated with voluntary upper limb movements during an upright stance. While numerous previous studies have demonstrated that APA parameters are related to the magnitude and direction of the translational force associated with upper limb movements, it remains unclear how APAs compensate for the moments around the body center of mass (CoM) in the horizontal plane. For instance, we considered a task where a handle is pulled with the left or right arm at 45 degrees leftwards. Although the magnitude and direction of the translational force are identical for left and right arm conditions, the moment generated around the CoM differs. We hypothesized that the direction of the ground reaction forces (GRFs) under the left and right feet would be functionally adjusted to counteract the horizontal moment. We measured the GRFs under each foot as twelve right-handed, able-bodied adults (23 ± 2 years, 10 males, 2 females) performed an isometric pulling task at 5% weight strength with either hand. The direction was either inwards or outwards (45 degrees). The GRFs at the onset of the pulling force were quantified as the APA. While the sum of the GRFs of the left and right feet was comparable across the hand conditions, the horizontal GRFs of each foot were oriented in different directions depending on the arm pulling the handle. The anteroposterior (AP) component of the GRF was larger when pulling outwards with the left hand compared to pulling inwards with the right hand (Left hand: 0.779 ± 0.382; Right hand: 0.404 ± 0.309 (%Weight), mean ± SD, $p = 0.002$). Conversely, the right foot AP GRF was smaller when pulling outwards with the left hand compared to pulling inwards with the right hand (Left hand: -0.009 ± 0.165; Right hand: 0.352 ±

0.135 (%Weight), $p < 0.001$). When the participants pulled the handle in the outward direction (i.e., the upper limb force vector does not pass through the CoM), the horizontal GRFs under the left and right feet during the APA phase oriented in different directions. The distinct direction of the GRFs under the left and right feet suggests a functional APA to counteract the moments in the horizontal plane generated by the upper limb forces.

Disclosures: M. Shinya: None. **T. Akamatsu:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.19/I37

Topic: E.06. Posture and Gait

Support: NIH, Grant R01HD083314

Title: Neuromodulation through spatiotemporal transcutaneous spinal cord stimulation improves walking in humans with spinal cord injury

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Abstract: Objective: The goal of this study was to determine whether the application of spatial-temporal transcutaneous spinal cord electrical stimulation will facilitate walking in humans with SCI. Ten subjects with chronic (>1 year) SCI were recruited in this study. They were tested in 3 spinal stimulation conditions: swing phase, stance phase, and combined stance and swing phases as well as a control condition. For the swing phase condition, two self-adhesive round electrodes (5cm in diameter) were placed ~2 cm laterally from midline at the level of L1 and L4 spinous processes on the weaker leg side as active electrodes. The stimulation waveform used monophasic, rectangular, 100 us pulses at a frequency of 80 Hz filled with a carrier frequency of 9.5 kHz. Each participant was tested in 4 sessions, L1, L4 phasic, and L1, L4 continuous. Participants walked on a treadmill as the stimulation was applied to L1 or L4 segment, and each session lasted ~2 minutes. For the stance phase condition, a protocol that was comparable to the swing phase condition was used except the active electrodes were placed 2cm laterally from the midline at the level of L3 and S1 spinous process and the stimulation frequency was 30 Hz. Each participant was tested in 4 sessions, S1, L3 phasic, and S1, L3 continuous. For the phasic stimulation condition, the stimulation was delivered only during the swing or stance phase of the weaker leg for the swing and stance phase sessions, respectively. For the combined condition, a protocol that was comparable to the swing phase condition was used except that the stimulation

was applied to S1 during stance phase with frequency at 30 Hz, and to L4 during swing phase with frequency at 80 Hz. For the control condition, no stimulation was applied. Results: For the swing phase condition, the step height of the weaker leg was significantly greater with phasic stimulation at L4 ($p = 0.02$) and L1 ($p = 0.045$) than the control, and the step height of the weaker leg tended to be greater for the phasic stimulation at L4 ($p = 0.058$) and L1 ($p = 0.05$) than that of continuous stimulation. For the stance phase condition, step height of the weaker leg was significantly greater with phasic stimulation at S1 than the control ($p = 0.0498$), and than that of the continuous stimulation ($p = 0.038$). The application of stimulation at L3 had no significant impact on the step height of the weaker leg ($p = 0.16$). For the combined condition, the step height of the weaker leg was significantly greater for the combined condition that the control ($p = 0.04$). Conclusion: The application of spatial-temporal transcutaneous spinal cord stimulation may be more effective than continuous stimulation in facilitating walking in people with SCI.

Disclosures: M. Wu: None. **S. Yan:** None. **H. Lim:** None. **I. Hameeduddin:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.20/I38

Topic: E.06. Posture and Gait

Title: Surface electromyographic and biomechanical analysis in weightlifting executions by artificial vision

Authors: *G. D. LOPEZ-ARMAS[1], N. CASTILLO-GUTIERREZ[2], G. NUNEZ ROBLES[2], A. SALINAS ACEVES[2], C. LÓPEZ RAMÍREZ[2], O. ANDRADE[3], B. LLANES CERVANTES[4]; [1]Subdirección de Investigación/Ingeniería Mecatrónica-Biomédica, [2]Ingeniería Mecatrónica, Ctr. De Enseñanza Técnica Industrial, Guadalajara, Mexico; [3]Ingeniería Mecatrónica, Ctr. De Enseñanza Técnica Industrial, guadalajara, Mexico; [4]Nutrición, Univ. de Guadalajara Ctr. Universitario de Ciencias de la Salud, Guadalajara, Mexico

Abstract: Introduction: Weightlifting is a sport where a significant muscular effort is employed and precision in movement performance is required. However, the measurement of muscle yield is difficult to obtain and requires a lot of work. As time passes, it becomes difficult for the athlete to reach the target lift. This may be related to improper training, excessive force or intensity, or work overload, which can increase the risk of injury. The biomechanics of the executed movement are intimately linked to the muscle groups and force used to perform a valid execution. This work addresses surface electromyography (sEMG) measurement and the biomechanical analysis of the angular movements executed in deep squats by artificial vision.

Objective: To measure deep squat execution in weightlifting using sEMG and biomechanical analysis by computer vision. Methodology: We enrolled 20 participants between 18 and 30 years of age of both sexes. Two study groups were formed, with 10 participants each. The first group included high-performance weightlifting athletes, and the second group were amateur athletes. All patients gave written informed consent, and Helsinki guides were followed. To analyze deep squat execution, we considered the quadriceps femoris, erector spinae, and gastrocnemius muscles. All participants underwent muscle signal acquisition with BIOPAC MP36 equipment and training video acquisition using a webcam to develop artificial vision software with the Python programming language and OpenCV, using the open-source Mediapipe library. Four crucial moments were established: initial upright 160° to 170°, spine-hip flexor moment of 50°-90°,knee-ankle flexor moment 60°- 90° and final upright 160°-170° with ±3° of freedom for complete or incomplete execution. Results: The deep learning algorithm detected errors in the execution of the deep squat effectively based on the threshold deviation between body angles; this was reflected by the acquisition of sEMG, which showed more activity in lumbar muscle groups when a wrong execution was performed. In the future, the system could be used by coaches and athletes for monitoring, correct execution of the low squat as well as injury avoidance.

Disclosures: G.D. Lopez-Armas: None. **N. Castillo-Gutierrez:** None. **G. Nunez Robles:** None. **A. Salinas Aceves:** None. **C. López Ramírez:** None. **O. Andrade:** None. **B. Llanes Cervantes:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.21/I39

Topic: E.06. Posture and Gait

Support: Texas Health Catalyst Award Program

Title: Personalized Optimization of Omnidirectional Assistive Exosuits for Individuals with Neurodegenerative Disorders Through Neuromusculoskeletal Simulation

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Abstract: Soft exosuits hold promise as assistive technology for people with gait deficits owing to a variety of causes, such as neurodegenerative disorders like multiple sclerosis (MS) where the adjustability of such a device could cater to the functional changes these individuals experience. A key aspect of providing useful assistance is to keep the human user at the center of all considerations made in the design and configuration of assistive device. In this work we explore

a method for informing the configuration of a soft hip exosuit that can be configured to assist variably in flexion, extension, and abduction through neuromusculoskeletal simulation and 3D modeling of the user's body surface. This exosuit uses bilateral elastic bands that span the hip joint in a desired configuration to alter user biomechanics. By modeling the physical interface of the exosuit and the user, assistive forces provided by the device are calculated and added to simulations of the user's movement. These simulations then estimate the muscle excitations needed to accomplish the given movement under the specific device configuration. This system provides the ability to use neuromuscular signals to inform device configuration that would otherwise be invasive or impossible to obtain directly from the user. In our study, 4 participants with MS and 4 healthy participants were recorded at a comfortable walking pace while motion capture and forceplates were used to obtain kinematics and kinetics. Because the MS group had primarily unilateral gait deficits, we chose our optimization goal to be the minimization of muscle excitation asymmetry across pairs of muscles in the legs. The estimated metabolic cost of the movement was also calculated to compare the energy required to move with and without the optimized exosuit. We hypothesized that the system would reduce asymmetry in pairwise muscle excitations, and that optimal device configurations would vary amongst individuals. Bayesian optimization was used to search the design space for configurations that minimized asymmetry in few iterations. Results demonstrated device configurations that reduced excitation asymmetry in all participants, with greater reductions in those with multiple sclerosis. In support of our hypothesis, optimal configurations varied across participants. Metabolic cost of walking positively correlated with reduced asymmetry for 3 of the 4 MS group, and negatively for 3 of the 4 controls, suggesting that this optimization technique could be beneficial to those with unilateral gait deficits due to neurological conditions such as MS. Future physical experiments should be conducted to validate this system's potential.

Disclosures: R. Neuman: None. **N.P. Fey:** None.

Poster

PSTR408

Human Posture and Gait: Kinematics, Muscle Activity, Exercise and Fatigue, and Biomechanics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.22/I40

Topic: E.06. Posture and Gait

Support: Departmental ICR funds for Dr. Tanvi Bhatt

Title: Does unilateral quadriceps muscle fatigue worsen the reactive stability during walk slips?

Authors: *U. SAHU[1], S. WANG[2], T. VAN CRIEKINGE[3], T. S. BHATT[2];
[1]Rehabil. Sci., Univ. of Illinois Chicago, Chicago, IL; [2]Physical Therapy, Univ. of Illinois at Chicago, Chicago, IL; [3]Rehabil. Sci., KU LEUVEN, Brugge, Belgium

Abstract: Background: Muscle fatigue (MF) is an independent and modifiable risk factor for falls in older adults. Previous studies have shown that acutely induced bilateral quadriceps MF decreases reactive balance during motorized and over-ground slips in young individuals. However, it is known that for recovery from slips the slipping and trailing limbs play different roles, the former provides limb support whereas the latter is involved in recovery stepping and restores stability. Hence, there is an identified gap to understand specifically the effect of unilateral limb fatigue on reactive balance control and falls. **Aim**: To investigate the effect of unilateral quadriceps MF of the slipping limb (right) on reactive balance control in response to an unexpected overground gait-slip in healthy young adults. **Methods**: 30 healthy individuals, 16 *Fatigue* (24.18±3.61, 9 males) and 14 *No Fatigue* (24.0±2.53, 5 males) participated. To induce isolated MF, the right quadriceps muscle underwent isokinetic fatigue through repeated leg extension using a Biodex device in sitting. Followed by immediate exposure to a novel right-sided overground slip (distance=90cm) in gait. Reactive stability (shortest distance from this theoretical boundary for backward balance loss to the instantaneous center of mass state) and slip velocity was calculated at post-slip recovery liftoff (LO) and touchdown (TD), and step length (distance between heels) was calculated at TD. Independent samples t-test with effect sizes compared the measures between the two groups. **Results**: *Fatigue* group had a higher slip velocity at LO (p=0.003), lower reactive stability at LO (p=0.018), and increased step length (p=0.04) compared to *No Fatigue* group, resulting in a higher fall rate (31.25% vs 7.17%).**Conclusion**: Our results suggested that unilateral quadriceps MF could worsen the control of slip intensity, resulting in lower reactive stability at LO and increased fall risk in the *Fatigue* group. As slipping limb quadriceps play an important role in heel contact dynamics, the MF may increase the heel contact velocity and thereby increase the slip velocity. To compensate for this instability, the Fatigue group took a recovery step with a longer step length to improve the stability at TD. However, this reactive response could reduce the vertical support force against limb collapse, potentially increasing the risk of falls. Our study results align with the evidence that unilateral quadriceps MF significantly affects recovery responses and increases fall risk. Preliminary findings in young individuals suggest a potential escalation of fall risk in older adults, which needs further investigation.

Disclosures: U. Sahu: None. **S. Wang:** None. **T. Van Criekinge:** None. **T.S. Bhatt:** None.

Poster

PSTR409

Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR409.01/J1

Topic: E.09. Motor Neurons and Muscle

Support: NIH BRAIN Initiative R01NS109552-01
This research used resources of the Advanced Photon Source, Contract No. DE-AC02-06CH11357

This research used resources of the Argonne Leadership Computing Facility, Contract DE-AC02-06CH11357

Title: Optimizing deep learning for segmentation of feline spinal cord microtomography images

Authors: *M. GARCIA[1], D. DEMATTIES[2], R. VESCOVI[3], V. SAMPATHKUMAR[4], C. HECKMAN[5], M. K. CHARDON[6];
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Abstract: The ability to map the three-dimensional (3D) structure of the spinal cord's internal networks is crucial for understanding motor function and developing effective therapies for spinal cord injuries. Electrical stimulation has shown promise but lacks a detailed stimulation map able to predict the effect of spinal cord electrical stimulation on the underlying motor circuits. In order to construct this stimulation map, a model based on the 3D structure of the spinal cord internal network needs to be built. This study aims to improve the segmentation of motoneuron (MN) somas (neural cell bodies) from high-resolution feline spinal cord images using deep learning techniques to help build such a map. We are using high-energy X-Rays at the Advanced Photon Source, a U.S. Department of Energy Office of Science User Facility at Argonne National Laboratory, to image cross sections of the feline lumbar spinal cord. Our unique dataset comprises 1920 images (1920 x 3933 pixels) obtained at a 0.586 microns/pixel resolution from one sample of 1 mm of thickness containing intact circuitry. After preprocessing, we manually annotated a subset of images to create ground truth labels for supervised learning. Then, a 2D U-Net convolutional neural network was trained to segment MN somas. Our goal was to evaluate the impact of various hyperparameters, including optimizers (e.g., Adam, SGD), schedulers (e.g., LambdaLR, OneCycleLR,), batch sizes, and image augmentations (e.g., rotations, crops, flips) on soma detection accuracy. Our preliminary results show that AdamW optimizer with a constant learning rate and flips, rotations and transposes outperformed other configurations with 30% higher accuracy on the validation set than other optimizers. These findings underscore the importance of effective hyperparameter tuning for deep learning models applied to biomedical image segmentations. Accurate MN soma detection is a crucial first step toward constructing a comprehensive 3D spinal cord simulation that can elucidate motor unit firing patterns and motor function mechanisms. Our study contributes to a larger ongoing project aimed at developing advanced supercomputer-based reserve engineering techniques for this purpose. Future work will focus on extending the deep learning approach to segment other critical spinal cord components, as well as exploring transfer learning strategies to leverage insights across different imaging modalities. By integrating these efforts, we aim to establish a powerful computational framework for decoding the intricate neural circuitry underlying motor control and pave the way for innovative therapeutic interventions in spinal cord injuries.

Disclosures: M. Garcia: None. **D. Dematties:** None. **R. Vescovi:** None. **V. Sampathkumar:** None. **C. Heckman:** None. **M.K. Chardon:** None.

Poster

PSTR409

Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR409.02/J2

Topic: E.09. Motor Neurons and Muscle

Title: End plate morphology and glial cell line-derived neurotrophic factor content during postnatal development in skeletal muscle in male and female rats

Authors: *A. B. GALENTINE, J. M. VANGYSEGHEM, J. M. SPITSBERGEN;
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Abstract: Neurotrophic factors are important molecules that support the survival and maintenance of neuronal cells by regulating cell growth, promoting neuronal survival, and aiding in synaptic plasticity. Glial cell line-derived neurotrophic factor (GDNF) is known to be the most potent trophic factor for somatic motor neurons, making it crucial in maintaining neuromuscular health. Previous studies from our lab have shown decreases in GDNF expression and alterations in NMJ morphology in males and females with age, however, this has not yet been examined in developing animals. The aim of this study is to elucidate the relationship between GDNF content and end plate morphology in male and female rats during postnatal development. Hindlimb muscles of male and female Long-Evans rats were harvested at 3 and 8 weeks of age. Soleus muscles were sectioned longitudinally at 50 μm and bound with α-bungarotoxin to visualize acetylcholine receptors at motor end plates. Sections were then viewed using a confocal microscope and analyzed for end plate area using ImageJ software. To measure GDNF content, soleus and plantaris were processed and protein levels measured by ELISA. In plantaris muscle, GDNF content declines with age (2.094±0.265 pg/mg vs 0.639±0.075 pg/mg) between 3 and 8 weeks in males but not in females. Soleus muscle, however, shows declines in GDNF content (4.869±0.280 pg/mg vs 3.624±0.197 pg/mg) between 3 and 8 weeks in females with no change observed in males. A comparison between sexes showed that GDNF in plantaris was significantly higher in 3-week-old males compared to females (0.804±0.070 pg/mg). There were no significant sex differences in soleus for either age groups. End plate area in soleus muscle increased significantly in both males (114.162±27.437 μm^2 vs 283.994±62.582 μm^2) and females (83.567±19.905 μm^2 vs 229.520±62.828 μm^2) from 3 to 8 weeks of age. When comparing sexes, end plate area in the 3-week groups was higher in males compared to females. At 8 weeks, there were no significant sex differences in end plate area. In conclusion, our data showed sex differences for both end plate morphology and GDNF protein content. GDNF levels decreased in female soleus muscles with age, whereas this occurred in plantaris in males. This may be due to differences in muscle fiber type composition where soleus is a primarily slow twitch muscle and plantaris is a primarily fast twitch muscle. Previous studies have shown a correlation between higher end plate area and increased GDNF levels. Here, we observe an opposite trend, suggesting differences in the effects of GDNF on the NMJ in developing animals.

Disclosures: A.B. Galentine: None. **J.M. Vangyseghem:** None. **J.M. Spitsbergen:** None.

Poster

PSTR409

Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR409.03/J3

Topic: E.09. Motor Neurons and Muscle

Title: Modulation of spinal motor neuron excitability by transcranial stimulation

Authors: P. YADAV[1], J. A. BEAUCHAMP[2], L. BORDA[3], M. FORSSELL[4], J. KIM[2], V. JAIN[4], P. GROVER[5], G. F. WITTENBERG[6], *D. WEBER[7];
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Abstract: Modulation of corticospinal excitability can be achieved through transcranial magnetic stimulation (TMS) and has been used to promote motor rehabilitation. Furthermore, repeated bouts of cortical stimulation have been shown to additionally modulate monoaminergic reticular nuclei and descending monoaminergic projections. Indeed, the use of TMS to treat depression often hinges on changes in monoamines (serotonin, norepinephrine). Interestingly, monoamines have profound effects on spinal motoneurons (MNs), controlling their intrinsic excitability via dendritic persistent inward currents (PICs). Consequently, this raises the question, could the rehabilitative motor effects observed from TMS be facilitated by monoamine induced changes to intrinsic MN properties?TMS studies typically measure motor evoked potentials (MEPs). While MEPs represent clear and direct effects of TMS on corticospinal activity, they do not provide sufficient information about modulation of intrinsic MN excitability. However, estimates of PICs from MN firing rates can provide insight into the modulation of intrinsic MN excitability and, subsequently, the modulation of monoaminergic brainstem nuclei by TMS.We used high-density surface electromyography (HDsEMG) and motor unit decomposition to estimate the discharge of motor units from individuals' forearm flexor muscles during isometric wrist flexion tasks. Participants performed two successive linear isometric ramp contractions, with a 30 second separation between the two contractions. In this 30 second resting interval, the participants were stimulated with TMS over the contralateral motor cortex to evoke MEPs or at a sham stimulation site which did not evoke MEPs. From this HDsEMG data, we decomposed the estimated firings of individual motor units (MU) using a Convolution Kernel Compensation (CKC) algorithm and estimated MN excitability with common metrics of PICs (i.e., delta-F, brace height). Early findings indicate that PICs may increase in response to TMS over the motor cortex, but not in the control (no stimulation) or sham stimulation conditions. This early finding suggests that TMS may modulate intrinsic spinal MN properties.

Disclosures: P. Yadav: None. **J.A. Beauchamp:** None. **L. Borda:** None. **M. Forssell:** None. **J. Kim:** None. **V. Jain:** None. **P. Grover:** None. **G.F. Wittenberg:** None. **D. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroOne, NeuronOff, Reach Neuro, Iota Biosciences, Bionic Power. Other; cofounder of Reach Neuro.

Poster

PSTR409

Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR409.04/J4

Topic: E.09. Motor Neurons and Muscle

Support: U19 grant U19MH114830
Paul G Allen

Title: Enhancer AAV toolkit targeting descending motor pathways in rodent and macaque

Authors: *E. A. KUSSICK[1], T. L. DAIGLE[6], N. JOHANSEN[11], B. WYNALDA[12], Z. YAO[13], B. TASIC[7], B. P. LEVI[8], R. D. HODGE[9], T. BAKKEN[10], H. ZENG[13], E. LEIN[8], J. T. TING[8], M. G. FORTUNA[17], N. TASKIN[1], K. GUDSNUK[1], Y. GAO[2], K. SMITH[2], N. DEE[13], T. CASPER[14], M. CLARK[3], W. HO[2], K. RONELLENFITCH[2], C. M. SOBIESKI[4], R. FERRER[2], S. YAO[15], M. BERG[18], M. REDING[2], E. LIANG[2], C. HUANG[2], B. THYAGARAJAN[2], A. CHAKKA[2], J. GOLDY[10], A. TORKELSON[2], J. GUZMAN[5], R. CHAKRABARTY[2], N. GUILFORD[16], T. PHAM[2], C. VAN VELTHOVEN[5], N. GUILFORD[16], D. NEWMAN[5]; [1]Gene Therapy, [2]Allen Inst., Seattle, WA; [3]Allen Inst., seattle, WA; [4]Allen Inst., Charleston, WV, ; [5]Allen Inst., Seattle, WA, ; [6]Gene Therapy, [7]Cell and Circuit Genet., [8]Human Cell Types, [9]Cell Types Program, [10]Allen Inst. For Brain Sci., Seattle, WA; [11]Human Cell Types, [12]Mol. Genet., [13]Allen Inst. for Brain Sci., Seattle, WA; [14]Allen Inst. for Brain Sci., seattle, WA; [15]Allen Inst. for Brain Sci., Seattle, WA, ; [16]Allen Inst. for Brain Sci., Portland, OR, ; [17]Cognitive Neurosciences Lab., German Primate Ctr., Goettingen, Germany; [18]Washington Natl. Primate Res. Ctr., Seattle, WA

Abstract: The spinal cord facilitates the exchange of information between the brain and the periphery, implicating it in musculature control. This circuit is called the descending motor neuron pathway and is comprised of upper motor neurons (UMNs) in the brain that transmit signals to spinal motor neurons (SMNs) through a direct synapse in humans and non-human primates (NHP), or through an interneuron intermediate synapse in mouse and rat. Spinal motor neurons can be subdivided into alpha, beta and gamma types based on their connectivity, physiology and morphology and have been shown to selectively degenerate in devastating CNS disorders, such as amyotrophic lateral sclerosis (ALS) or spinal muscular atrophy (SMA). Despite their importance, few genetic tools exist to selectively target spinal motor neurons in

mouse and beyond. Therefore, to generate cell type-specific genetic tools, we generated 10X multiome datasets from mouse and NHP spinal cords and discovered putative enhancers for spinal motor neuron types using a biased, marker gene-based approach. We cloned the putative enhancers using standard molecular cloning techniques into an rAAV backbone, upstream of a beta-globin minimal promoter and driving a reporter fluorophore, packaged them into PHP.eb capsids, and delivered them via retro-orbital route of administration. Enhancer AAVs were validated using various molecular techniques (IHC, RNAscope), and transgenic tools to determine the specificity and completeness of labeling. We also evaluated viral infectivity in select peripheral organs to support future work. Promising viruses were further characterized in rat and NHP and showed conservation of labeling as well as areas of species divergence. We also evaluated multiple clinically relevant routes of administration in NHP and found highly specific labeling throughout all levels of cord. Lastly, to enable therapeutic applications in diseases such as ALS, we created a single viral vector that can simultaneously target both UMNs and SMNs by stitching enhancers together. This motor neuron AAV toolkit will enable future explorations into the specific properties of disease-relevant motor neuron cell types in the brain and spinal cord across species and may enable future therapeutic interventions for human neurodegenerative diseases.

Disclosures: E.A. Kussick: None. **T.L. Daigle:** None. **N. Johansen:** None. **B. Wynalda:** None. **Z. Yao:** None. **B. Tasic:** None. **B.P. Levi:** None. **R.D. Hodge:** None. **T. Bakken:** None. **H. Zeng:** None. **E. Lein:** None. **J.T. Ting:** None. **M.G. Fortuna:** None. **N. Taskin:** None. **K. Gudsnuk:** None. **Y. Gao:** None. **K. Smith:** None. **N. Dee:** None. **T. Casper:** None. **M. Clark:** None. **W. Ho:** None. **K. Ronellenfitch:** None. **C.M. Sobieski:** None. **R. Ferrer:** None. **S. Yao:** None. **M. Berg:** None. **M. Reding:** None. **E. Liang:** None. **C. Huang:** None. **B. Thyagarajan:** None. **A. Chakka:** None. **J. Goldy:** None. **A. Torkelson:** None. **J. Guzman:** None. **R. Chakrabarty:** None. **N. Guilford:** None. **T. Pham:** None. **C. van Velthoven:** None. **N. Guilford:** None. **D. Newman:** None.

Poster

PSTR409

Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR409.05/J5

Topic: E.09. Motor Neurons and Muscle

Support: NSERC Discovery Grant RGPIN-2015-06403
NSERC PGS-D 569969-2022

Title: Developmental changes in the expression and modulation of a persistent subthreshold potassium current in primary motoneurons of larval zebrafish

Authors: *S. F. GAUDREAU, T. V. BUI;
Biol., Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Locomotion is invaluable to vertebrates. Crucially, animals undergo refinement of spinal circuits early in life to locomote effectively. Stereotyped transitions to progressively more refined locomotor behaviours are observed in larval zebrafish in the first few days of development. During this time, new neurons are being incorporated into existing circuits, new connections are being formed, and intrinsic properties of individual neurons are maturing. How changes in intrinsic properties of individual neurons during development of spinal networks may influence circuit-wide locomotor output is currently unknown in zebrafish. We have identified, for the first time, the persistent subthreshold potassium current I_M – a current with known involvement in dampening neuronal excitability – in larval zebrafish spinal circuits for locomotion. Specifically, we demonstrate via patch-clamp electrophysiology that primary motoneurons involved in fast and large amplitude movements express I_M. Interestingly, our data demonstrate that I_M is expressed differentially at 3 days post-fertilization compared to 4 days post-fertilization and older. We find that the amplitude of I_M in primary motoneurons is reduced by just over 50% by 4 days and nearly 70% by 5 days post-fertilization. Furthermore, ongoing data collection seems to suggest that serotonin (20 μM) may hyperpolarize the activation voltage of I_M to a lesser degree at 3 days compared to 4-5 days post-fertilization. Lastly, our behavioural data of tactile-evoked escape responses, a fast large amplitude movement, suggest that the expression of I_M may influence primary motoneuron recruitment for execution of the movement. We find that pharmacological enhancement of I_M reduces the number of swimming bouts produced during touch evoked escapes. Altogether, we propose a role for I_M in primary motoneuron recruitment during the escape response that may be influenced by neuromodulators differentially throughout early development. Understanding how intrinsic properties of individual neurons change with the progression of movement execution throughout development is important to piece together fundamental mechanisms of operation of spinal circuits. How these circuits are modulated sheds light onto the mechanisms by which circuits appropriately adjust their locomotor output given the context – an essential feature of locomotor circuits across species.

Disclosures: S.F. Gaudreau: None. **T.V. Bui:** None.

Poster

PSTR409

Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR409.06/J6

Topic: E.09. Motor Neurons and Muscle

Support: NSERC Discovery 2020-04157

Title: Modulation of persistent inward currents with changes in posture and cutaneous sensory input

Authors: *R. W. WELLER[1], J. M. KALMAR[2];
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Abstract: Persistent inward currents (PIC) increase and sustain motor neuron (MN) output. PIC is amplified by descending monoamines and reduced by afferent inhibition. Given that monoaminergic drive is altered by posture, and there is coupling between plantar cutaneous sensory input and lower limb MNs, our purpose was to assess posture-dependent plantar cutaneous sensory modulation of tibialis anterior (TA) MNs. We hypothesized that plantar cutaneous sensory input in the heel region, via either heel pressure (HP) or electrical stimulation (ES) would decrease PIC, and that PIC would be lower in a supine position. Twenty participants (10 female) performed isometric dorsiflexion contractions with (HP) and without (noHP) heel pressure in seated upright (SE) and supine (SU) positions with the hip, knee, and ankle at 90°. Cutaneous ES (5x1ms pulses, 300Hz, 2x perceptual threshold) was applied to the heel during half of these contractions. Intramuscular EMG was recorded from the TA, and surface EMG (sEMG) from the TA and soleus (SOL). The paired motor unit technique was used to estimate PIC as the difference between control MN firing rate (Hz) at test MN recruitment and decruitment. Middle latency reflex (MLR) inhibition elicited via cutaneous ES was quantified as %decline in TA sEMG 70-140ms after each stimulus (80 stimuli). Repeated-measures ANOVA was used to assess PIC, muscle activation, and MLR. We found that PIC was lower when the heel was in contact with the dynamometer to generate plantar cutaneous pressure (noHP: 4.57 ± 1.91Hz, HP: 3.76 ± 1.67Hz, p=0.02). However, ES of the plantar aspect of the heel did not alter PIC (ES: 4.20 ± 1.82Hz, no ES: 4.36 ± 1.86Hz) and PIC did not differ between postures (SE: 4.24 ± 1.94Hz, SU: 4.33 ± 1.74Hz). TA and SOL sEMG was assessed to determine whether lower PIC in the HP condition was due to changes in muscle activation. There were no differences in TA (noHP: 15.25 ± 6.32%maxEMG, HP: 15.28 ± 7.23%maxEMG) or SOL activation (noHP: 6.89 ± 5.60%maxEMG, HP: 5.91 ± 4.19%maxEMG). Although ES did not reduce PIC amplitude, it was sufficient to evoke an inhibitory MLR. There were no differences in the magnitude of TA MLR elicited by ES between postures (SE: 40.96 ± 18.84%decline, SU: 42.21% ± 14.94%decline) or heel pressure conditions (noHP: 41.81 ± 16.30%decline, HP: 41.81 ± 16.63%decline). Our findings suggest that plantar cutaneous pressure shapes MN excitability via modulation of PIC, but this modulation is not posture-dependent and was not reproduced using a conventional intermittent ES paradigm. This study adds to our understanding of reflex control of lower limb PIC in tasks associated plantar cutaneous pressures, such as locomotion or postural stability.

Disclosures: R.W. Weller: None. **J.M. Kalmar:** None.

Poster

PSTR409

Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR409.07/J7

Topic: E.09. Motor Neurons and Muscle

Support: Tohoku Initiative for Fostering Global Researchers for Interdisciplinary Sciences (TI-FRIS)
Japan Society for the Promotion of Science Grant-in-Aid for Early-Career Scientists (No. 22K15626)
Young Leaders Overseas Program at Tohoku University

Title: Natural finger-tapping tempo reflects intrinsic neuropsychological characteristics

Authors: *S. SUN[1], D.-A. WU[2], M. H. SHEHATA[2], S. SHIOIRI[1], S. SHIMOJO[2];
[1]Tohoku Univ., Sendai, Japan; [2]Caltech, Pasadena, CA

Abstract: Individuals have a natural pace or rhythm in their daily activities, known as their personal tempo. This intrinsic tempo is evident when individuals perform repetitive rhythmic tasks comfortably and spontaneously. Previous studies have primarily focused on the average tempo; for instance, walking typically exhibits a rhythm of approximately 2 Hz in young adults, while finger tapping, another spontaneous rhythmic motor action, tends to have a preferred tempo of 1.7 Hz. Speech generally peaks at a frequency of 5Hz, while music is typically at 2Hz. However, the significance of personal tempo and the variations among individuals are not well understood. Additionally, how the brain generates and represents this natural tempo to modulate sensory-motor functions remains unclear.We conducted a series of finger-tapping tasks, both spontaneous and constrained, to capture an individual's natural motor tempo and brain activity using electroencephalography (EEG). Our hypothesis was that each person's tapping tempo would be consistent across sessions, stable within themselves, and unique compared to others. With EEG, we aimed to identify specific neural patterns associated with natural states and link these patterns to individual differences in tempo preference. Our focus was on alpha oscillations during resting and tapping (such as individual alpha peak frequency (IAPF) and amplitude) and activities in the default mode network (DMN), which are believed to be intrinsic to individuals and reflect spontaneous brain function. We found that each participant had a uniquely preferred motor tempo that remained consistent across multiple sessions and within individuals. Tapping at a natural tempo led to enhanced alpha power and activity in the default mode network, suggesting less cognitive control. Additionally, the individual's uniquely preferred tempo was negatively correlated with resting state individual alpha peak frequency, tapping-evoked frontal MEP signals and default mode network activity. These findings provide compelling evidence that natural tapping tempo could function as an intrinsic neuropsychological "fingerprint" for individuals.

Disclosures: S. Sun: None. **D. Wu:** None. **M.H. Shehata:** None. **S. Shioiri:** None. **S. Shimojo:** None.

Poster

PSTR409

Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR409.08/J8

Topic: E.09. Motor Neurons and Muscle

Support: Royal Society Newton International Fellowship (NIF\R1\180091)
Canadian Institute for Health Research PDF (202012MFE-459188-297534)

Title: Spinal neurons with a biophysical signature consistent with a gamma motoneuron identity emerge during the third week of postnatal development in mice

Authors: *S. A. SHARPLES[1], G. B. MILES[2];
[1]Neurosurg., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [2]Sch. of Psychology & Neurosci., Univ. of St Andrews, St Andrews, United Kingdom

Abstract: The establishment of functionally defined motoneuron subtypes is a key process that contributes to the emergence of complex motor behavior during postnatal development. Gamma motoneurons are a subtype of spinal neuron that innervate muscle spindles and are critical for maintaining the spindle sensitivity during muscle contractions. Recent work has identified that ERR2/3 are key transcription factors for defining the biophysical properties of gamma motoneurons and are important for the generation of complex movement. However, gamma motoneurons are not functionally diversified at birth and it is not known when this occurs during postnatal development as complex motor behaviors emerge. To address this gap, we analyzed open access data obtained using whole cell patch clamp electrophysiology from lumbar motoneurons studied across the first three weeks of postnatal development in mice (1-3). From this analysis, we identified a cluster of low-threshold, high firing gain motoneurons with intrinsic properties that are consistent with gamma motoneurons that emerges during the third week of postnatal development. Importantly, this cluster could not be identified in a sample of motoneurons studied in Hb9::GFP mice (3), which would be expected to be devoid of gamma motoneurons. Compared to putative slow alpha motoneurons, this cluster of putative gamma motoneurons presented with higher firing rates across the frequency-current range and was supported by shorter action potentials and smaller afterhyperpolarizations but not differences in persistent inward current amplitude. In addition, 92% of putative gamma motoneurons present with a sodium pump-mediated ultra-slow afterhyperpolarization - a previously established marker of gamma motoneurons. These data suggest that the establishment of a functional gamma motoneuron identity may be a key developmental process that leads to the emergence of complex motor behavior during the third week of postnatal development in mice.
References: 1.) Sharples and Miles, 2021. Elife; 2.) Sharples et al., 2023, JPhysiol.; 3.) Smith and Brownstone, 2020, JPhysiol.

Disclosures: S.A. Sharples: None. **G.B. Miles:** None.

Poster

PSTR409

Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR409.09/J9

Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant 4R00NS114179

Title: Building a Genetic Library for Motor Circuits Controlling an Active Sensor in Drosophila

Authors: *E. KOPHS, M. P. SUVER;
Dept. of Biol. Sci., Vanderbilt Univ., Nashville, TN

Abstract: Animals actively sense their surroundings to respond appropriately to environmental cues and stimuli. This dynamic acquisition of sensory information is enabled by active positioning of sensors and facilitates motor control essential for behavioral responses to evolving and sometimes unpredictable environments. Yet how these active movements are controlled during behavior and coordinated with ongoing sensory acquisition is not fully understood. In the fruit fly *Drosophila melanogaster,* the antennae are crucial sensors capable of extracting important information from the environment including mechanosensory, olfactory, and auditory signals. Four distinct muscles command movement of the antennae, yet we know very little about the motor circuits controlling these muscles. We are characterizing the complete set of antennal motor neurons used by *Drosophila* for the active positioning of their antennae. Using genetic tools to stochastically label a subset of neurons in individual flies in combination with optogenetic activation experiments, we are elucidating the role of single, genetically-identifiable motor neurons for coordinating active antennal movements. This study generates a library of genetic driver lines labeling individual and specific subsets of antennal motor neurons, enabling precise optogenetic manipulation of an active sensor for future studies identifying the function of active antennal movements. Together, this work contributes to a more wholistic understanding of neural circuits underlying active sensing in a multimodal sense organ.

Disclosures: E. Kophs: None. **M.P. Suver:** None.

Poster

PSTR409

Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR409.10/J10

Topic: E.09. Motor Neurons and Muscle

Support: JP23H05488
JP19H04997

Title: Mapping the Gamma Motor Neurons in the Lower Cervical Spinal Cord of Macaque Monkeys Using ERR3

Authors: S. NAKAMURA[1], M. KUDO[2], S. KIKUTA[2], ***K. SEKI**[2];
[1]Univ. of Texas, Austin, TX; [2]Natl.inst.Neurosci., Tokyo, Japan

Abstract: Alpha motoneurons innervate extrafusal muscle fibers and are responsible for muscle contraction that generates joint movement. In contrast, gamma motoneurons regulate the gain of the stretch reflex by adjusting the tension in intrafusal muscle fibers within muscle spindles. Gamma motoneurons comprise about one-third of the motoneuron pool, and their functional disruption significantly impairs motor control. Historically, the anatomical distinction between motoneurons has relied on cell size differences, with larger alpha and smaller gamma motoneurons. Recent research, however, identifies molecular markers that differentiate these types: gamma motoneurons express high levels of the orphan nuclear hormone receptor Err3 and lack the neuronal DNA binding protein NeuN, whereas alpha motoneurons exhibit low or negligible Err3 levels and high NeuN expression. Fiense et al. (Friese et al., PNAS 2009) successfully distinguished gamma and alpha motoneurons in rodent spinal cords based on Err3 expression levels.This study aims to explore whether this method can be applied to non-human primates and to examine the distribution of presumptive gamma motoneurons in the cervical spinal cord. A macaque monkey was deeply anesthetized and perfused with formalin in phosphate buffer. The spinal cord was then removed, sectioned coronally at 50-μm thickness using a freezing microtome, and stained with Err3 and choline acetyltransferase (ChAT) antibodies. We measured the size and fluorescence intensity of 4,934 labeled cells, and performed Gaussian fitting to analyze the distribution of cell size and intensity. Our findings revealed a bimodal distribution in both parameters, suggesting that Err3 can differentiate gamma from alpha motoneurons, although the peak separation was less distinct than previously reported in rodent lumbar spinal cords. Preliminary analyses of the ratio of alpha to gamma motoneurons across different intraspinal locations, corresponding to the motoneuron pools that innervate various body parts, indicate that gamma motoneurons are less prevalent in distal muscles and more common in proximal muscles such as the trunk.

Disclosures: S. Nakamura: None. **M. Kudo:** None. **S. Kikuta:** None. **K. Seki:** None.

Poster

PSTR409

Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR409.11/J11

Topic: E.09. Motor Neurons and Muscle

Support: Alfred Deakin Postdoctoral Research Fellowship for Dr Lucas Orssatto

Title: Is intrinsic motoneuron excitability different between older females and males?

Authors: *L. B. R. ORSSATTO[1], D. SCOTT[1], B. CLARK[2], R. DALY[1];
[1]Inst. for Physical Activity and Nutr. (IPAN), Deakin Univ., Melbourne, Australia; [2]Deparment of Biomed. Sci., Ohio Univ., Athens, OH

Abstract: Persistent inward currents (PICs) are depolarizing currents generated by motoneuron voltage-gated dendritic sodium and calcium channels. PICs increase cell excitability, accelerating, amplifying, and prolonging the firing output activity of motoneurons according to the physical task demand. PICs are facilitated by monoaminergic neuromodulation and hampered by inhibitory inputs onto the motoneurons. Estimates of PICs are known to be larger in young adult females than young adult males. However, ageing is known to induce changes within the neuromodulatory, inhibitory, and hormonal systems, which can impair the generation of PICs, contributing to motoneuron firing frequency reductions and motor impairments. However, it remains unclear if the sex-related differences in PICs observed in young adults persists at older age. This study investigated sex-related differences in intrinsic motoneuron excitability (which is in part reflective of PICs), and in the neuromodulatory and inhibitory drives onto the motoneurons in older adults. 14 females, 74±7y; 12 males, 73±6y, had high-density surface electromyography signals (128-channels) recorded from their tibialis anterior during ramp-shaped contractions to 20%, 40%, and 60% of maximal dorsiflexion isometric force. A blind-source-separation algorithm was used to decompose the recorded signals into individual motoneuron spike trains, allowing the: i) estimation of PICs, ii) quantification of the neuromodulatory drive onto the motoneuron with brace height calculation, and iii) estimation of inhibitory input onto motoneurons with attenuation slope calculation. Estimates of PICs were higher in females [4.08 (95%CI:3.70, 4.47) pps] than males [3.35 (95%CI:2.94, 3.76) pps], with an effect size d = 0.8 (95%CI: 0.2, 1.4), irrespective of contraction levels. Brace height was higher in females [36.1 (95%CI:34.4, 37.7) %] than males [32.0 (95%CI:30.3, 33.8) %], d = 0.4 (95%CI: 0.2, 0.6), irrespective of contraction levels. Attenuation slope did not increase with contraction levels in females but increased for males from 20% to 40% [d = 0.3 (0.02, 0.5)] and 40% to 60% [d = 0.6 (0.3, 0.9)]. Also, it was lower in females than males at 40% [d = -0.3 (-0.6, -0.1)] and 60% [d = -0.8 (-1.1, -0.5)] contraction levels, but not at 20% [d = -0.2 (-0.5, 0.1)]. In conclusion, we observed higher intrinsic motoneuron excitability in older females compared to males. Female motoneurons seem to receive greater neuromodulatory drive, irrespective of contraction level, and lower inhibitory input at higher contraction levels than males, which may play a critical role in the sex-related differences in motoneuron excitability modulation.

Disclosures: L.B.R. Orssatto: None. **D. Scott:** None. **B. Clark:** None. **R. Daly:** None.

Poster

PSTR410

Song Learning and Vocal Production

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR410.01/J12

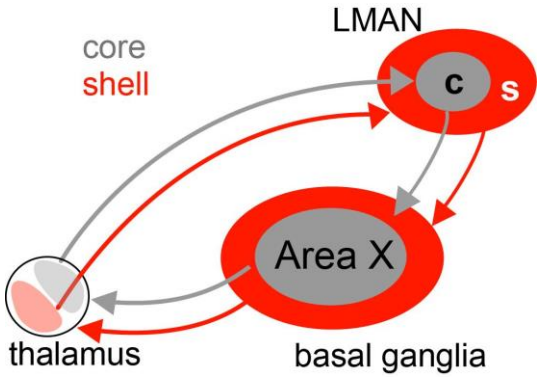
Topic: E.03. Basal Ganglia

Support: NSF 1940957

Title: Refining the reciprocal projections between basal ganglia and thalamus in songbirds

Authors: H. GAO, *S. W. BOTTJER;
USC, Los Angeles, CA

Abstract: Vocal learning in songbirds provides a powerful model for studying mechanisms essential for goal-directed acquisition of a stereotyped behavior. Parallel CORE and SHELL cortico-basal ganglia-thalamo circuits mediate vocal learning in juveniles during sensorimotor learning, but a strong barrier to progress is lack of complete knowledge of the circuits that contribute to learning. Area X is an anatomically localized and song-specialized basal ganglia region that contains both striatal and pallidal neurons. The complete efferent targets of pallidal cells in CORE versus SHELL regions of Area X, as well as their further downstream targets, are largely unknown. We made small iontophoretic injections of fluorescent dextrans into CORE versus SHELL regions of Area X in juvenile zebra finches (*Taeniopygia guttata*) to study these efferent connections. Preliminary results show that CORE and SHELL neurons in Area X form calyceal terminals onto single thalamic neurons, and extend large bouton endings and thick local branches to adjacent neurons. This novel discovery indicates that pallidal projection neurons in Area X not only innervate single post-synaptic DLM neurons via calyceal endings but also provide strong inhibitory inputs onto neighboring neurons. Injections into Area X also produced retrogradely labeled neurons in the thalamus; these reciprocal-projecting neurons never received afferent inputs from Area X. This pattern suggests two different classes of thalamic neurons, one that receives strong inhibitory inputs from pallidal neurons in Area X and another that conveys feedback to Area X. As shown previously, collaterals of CORE and SHELL pallidal neurons in Area X form fine terminal branches in adjacent regions of ventral pallidum (VP). We also observed terminal labeling in substantia nigra pars compacta (SNc) following Area X injections. Mapping the connections of CORE and SHELL pathways in Area X serves as an essential prerequisite to study the functional roles of these two parallel pathways.



Disclosures: H. Gao: None. **S.W. Bottjer:** None.

Poster

PSTR410

Song Learning and Vocal Production

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR410.02/J13

Topic: F.01. Neuroethology

Support: NIH Grant R01 NS102488
NIH Grant R01 DC020333

Title: Functional Dissociation of Skilled Vocal-Orofacial Behaviors in Zebra Finch Corticobulbar and Midbrain Circuits

Authors: *Z. ZHAO[1,2], M. TRUSEL[3], S. KILIC[4], A. GRASSLER[4], J. T. O'BRIEN[3], S. J. SOBER[4], B. G. COOPER[5], T. F. ROBERTS[3];
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Abstract: Coordination of respiratory, throat, and orofacial motor systems is vital for eating, drinking, and vocalizing. Brainstem circuits directing these various motor systems are largely conserved among tetrapods. Yet, it is poorly understood how top-down cortical coordination of these circuits is synaptically and functionally achieved. Finches are renowned for both their learned song(s) and their highly adapted beaks used for skillfully manipulating, cracking, husking, and eating seeds and nuts. Zebra finches have a well-characterized descending pathway from a specialized region of the avian motor 'cortex' (robust nucleus of the arcopallium (RA)) controlling production of their learned song. This pathway innervates the ventral respiratory column (VRC), the tracheosyringeal part of the hypoglossal motor nucleus (nXIIts), and the dorsomedial periaqueductal gray (PAGDM), which is thought to help control innate vocalizations. Another set of neurons in the lateral arcopallium (Ail) are speculated to help control beak movements via projections onto brainstem facial motor regions. Using viral and chemical tracing, we show that Ail also strongly innervates the VRC, nXIIts, the lingual part of XII and PAGDM, suggesting that Ail may be capable of controlling respiratory, throat, and beak movements. Consistent with this anatomy, electrical stimulation of Ail during anesthesia induced tongue movements. Pharmacological lesions and genetic decoupling of Ail from brainstem circuits, using viral expression of tetanus toxin (TeNT), results in profound disruptions in coordinated movements needed for grasping, manipulating, cracking and husking seeds. Since RA and Ail each project to PAGDM we next examined the role of this midbrain region in the control of song and eating. We identified a molecular handle to selectively lesion PAGDM neurons. This revealed that PAGDM is necessary for production of learned and innate vocalizations but not for eating. Moreover, selective expression of TeNT in VRC-projecting PAGDM neurons also muted birds of both their innate calls and their learned song, suggesting

that the descending pathway from RA relies on midbrain circuitry to facilitate song production. Together, our findings reveal how distinct cortical regions innervating largely overlapping brainstem circuits allow for the specialized control of complex behaviors essential for communication and survival.

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Poster

PSTR410

Song Learning and Vocal Production

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Topic: F.01. Neuroethology

Support: NIH Grant R01 NS102488
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Title: Thalamo-cortical control of vocal variability in the Zebra Finch

Authors: *R. MISHRA[1], J. T. O'BRIEN[1], M. TRUSEL[2], T. F. ROBERTS[2];
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Abstract: Computations within the basal ganglia and their thalamo-cortical output are hypothesized to play important roles in learning and production of skilled behaviors. However, the functional role of these circuits in the acute control of behavior remains poorly explored. Here we report on the influence of thalamo-cortical circuitry in the control of zebra finch song. The premotor song nucleus lMAN, through its projections onto the primary song motor nucleus RA, is an important source of vocal variability, allowing for vocal exploration and song learning. lMAN is the target of the vocal basal ganglia's thalamo-cortical pathway via the thalamic nucleus DLM. To understand the role of the DLM-to-lMAN pathway in vocal performance we made use of somatic and axonal expression of the opsin eGtACR1. GtACRs are light-driven Cl^- channels that, while strongly hyperpolarizing at the soma and dendrites, are depolarizing at axon terminals due to the shifted internal Cl^- concentration in axonal compartments. Here we use viral expression of eGtACR1 to achieve bidirectional control of the DLM-lMAN pathway. We expressed eGtACR1 in DLM neurons and implanted fiber optics bilaterally over DLM and lMAN, permitting optogenetic silencing of DLM neurons and excitation of DLM axon terminals in lMAN in the same birds. Song-contingent optogenetic manipulations revealed that this pathway controls vocal variability through temporally precise control of the spectral characteristics of targeted syllables. Individual syllables could be made less noisy or noisier on individual trials by either silencing DLM_{lMAN} neurons or exciting their axon terminals in lMAN, respectively. This indicates that levels of vocal variability are actively controlled by this basal

ganglia thalamo-cortical pathway. In other experiments, using expression of the excitatory opsin ChRmine, we find that excitation of either DLM_{lMAN} neurons or $lMAN_{RA}$ neurons mirrors the effects of stimulating DLM axon terminals in LMAN. Our findings offer novel insights into the neural circuitry governing skilled motor behaviors and provide a concrete example of how bidirectional control of neural pathways can be achieved in the songbird.

Disclosures: R. Mishra: None. **J.T. O'Brien:** None. **M. Trusel:** None. **T.F. Roberts:** None.

Poster

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Topic: F.01. Neuroethology

Support: NIH Grant R01 NS102488
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Title: Connectivity and electrophysiological characteristics of mman neurons in the zebra finch

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Abstract: The medial magnocellular nucleus of the anterior neostriatum (mMAN), is a poorly understood region in the zebra finch brain. In Bengalese finches, mMAN has recently been shown to contribute to song sequence variability. In zebra finches, who produce a less variable song, mMAN is not required for adult song production but may be important for song learning. We have recently found that mMAN not only projects to HVC, a premotor cortical analog crucial to song learning and production, but also to Avalanche, an area involved in auditory processing that is reciprocally connected with HVC. We wanted to further investigate the potential role mMAN plays in song by characterizing its circuitry and neuronal properties. To quantify and characterize the HVC and Avalanche projections from mMAN, we used retrograde fluorescent tracing combined with in-situ hybridization labeling against SLC17A6 and GAD1, markers of glutamatergic and GABAergic neurons, respectively. We found two exclusively glutamatergic neuron populations in mMAN — one projecting to HVC and another projecting to Avalanche — and a third population of neurons that tended to express SLC17A6 and GAD1 and projecting to both HVC and Avalanche. We are following up by assessing the electrophysiological properties of these three mMAN populations and their connectivity, using opsin-assisted circuit mapping approaches. In addition, we aim to understand if subpopulations of neurons in the song circuitry co-release glutamate and GABA, as has been described in other regions of the vertebrate brain.

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Poster

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Support: NIH Grant NS084844
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Emory Woodruff Fellowship

Title: Development of precise neural codes during vocal learning

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Abstract: Motor skills learned early in life, from speaking to reaching, play an important role in developing motor systems. Learning motor skills involves building a repertoire of distinct motor actions ("motor gestures") followed by refining these gestures. To build this "vocabulary" of refined motor gestures, the nervous system's task is to develop reliable and precise coordination of muscle activations to execute each motor gesture. We hypothesize that the nervous system learns motor gestures by first creating assemblies of co-active neurons specific to each gesture and then by refining the precisely timed spike patterns within that ensemble. To test this, we examined changes in the activity patterns of individual motor cortical neurons across song learning in juvenile Bengalese finches - a period in which the young songbird acquires new units of vocalizations ("syllables") which then undergo refinement until high levels of acoustic precision is reached in adulthood. Our chronic extracellular recordings from neurons in the robust nucleus of the arcopallium (RA, the songbird analog of the mammalian motor cortex which shape syllable acoustics) span multiple weeks of vocal learning in individual animals, which we then examine with novel mathematical tools. Consistent with prior results in the zebra finch, our preliminary results show gross changes in the statistics of RA spiking activity during song across learning. Beyond quantifying gross spiking statistics across entire song motifs, we also analyzed how the activation patterns of RA neurons change during 1) syllable emergence and 2) syllable refinement. As new syllables emerge, neurons transition from undifferentiated firing during many different syllables to producing syllable-locked activity in a smaller number of syllables. As syllables become refined, individual RA neurons decrease spiking variability for each syllable. Furthermore, we examine how the timescale of motor coding changes during learning -that is, whether syllables undergoing refinement is impacted by variations in spike patterns on the scale of 1-millisecond, as it has been demonstrated in adult song, or by variations on slower timescales. Results from these analyses will be the first to quantify how the neural

representation of individual vocal gestures emerges during learning. Lastly, we will quantify the extent to which RA neurons changes in the motor code during development reflects sensorimotor practice versus merely age. This study provides new insights and a framework for how the nervous system transforms activity patterns to enable reliable and precise execution of skilled behavior across species.

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Poster

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Topic: F.01. Neuroethology

Support: NIH Grant 5R01NS084844-10

Title: The Role of Multifunctional Muscles During Vocalization and Feeding Behaviors in Songbirds

Authors: *A. L. GRASSLER[1], Z. ZHAO[2], M. TRUSEL[3], L. PASCUAL[1], T. F. ROBERTS[2], S. J. SOBER[1];
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Abstract: Many muscles throughout the body are multifunctional, producing kinematically similar behaviors with different performance outcomes. Identifying the effect of shared motor behaviors on a single muscle ensemble is critical to understanding how compensatory consequences in other behaviors occur. In songbirds, control of spectral characteristics of learned vocalizations requires precise coordination of muscles in the upper vocal tract, including the beak and tongue. Because feeding behaviors also rely on movement of the beak and tongue, the feeding circuit has previously been hypothesized to control beak movement. Muscles of the suprasyringeal vocal tract receive projections from premotor and motoneurons identified outside the song system, supporting this claim. Examining both neural and electromyographic activity is necessary to understand how multifunctional muscles are utilized in motor control strategies for the performance of different behaviors. We have therefore designed multielectrode arrays to chronically record activity from adductor and depressor muscles of the beak. Electrophysiology, acoustic, and multi-video recording systems have been successfully synchronized to precisely correlate timing of neural activity, muscle activity, and behavior. In current and future experiments, high-resolution chronic neural recordings from motor cortical areas will be collected alongside electromyographic recordings from jaw muscles and 3D kinematics to characterize the neural-muscular pathways underlying skilled beak movement across behaviors. In parallel with these behavioral and electrophysiological studies, viral labeling methods

identified projections from the lateral arcopallium (Ail), a motor cortical region with indirect projections to facial motoneurons, to areas involved in innate vocalizations including the ventral respiratory column (VRC), the tracheosyringeal part of the hypoglossal nucleus (nXIIts), and the dorsomedial periaqueductal gray (PAGDM), suggesting shared circuitry may exist between feeding and vocalization systems. Overall, this work aims to characterize the control of two functionally distinct behaviors requiring use of the same muscles.

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Poster

PSTR410

Song Learning and Vocal Production

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Support: NIH-NINDS-R01NS108424

Title: An experimental test that introductory notes are a preparatory motor program for singing in zebra finches

Authors: R. S. ABRAM[1], M. NGUYEN[1], A. R. MAGEE[1], R. ARNOLD[1], S. ASADOORIAN[1], K. BIEN[1], H. E. SCHEFFER[1], H. R. STOCKTON[1], S. K. SHAH[1], J. M. MENDEZ[2], ***B. G. COOPER**[1];
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Abstract: Motor preparation is a fundamental feature of learned vocal behaviors, including speech and language; respiratory preparation is commonly observed prior to language production. Experimental manipulations that disrupt preparatory neural activity cause an abnormal delay in behavioral execution. Songbirds are an animal model for the study of learned vocal motor preparation. Zebra finches (*Taeniopygia guttata*), produce a variable number of introductory notes before they sing a stereotyped syllable sequence (song). It has been postulated that their introductory notes are preparatory motor patterns; however, an experimental test of this hypothesis has not been conducted. Song respiration was recorded (n=7) and multiple features of respiratory motor gestures across the sequence of 4 introductory notes preceding song were quantified. The Euclidean distance between the terminal introductory note and the preceding notes decreased as did the cluster variance. In particular, the inspiratory phase of respiration became shorter in duration, typically deeper in amplitude, and more stereotyped; all of which are motor gestures that closely approximate song inspirations (mini-breaths). To test the hypothesis that introductory notes are preparatory motor patterns, white-noise playback (WNP) was triggered based on acoustic features that identified introductory notes (n=6). Baseline song was recorded, and then one-hour, twice daily WNP sessions occurred that targeted introductory notes

or song syllables. Playback amplitude (75 or 95 dB) and duration (100 or 500ms) were varied. Compared to baseline and collapsing across WNPs, there was a 70% increase in changes to the expiratory amplitude or insertion of abnormal delays into the execution of the introductory notes. During song, WNP increased interruption by 20-30% compared to baseline. In some cases, the song interruption occurred within 45-55 ms and caused a cessation of respiration during the execution of an ongoing syllable. Higher amplitude WNP was associated with an increased likelihood for premature song termination. Following song interruption or termination, birds restarted their song from the introductory note(s). In sum, external stimuli can abort the song motor program, and the increased likelihood of motor interruption during introductory notes compared to song strongly suggests that they are a preparatory motor pattern. We postulate that introductory note respiratory patterns help to overcome the inertia of the thoracic cavity before the bird engages in song respiration, especially those movements producing the mini-breaths that enable rapid air resupply during the acoustic performance.

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Poster

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Topic: F.01. Neuroethology

Support: JSPS KAKENHI JP21K18265
JSPS KAKENHI JP21H02456

Title: The role of dopamine receptors in vocal sequence regulation of during song production in Bengalese finch

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Abstract: Sequential motor skills require the proper function of the basal ganglia, where dopamine receptors, D1 and D2 families, are highly expressed. D1 and D2 families have distinct functions in cell signaling, increasing or decreasing the cAMP levels and exhibiting low or high affinity to dopamine, respectively. Moreover, both families have multiple subtypes, such as DRD1 and DRD2. However, the specific regulatory mechanisms of DRD1 and DRD2 in learned motor skills, such as vocal communication, remain unknown. Similar to human speech, songbirds offer a compelling model for studying the development of complex vocalizations in juveniles and maintenance in adults. The song nuclei Area X, a homologous region to the

mammalian basal ganglia, exhibits species-specific differences in neural function related to vocal regulation. Zebra finches (*Taeniopygia guttata*) with lesions in Area X at the adult stage show no drastic changes, whereas Bengalese finches (*Lonchura striata* var. *domestica*) with Area X lesions exhibit increased abnormal repetition of syllables in their songs. In this study, we investigated the role of dopamine receptor subtypes DRD1 and DRD2 in regulating adult Bengalese finch songs in Area X. Initially, we conducted single-cell RNA sequencing to unveil the expression patterns of DRD1 and DRD2 in Area X. Our analysis revealed that 49% of Area X comprises medial-spiny neurons, with DRD1 expressed in 34%, DRD2 in 39%, and both in 13%. Additionally, 16% of Area X comprised pallidal-like neurons with DRD1 expressed in 10%, DRD2 in 19%, and both in 1%. To modulate the expression, we utilized AAV mediated RNA interference, which allows a 40-50% knockdown of DRD1 or DRD2 expression in vivo. We focus on the transition and interval duration between song chunk structures, comparing pre- and post-knockdown of DRD1 and DRD2, respectively.

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Poster

PSTR410

Song Learning and Vocal Production

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Topic: F.01. Neuroethology

Support: NSF IOS Award # 2154646

Title: Temporal precision and the biophysics of a synapse in motor cortex analogue neurons in songbirds

Authors: *L. E. S. TAVARES[1], B. ZEMEL[1], C. V. MELLO[2], H. P. VON GERSDORFF[1]; [1]Vollum Institute, OHSU, Portland, OR; [2]Oregon Hlth. and Sci. Univ. Sch. of Med., Portland, OR.

Abstract: Songbirds possess impressive fine vocal motor control for singing. Song production relies on a specialized cortical circuit consisting of pre-motor nucleus HVC, which orchestrates temporal progression of the song, and motor nucleus RA, responsible for the song's spectral content. Projection neurons in RA, RAPNs, fire tonically at 40Hz and are silenced by local inhibition immediately prior to song onset. HVC-RA projections subsequently drive RAPNs into a burst-pause-burst mode, with burst onsets time-locked to specific song elements at sub-millisecond precision (Yu & Margoliash, 1996, Leonardo & Fee, 2005). RAPNs thus face the challenge of integrating several high-frequency bursts from HVC (~330-890 Hz (Kozhevnikov & Fee, 2006)) and responding with short, stereotyped bursts. Although much is known of the intrinsic properties that allow RAPNs to display such temporal acuity (Zemel et al, 2021, Zemel et al, 2023), less is known about the HVC-RAPN synapse. Previous recordings in slice have

shown that HVC-RAPN synaptic currents in adult finches are mostly composed of a fast CNQX-sensitive component (Mooney & Konishi, 1991, Stark & Perkel, 1999, Garst-Orozco et al, 2014). Otherwise, the biophysics of this synapse remains largely unexplored. Using afferent fiber stimulation and whole-cell patch clamping recordings, we characterized short-term plasticity and the readily releasable pool of vesicles in the HVC-RAPN synapse. Using a recently developed detailed conductance-based RAPN model with minimal morphology informed by our recordings in slice, we were able to reproduce spiking patterns similar to those we observed in current-clamp during afferent fiber stimulation. Finally, we used this model to simulate extracellular recordings and match it to those seen in the behaving animal. These data provide an unprecedented insight into how a vocal motor circuit achieves the temporal precision necessary to underly a rapid, precise and complex behavior.

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Poster

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Song Learning and Vocal Production

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Title: Basal ganglia lesions induce stuttering in canaries

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Abstract: Two recurrent neural circuits are involved in birdsong learning and production: the Song Motor Pathway (SMP) and the Anterior Forebrain Pathway (AFP). The SMP is a cortico-thalamic loop that controls the brainstem motor neurons. The Anterior Forebrain Pathway (AFP) is a cortical-basal-ganglia-thalamic loop involved in song learning in juvenile birds. In Zebra finches, the AFP is not required for adult song, but for a more complex singer, the Bengalese finch, transient stuttering occurred after partial basal ganglia lesions of adult birds. We hypothesize that nuclei in the AFP contribute to encoding syntax and timing in adult birds with complex song. We further examined the role of basal ganglia (Area X) in adult song syntax by studying the behavioral impact of lesioning this nucleus in adult canaries. It is not known how the song circuits encode the transition between phrases, and even the basic circuit mechanisms of syllable production are not well defined. Canary song syllables are delivered in strings of repeats called phrases. A phrase transition is defined by a switch to a new syllable type. These transitions are governed by stochastic rules with long history dependence. Previous calcium imaging found

that multiple distinct groups of projection neurons in the song motor pathway fire during a given syllable. The set of active cells depends on the position of the syllable in a phrase - a *temporal-syllabic*, rather than purely syllabic code. This unexpected temporal coding underscores that the neuronal basis of syllable identity remains to be defined in canaries. These finding suggest that other regions, such as nuclei in the AFP, may signal complex transition timing to the SMP. We investigated the role of Area X in syntax by comparing phrase transition timings pre/post lesions. Baseline behavior was recorded in individual sound-proof chambers from adult male canaries. Behavioral impacts were assessed by comparing the number of different syllable types per song, song duration, and the number of repeats for each syllable type pre/post lesion. Song syllables were annotated using a custom deep neural network (TweetyBERT). This self-supervised Transformer model is capable of unsupervised clustering of canary syllables, allowing for high throughput annotation and analysis. The lesion borders were assessed histologically using Nissl staining. Post-lesion, we found a significant increase in the number of repeats for short-duration syllables. These results indicate that lesions to Area X produce stuttering in canaries, implicating the basal ganglia in real-time song control of adult canaries.

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Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

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Goldstein Innovation Award

Title: Impaired social behavior following early life adversity coincides with changes in the BLA transcriptome and peripheral blood metabolome

Authors: *C. A. MEDINA[1,2], M. SONG[1], P. BENDALE[1], M. OPENDAK[3,4];
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Abstract: Early life adversity (ELA) is a major risk factor for neuropsychiatric diseases such as anxiety and depression. Previous work using the maternal separation (MS) and scarcity-adversity via limited bedding (LB) models of ELA suggest that the long-term behavioral consequences of ELA such as asocial behavior and impaired threat processing coincide with increased c-Fos

expression in the basolateral amygdala (BLA) and changes in the transcriptomic profile of the BLA. However, because MS and LB are both social manipulations (i.e., alter maternal care), our lab developed the deconstructed adversity model (DAM-ELA) to disentangle the role of social and nonsocial effects of ELA on the pup. This approach also permits us to ensure each pup receives a consistent treatment, as well as distinguish pup stress effects from effects on the dam. Briefly, DAM-ELA involves removing pups from the nest for 90 minutes each day from P8 through P12 and either leaving them alone (control), repeatedly shocking (0.5 mA tail shock every 5 mins) them alone (nonsocial adversity), or repeatedly shocking them in the presence of an anesthetized lactating dam (social adversity). Using this paradigm, we had previously found that social adversity but not nonsocial adversity reared pups exhibit reduced social behavior similar to the LB rearing paradigm during a social approach test at P13. We hypothesized that changes to the transcriptomic profile of the BLA in social adversity reared pups underlies these social deficits and that physiological readouts of these changes are reflected in the blood metabolome. To test this hypothesis, we performed RNA-sequencing of the BLA and metabolomics of peripheral blood following a maternal social approach test at P13 or 2-choice social preference test at P45. We found that social adversity produced lasting social deficits and changes in the BLA transcriptome or peripheral blood metabolome at both timepoints while other treatments spared social behavior.

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Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

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Topic: F.03. Stress and the Brain

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Title: The functional contribution of CRH in the CRH/GABA BLA to NAc pathway

Authors: *M. BIRNIE[1], G. B. DE CARVALHO[2], L. TANIGUCHI[3], B. G. GUNN[2], G. ANGELES[1], L. Y. CHEN[2], Y. CHEN[4], N. J. JUSTICE[5], T. Z. BARAM[6];
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Abstract: Disrupted operations of reward circuits underlie major emotional disorders, including depression, which commonly arise following early life adversity (ELA). The nucleus accumbens (NAc) is a major component of the reward circuit and key structure mediating pleasure, motivation, and emotional processes. Multiple inputs converge onto the NAc to modulate reward behaviors, including from the basolateral amygdala (BLA). We recently reported a GABAergic projection from the BLA to the NAc that co-expresses the stress-sensitive neuropeptide corticotropin-releasing hormone (CRH) (Birnie et al., *Nature Commun*, 2023). Specifically, stimulating this projection suppressed reward behavior in CTL mice, and inhibition of this projection rescued reward deficits in ELA mice. However, whether this adverse outcome following ELA is mediated by aberrant CRH release, GABA release, or both, remains unknown. Here, we seek to identify the individual and collective functional contributions of CRH and GABA release mediated by this projection during reward behavior. In CRH-cre mice, we pair viral-genetic (chemogenetic, optogenetic, photometry) methods with physiological, pharmacological (PTX; NBI30775) and genome-editing (CRISPR/Cas9) approaches to delineate neurotransmitter and neuropeptide regulation of reward behavior in adult mice. First, using ex vivo slice physiology, an optogenetic virus (DIO-ChR2) is injected into the BLA, followed by light stimulation in the NAc, and sIPSCs are measured. Second, in freely behaving mice, ChR2 is injected into BLA and GRABCRF3.0 is injected into the NAc. Endogenous CRH release is measured in the NAc during reward behavior and following stimulation of the BLA-NAc projection. Finally, a chemogenetic virus (DIO-hM3Dq) is injected into BLA, followed by medial NAc shell targeted stimulation (with and without CRH1 antagonism / knockout). Outcomes are measured using two reward tasks (free access and operant (FED3)). Viral-genetic tracing combined with slice physiology has identified a CRH/GABA projection from the BLA to the medial NAc shell that is inhibited with PTX in CTL and ELA mice, and that mediates the effects of ELA in adult males. To resolve the relative roles of CRH and GABA in the physiological and behavioral functions of the projection, physiological frequency stimulation of this projection is ongoing. During behavior, measurements of CRH release, with and without BLA-NAc stimulation, and its effects on reward behavior are underway. Here, we delineate the roles of CRH and GABA on the recently described CRH/GABA BLA-NAc projection and establish a mechanism for mediating the enduring effects of ELA on adult reward behavior.

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Poster

PSTR411

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Trainee Professional Development Award
Alzheimer's Disease and Related Dementias T32 Training Program

Title: Lifelong consequences of adverse childhood experiences: the effectsof maternal deprivation on eyeblink classical conditioning and the perineuronal net

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Abstract: Adverse childhood experiences (ACEs) are faced by over 60% of children and are especially prevalent in Appalachia due to high levels of poverty. As these children move toward adulthood, only some display maladaptive physiology and behavior, including hyperarousal as seen in post-traumatic stress disorder (PTSD). This mismatch suggests differences between individuals in resilience to developing these maladaptive behaviors. PTSD is a disorder of learning and memory, in that an exposure to a stressful stimulus leads to maladaptive responding to both novel and previously encountered stimuli. Most animal models designed to study PTSD fail to measure both the learning-related and the hyperarousal symptoms seen in PTSD—however, by presenting rats with a series of periorbital pulses varying in intensity and duration, both before and after delay eyeblink conditioning (dEBC), we provide a way to quantify changes in responding to previously encountered stimuli (unconditioned responding) as a result of conditioning, or conditioning-specific reflex modification (CRM), successfully modelling both categories of PTSD-like symptoms in the rat. We posit that ACEs predispose individuals to CRM, and that ACE exposure disrupts normal development of the perineuronal net (PNN) in the deep cerebellar nuclei (DCN), the integration center of dEBC stimuli. This may cause aberrant regulation of PNN plasticity during the critical period of development, and on through adolescence and adulthood during learning. To model ACEs, we exposed rat pups to daily maternal deprivation (MD) from post-natal day 1 (P1) to P12 and assessed for differences in dEBC behavior and CRM during conditioning at P40 and P90. We expect MD-exposed rats to display CRM at greater frequency and severity when compared to control groups. To explore ACE-induced changes to PNN plasticity in the DCN, we stained DCN tissue for neurons and PNN in MD and control animals; we expect a lower density and more diffuse distribution of DCN PNN in MD subjects, indicative of a prolonged period of plasticity. These studies will delineate a connection between ACEs and PTSD-like hyperarousal later in life and may also provide a mechanism for the differences in susceptibility to PTSD-related hyperarousal seen among ACE-exposed individuals.

Disclosures: M.D. Smith: None. **L. Bays:** None. **E. Irewole:** None. **D. Wang:** None. **B.G. Schreurs:** None.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.04/J26

Topic: F.03. Stress and the Brain

Support: NIH Grant DA059966
NIH Grant DA056534

Title: Early resource scarcity in rats leads to female-specific changes in perivascular gene expression in the prefrontal cortex

Authors: *E. ANDREWS[1], E. P. HARRIS[2], S. CHEHIMI[3], R. CRIST[4], M. E. WIMMER[6], D. A. BANGASSER[7], B. C. REINER[5];
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Abstract: Early resource scarcity is a risk factor for psychiatric disorders. In rats, this is modeled through a limited bedding and nesting manipulation (LBN) where dams and offspring are exposed to limited resources from PND 2-9. This is a sensitive period of brain development where changes in the environment have lasting effects into adulthood. In fact, we have previously demonstrated that LBN changes cognition and motivated behavior that relies on medial prefrontal cortex (mPFC) in adult rats, often in sex-dependent ways. To investigate the persistent effects LBN has on mPFC, we used single nucleus RNAseq to measure changes in cell-type gene expression compared to control male and female rats. Nuclei are clustered into 18 known cell types. Clusters included excitatory neurons, inhibitory neurons, neuroglia, and vasculature. We compared differentially expressed genes (DEGs) within sex such that control vs LBN were compared for males and separately for females. In general, we found LBN caused more transcriptional changes in females than males across most clusters. One cluster that showed transcriptional changes in LBN females was vasculature. To follow up on this data we plan to use qPCR to investigate blood brain barrier (BBB) integrity. These results provide insight into blood brain barrier (BBB) permeability suggesting an impact of LBN on BBB integrity in females. Few studies have investigated early life adversity and its effects on the developing neurovascular environment, which may be an important yet unexplored mechanism of female vulnerability to stress.

Disclosures: E. Andrews: None. **E.P. Harris:** None. **S. Chehimi:** None. **R. Crist:** None. **M.E. Wimmer:** None. **D.A. Bangasser:** None. **B.C. Reiner:** None.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.05/J27

Topic: F.03. Stress and the Brain

Support: Serotonin & Beyond MSCA Grant 953327

Title: Altered prefronto-thalamic loop connectivity and PFC dependent cognitive behaviors in response to early-lifechanges in serotonin signaling

Authors: *N. N. SOTO, A. M. DE STASI, P. GASPAR, A. BACCI;
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Abstract: The prefrontal cortex (PFC) plays a significant role in the regulation of higher order cognitive functions and emotional processes, which are integrated through robust reciprocal connections with the mediodorsal nucleus of the thalamus (MD) among others. The prefronto-thalamic loop has received growing interest in the context of cognitive functions in health and disease. While cognitive functions are impaired in nearly all psychiatric disorders, including depression, their underlying circuit pathophysiology remains vastly unknown. Here, using anatomical labeling, *in vivo* and *in vitro* electrophysiology, and a battery of cognitive tests, we sought out to dissect the effects of early-life alterations of serotonin levels on the prefronto-thalamic loop and cognitive functions in adult mice. A critical developmental stage of the mouse PFC occurs during the first two postnatal weeks, during which environmental stressors, including exposure to selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, result in impaired emotional behaviors in adulthood. We found that perinatal fluoxetine treatment (PNFLX) in mice results in PFC hypo-excitability and altered firing of a specific subtype of deep-layer pyramidal neurons (PNs), transiently expressing the serotonin transporter (SERT). SERT+ PNs extensively connect with the MD, thereby forming an integral component of the prefronto-thalamic loop. PNFLX treatment results in significant reduction of corticothalamic inputs from SERT+ PNs onto MD neurons, as well as increased MD inputs onto L2/3 PNs. Moreover, PNFLX-treated male but not female mice exhibited impaired cognitive flexibility and rule-based learning in a rule-shift task, which is heavily dependent on the prefronto-thalamic loop. We are presently assessing global prefronto-thalamic network activity in-vivo after PNFLX-treatment, aiming to unravel the circuit's pathophysiology underlying the behavioral impairments. These prominent PFC - MD synaptic alterations may explain, at least in part, some of the cognitive deficits observed in this developmental model of depression.

Disclosures: N.N. Soto: None. **A.M. De Stasi:** None. **P. Gaspar:** None. **A. Bacci:** None.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.06/J28

Topic: F.03. Stress and the Brain

Support: CIHR grant (#162376)

Title: Region-dependent inputs differentially mediate early life stress induced changes in prefrontal glutamate release in pre-adolescent male rats

Authors: *J. SONG[1,2], H. LONG[3], M. YOUNUS[1,2], T. WONG[3], C.-D. WALKER[4,2];
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Abstract: Exposure to early life stress (ELS) can exert long-lasting impacts on emotional regulation. The corticolimbic system including projections from the basolateral amygdala (BLA) and ventral hippocampus (vHIP) to the medial prefrontal cortex (mPFC) plays a key role in fear learning. Previously, we reported that ELS tended to diminish fear-induced glutamate release in the prelimbic (PL) mPFC of pre-adolescent males but not females. In this project, we aimed to determine whether reduced glutamatergic inputs and/or elevated inhibitory tone might contribute to the diminished glutamate response in the mPFC following ELS in pre-adolescent male rats. We used a limited bedding paradigm (LB) between postnatal days (PND)1-10 to induce ELS in the offspring. We assessed presynaptic glutamate transmission in the PL mPFC by analyzing paired-pulse ratios of field excitatory postsynaptic potentials (fEPSP) evoked by electrical stimulations in layer 2/3 and layer 5, targeting the projections from BLA and vHIP, respectively. We found that LB exposure increased presynaptic glutamate release probability in layer 2/3 but decreased it in layer 5 of the PL mPFC. To estimate whether ELS alters the structural projections from BLA and vHIP to the PL mPFC, we injected a fluorescent retrograde tracer (CTb) in the PL mPFC and are currently quantifying CTb positive neurons in the BLA and vHIP. To examine fear-induced activation of local inhibitory interneurons, we conducted triple immunostainings of Fos, parvalbumin (PV), and somatostatin (SST) in the PL mPFC after fear conditioning. The activation in PV, but not SST interneurons, was elevated by LB exposure in fear-exposed pre-adolescent males. Our preliminary results suggested that ELS modifies the excitation/inhibition balance in the PL mPFC of pre-adolescent males and induces layer specific alterations in glutamatergic transmission, probably dependent on the origin of these projections.

Disclosures: J. song: None. **H. Long:** None. **M. Younus:** None. **T. Wong:** None. **C. Walker:** None.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.07/J29

Topic: F.03. Stress and the Brain

Support: Intramural Programs of the National Institutes of Mental Health (V.A.A.: ZIAMH002987; C.R.G.: ZIAMH002497)
Intramural Programs of the National Institute on Alcohol Abuse and Alcoholism (V.A.A.: ZIAA000421)

Title: Early life stress exposure impacts baseline risk avoidance, alcohol drinking, and striatal dopamine signaling in mice

Authors: *L. G. ANDERSON[1], M. BOCARSLY[2], V. A. ALVAREZ[3];
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Abstract: In humans, exposure to early life stress (ELS) significantly increases the risk for both mood and substance use disorders (SUDs), including alcohol use disorder (AUD). One hypothesis is that mood disorders may mediate the relationship between ELS exposure and vulnerability to SUDs. Rodent models of ELS are essential to understanding the neurobiological mechanisms underlying this connection. We previously found that wildtype C57BL6/J mice with higher ratios of dopamine D1 to D2 receptors in the striatum exhibit greater baseline risk avoidance and are more sensitive to the anxiolytic effects of alcohol. We now ask whether stress during a developmentally sensitive period can shift the wildtype population further towards this phenotype, altering the balance of striatal D1 to D2 receptors to elicit greater baseline risk aversion and AUD-like behaviors. Cross-fostered C57BL6/J mice born to timed-pregnant dams were exposed to the Baram Lab's Limited Bedding and Nesting (LBN) paradigm on postnatal day (PND) 3-10 (n = 100). After mice reached PND 60, we used a light-dark box (LDB) test to measure baseline risk avoidance and a repeated elevated zero-maze task to measure alcohol-relieved anxiety. An alcohol-induced locomotion task measured the acute stimulatory effects of alcohol while a self-paced, operant social drinking task using the Intellicage Testing System (TSE) assessed a variety of AUD-like behaviors including overall alcohol consumption, binge drinking, and punishment-insensitive drinking. Post-sacrifice, ongoing work using qPCR, radioligand binding, and ex vivo fast-scanning cyclic voltammetry techniques will assess whether ELS-exposed mice display alterations in striatal D1 and D2 receptor ratios and/or electrically-evoked dopamine transients in the striatum. Preliminary results show significant alterations to baseline risk avoidance and chronic alcohol drinking behavior in ELS-exposed mice. Confirming our hypothesis, mice that underwent the LBN paradigm spent less time in the open zones of a LDB ($U = 360$, $p = 0.0023$) and had significantly more alcohol licks/day in our self-paced, operant drinking task ($U = 5$, $p < 0.0001$). In all, this work will clarify the mediatory role that altered dopamine signaling and subsequent shifts in the anxiolytic potency of alcohol have in mediating the connection between ELS and AUD-like behavior.

Disclosures: L.G. Anderson: None. **M. Bocarsly:** None. **V.A. Alvarez:** None.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

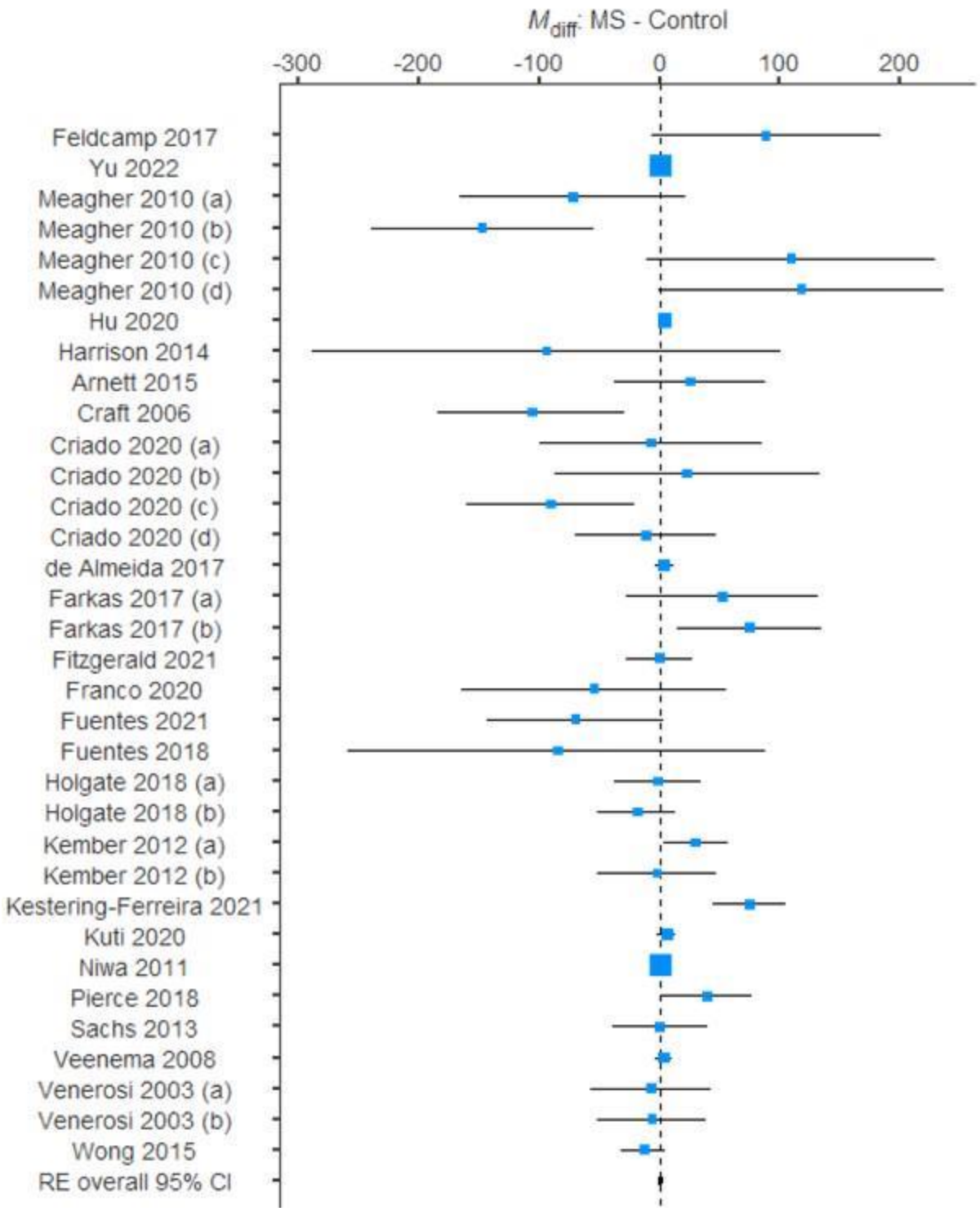
Program #/Poster #: PSTR411.08/J30

Topic: F.03. Stress and the Brain

Title: Meta-analysis of the effects of maternal separation on corticosterone levels in young mice

Authors: A. LEON, *A. KRAFNICK;
Dominican Univ., River Forest, IL

Abstract: Early life stress exposure can influence various behavioral and neurological mechanisms. These influences can ultimately result in an increased vulnerability to the development of psychiatric and neurological conditions (Fox et al., 2010). The HPA axis is a crucial mechanism involved in the biological response to stress, leading to the release of cortisol in humans and corticosterone in mice (Zhu et al., 2014). Despite extensive research on maternal separation in rodent models, there is a lack of consistency across the results observed in the topic. This meta-analysis sought to investigate the impact of maternal separation on the HPA axis in young mice, utilizing corticosterone levels following stress as a primary measure. The literature search was conducted through PubMed using the following keywords: maternal separation, HPA axis, and mice. From this initial search, 2048 papers were obtained. After removing duplicates, papers that included any animals other than mice or who utilized a different measure than corticosterone were excluded, yielding 58 total papers. For this preliminary analysis, we included papers with data only from mice who experienced maternal separation and were tested for corticosterone only at one discrete timepoint. For these criteria, this resulted in 34 samples from 24 papers. Using the esci module in jamovi, we conducted a mean difference meta-analysis, and found an overall raw mean difference of $Mdiff_{\text{MS-Control}} = 0.605$, 95% CI [-0.245, 1.46] (Figure 1). While this analysis suggests very little difference overall, $I^2(\%) = 73.205$ suggesting significant heterogeneity. Sex and PND of initial separation were examined as potential moderators. Both showed small, but non-significant differences: $Mdiff_{\text{female}} - Mdiff_{\text{male}} = 4.40$, 95% CI [-13.67, 22.5], $Mdiff_{\text{PND1}} - Mdiff_{\text{PND>1}} = 0.478$, 95% CI [-0.382, 1.34]. Our preliminary results suggest very little consistency across studies, but potential for moderators that may explain the heterogeneity. Future work will continue to add studies with relevant data, and search for moderators that explain the heterogeneity.



Disclosures: A. Leon: None. **A. Krafnick:** None.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.09/J31

Topic: F.03. Stress and the Brain

Support: R21ES035818
University of New England: Office of Research and Scholarship
The Salsbury Cove Research Fund
P20GM0103423
P20GM104318

Title: Hpi axis dysregulation via early life stress in zebrafish embryos

Authors: *W. SWIFT, T. MCGOLDRICK, M. A. BURMAN;
Univ. of New England, Biddeford, ME

Abstract: Early life pain and stress have long-term implications on neurological, endocrine, and behavioral functions that persist into adulthood. Zebrafish are used as an animal model for stress, as they share key neurological and endocrine features with humans. We focused on the zebrafish's hypothalamic-pituitary-interrenal (HPI) axis, a homologue of the human hypothalamic-pituitary-adrenal (HPA) axis, including sharing the stress hormone cortisol and the glucocorticoid receptor. Our lab has developed an early life stress (ELS) protocol, in which zebrafish embryos are subjected to alternating periods of darkness and bright light, followed by a strong vibration stressor each day over 0-3 days post fertilization (dpf). To accomplish this, they are placed in a "ZebraCube" apparatus. After 15 minutes of darkness, there are alternating 5-minute phases of illumination and darkness. Following 40 minutes of light/dark cycling, a 90-dB 440-hz stimulation occurs for 30-s intervals, with 30-s gaps between, for the last 10 minutes. On 4 dpf, individual zebrafish were separated into 24 well plates, placed into the ZebraCube, and exposed to the same variable light/dark program while tracking their general locomotion (mm). We found wild type ELS fish (WT ELS) produced significantly less light-induced locomotion compared to un-stressed wild type control fish (WT CTRL). To understand the mechanisms of this change in behavior, we utilized GR^{S357} zebrafish: genetically modified mutant (MUT) fish that have a point mutation which prevents the glucocorticoid receptor (GR) functioning as a transcription factor. We ran both MUT and WT CTRL alongside MUT and WT ELS, replicating our effects of ELS and found MUT zebrafish demonstrated significantly less light-induced locomotion than their respective WT counterparts. Next, in order to understand if hypercortisolism was responsible for the effects of ELS on light/dark induced locomotion, we tested WT stressed fish (WT Vehicle) alongside WT fish whose E-2 media was spiked with 10uM of Metyrapone (WT MET). Results from our experiment showed that WT MET fish were protected from the effects of ELS, suggesting that cortisol expressions is critical for the effects of ELS. Finally, to fully realize the extent of cortisol's relationship with ELS, we compared groups of both WT ELS and WT CTRL alongside WT fish whose media was spiked with 1 uM of Hydrocortisone (WT CORT). Contradictory to our hypothesis, WT CORT did not swim significantly less than WT CTRL, and thus did not mimic the effects of ELS. We are currently

conducting experiments with increased dosages of 5 and 10 uM alongside WT CTRL, to see if the effect of cortisol is dosage dependent.

Disclosures: W. Swift: None. **T. McGoldrick:** None. **M.A. Burman:** None.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: F.03. Stress and the Brain

Support: NIH Grant NS101104
NIH Grant MH127259
Tourette Association of America Young Investigator Award

Title: Early life stress produces sexually dimorphic phenotypes relevant to neurodevelopmental and neuropsychiatric disorders

Authors: *C. JIANG[1], I. RUIZ-SANCHEZ[2], C. MEI[2], C. J. PITTENGER[1];
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Abstract: Sex differences in predisposition to neurodevelopmental and neuropsychiatric disorders have been well documented. Autism spectrum disorder (ASD) is markedly more common in males, with a sex ratio of approximately 4:1. Conversely, major depressive disorder (MDD) and most anxiety disorders show a notable female preponderance. Early physiological and psychological stress have been implicated as significant environmental contributors to the pathogenesis of neurodevelopmental and neuropsychiatric disorders. For example, severe early life deprivation and neglect produce social deficits and repetitive behaviors similar to those seen in ASD. Likewise, childhood adversity is a strong predictor of episode onset of major depression. Despite the fact that early life stress (ELS) is an important pathogenic factor and significant sex biases exist in these disorders, how developmental stress contributes to these conditions, and how it interacts with sex, remains unknown. In our current study, we subjected the mouse pups to an ELS paradigm, consisting of 4-hour daily maternal separation and chronically reduced nesting materials, from postnatal day 2 to 10. We examined repetitive grooming, reciprocal social interaction, and sociability using the open field test and three-chamber sociability test as well as anhedonia and anxiety-like behaviors using the sucrose preference test and novelty-suppressed feeding test (NSFT), respectively. We also assessed the number of parvalbumin-expressing interneurons (PV-INs) in the medial prefrontal cortex (mPFC) in mice with or without ELS. We observed that ELS increases repetitive grooming behaviors and reduces reciprocal social interaction and sociability in male but not female adolescent mice. Social deficits, but not repetitive behavioral pathology, persisted into adulthood in males. On the other hand, ELS reduced sucrose preference and induced anxiety-like behaviors

in female but not male adolescents. ELS reduced the number of cortical PV-INs in both adolescent and adult males but not females, recapitulating PV-IN deficits seen in humans with ASD. Our data reveal the sexually dimorphic effects of ELS on ASD-, depression-, and anxiety-relevant behaviors as well as cortical PV-IN deficits in male and female mice. This paradigm may be used to delineate the neurobiological mechanisms underlying sexually dimorphic outcomes relevant to neurodevelopmental and neuropsychiatric disorders.

Disclosures: C. Jiang: None. **I. Ruiz-Sanchez:** None. **C. Mei:** None. **C.J. Pittenger:** None.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.11/J33

Topic: F.03. Stress and the Brain

Support: VIEP-BUAP 2023 to Academic group in Neuroendocrinology (BUAP-CA-288)
CONAHCYT

Title: Effects of pre- and post-natal exposure to bisphenol A in high- and low- yawing sublines of Sprague-Dawley rats.

Authors: *H. AQUINO[1], C. CORTES[2], J. R. EGUIBAR, Sr.[3];
[1]Benemerita Univ. Autonoma de Puebla, Heroica Puebla de Zaragoza, Mexico; [2]Inst. of Physiol., B. Univ. Autonoma de Puebla, Puebla, Mexico; [3]Behavioral Neurophysiol., Benemerita Univ. Autonoma De Puebla, Puebla, Mexico

Abstract: Effects of pre- and post-natal exposure to bisphenol A in high- and low-yawingsublines of Sprague-Dawley rats.H. AQUINO[1], C. CORTES[1], N. LIMA[1], J. MORALES[3], K. NAVA[4] & J. R. EGUIBAR[1,2].[1]Behavioral Neurophysiology Laboratory, Physiology Institute, BeneméritaUniversidad Autónoma de Puebla.[2]Internationalization Office, Benemérita Universidad Autónoma de Puebla.[3]Biomedical Research Institute, Universidad Nacional Autónoma de México.[4]Center for Atmospheric Sciences and Climate Change, Universidad NacionalAutónoma de México.Anxiety incidence around the world has increased significatively last years and hasworsened due to COVID-19 pandemia. Anxiety is a behavioral, physiologic, andpsychologic state induced by a potential o real threat for survival. This mental illnessinduces a fear-like behavioral responses such as increased vigilance, hypoactivity,increased heart rate and hypophagia. Bisphenol A (BPA) is an organic compoundproduced by plastic industry, which is found in food and drink containers so it couldbe ingested or inhaled. Studies on animals exposed to BPA have shown a positiveassociation with behavioral changes related to an increase in anxiety levels sinceBPA is an endocrine disrupting compound capable to modify mother´s hormonalstate and to induce short- and long- term effects

in the neurodevelopment in heroffspring. In our laboratory we have two sublines from Sprague - Dawley (SD) ratsone named high-yawning (HY) with 20 yawns/h and the other low-yawning (LY) with2 yawn/h. It has been shown by different psychobiological tests that LY rats are moreanxious with respect to HY rats. The aim of this study was evaluating BPA effects inanxiety levels in both sublines using the light-dark box (LDB) between 0800 and1000 h. We analyzed 65 HY and 57 LY of both sexes which are maintain understandard conditions. The BPA was administrated via drinking water with a 250µg/kg/day along the gestation and during lactation period. The litters were adjustedto 8 pups 4 male/4 female. Our results showed that HY male rats exposed to BPAsignificantly decreased the time spent in the light compartment with respect to controlconditions ($P<0.05$), and the latency to pass from light to the dark compartment($P<0.05$). In conclusion, BPA modify anxiety in adult HY male rats, but BPA did notaffect anxiety in LY male and female rats. Our results clearly show that the geneticbackground determine the anxiety in LDB.
Grant from VIEP-BUAP 2023 to Academic group in Neuroendocrinology (BUAP-CA-288). H. Aquino is a master's in science in Physiological Sciences with fellowshipCONAHCYT No. 1261033.

Disclosures: H. Aquino: None. **C. Cortes:** None. **J.R. Eguibar:** None.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.12/J34

Topic: F.03. Stress and the Brain

Support: FC2022-2024 PD

Title: Isoflavones neuroprotective effect to offspring of dams exposed to chronic unpredictable mild stress (CUMS)

Authors: A. GONZÁLEZ GARCIA, D. GONZALEZ-ESCAMILLA, *M. FUENTES-CANO, D. J. BUSTAMANTE VALDEZ, **P. DURAN**;
UNAM, Mexico City, Mexico

Abstract: Gestational stress causes changes in organization, plasticity, and adaptation during fetal development in mammals, and has been considered a nocive event to alter the central nervous system developmental critical periods. Stress during pregnancy increases the concentration of glucocorticoids and cytokines, as these can cross the placental barrier, and the fetus can also be affected by stress; it has also been linked to a higher incidence of psychiatric disorders in humans, such as Alzheimer's disease. On the other hand, gestational stress produces changes in the hypothalamic-hypophysis-adrenal axis, alterations in neuronal development in the hippocampus, and cognitive deficits. Due to the increased consumption of legumes such as

soybeans, the properties of isoflavones have been investigated. These plant proteins known as phytohormones, similar to 17β-estradiol (E2) in structure, can act as selective estrogen receptor modulators (SERMs). Likewise, depending on the dose, they may have neuroprotective qualities similar to those that E2 naturally possesses during pregnancy. The present study aims to evaluate the effects of isoflavones on early development and the learning and memory process in the offspring of mothers exposed to chronic unpredictable mild stress (CUMS). Female Sprague-Dawley rats were randomly distributed into four groups (control-C, stress-E, stress + isoflavone supplement-ES, and stress + isoflavones genistein and daidzein-EGD). The ES and EGD groups received 10 mg/kg of the "Pronat" supplement and pure extract of genistein and daidzein, respectively, and were subjected to the EAI protocol during the third week of gestation. The results show that ES and EGD females and males present less weight gain and a delay in the appearance of early developmental markers compared to the control and stress groups. Memory and learning tests showed that women in the ES group had lower retention compared to those in groups C, E, and EGD, while men in groups E and ES had lower retention than those in groups C and EGD. from the EGD group. Therefore, the administration of isoflavones during the third week of pregnancy has a neuroprotective effect on development, memory, and learning processes.

Disclosures: A. González Garcia: None. **D. Gonzalez-Escamilla:** None. **M. Fuentes-Cano:** None. **D.J. Bustamante Valdez:** None. **P. Duran:** None.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.13/J35

Topic: F.03. Stress and the Brain

Support: USDA0210553

Title: Prenatal exposure to plasticizers compromises metabolic function by probably affecting sympathetic innervation to the pancreas

Authors: *A. PALANIYAPPAN[1], M. ADNANE[2], P. S. MOHANKUMAR[3], S. M. MOHANKUMAR[4];
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Abstract: We have previously reported that prenatal exposure to plasticizers such as Bisphenol A (BPA) and diethylhexyl phthalate (DEHP) compromises metabolic function by increasing glucose excursions during the oral glucose tolerance test and promoting obesity. Glucose levels in the blood are regulated by Insulin and glucagon that are secreted by the pancreas. Moreover,

the secretion of these hormones is tightly regulated by a variety of factors and importantly by the sympathetic innervation to the pancreas. In the present study, we wanted to examine the impact of these exposures on sympathetic activity in the pancreas to see if that could possibly play a role in promoting metabolic dysfunction. Adult female Sprague Dawley rats were bred and exposed to 5ug/kg BW of BPA, 7.5mg/kg BW of DEHP or a combination of the two (BD) from day 6-21 of gestation. When the female offspring reached adulthood, they were challenged with a high-fat diet for 2-4 weeks. At the end of treatment, animals were sacrificed, and their pancreas was harvested and frozen. A part of the pancreas was homogenized in 40% trichloroacetic acid and centrifuged. The supernatant was analyzed for norepinephrine (NE) and epinephrine (E) concentrations using HPLC-EC. Prenatal exposure to BPA and BD dramatically decreased NE levels and prenatal exposure to BD reduced E levels in the pancreas. These results indicate that feeding a high-fat diet in adulthood compromises sympathetic activity in the pancreas and this probably contributes to metabolic dysfunction in these animals. Further analysis is underway to determine if this effect is sex-specific.

Disclosures: A. Palaniyappan: None. **M. Adnane:** None. **P.S. Mohankumar:** None. **S.M. Mohankumar:** None.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.14/J36

Topic: F.03. Stress and the Brain

Support: NIH R01 MH104603
NIH R01 AA030256

Title: Environmental and genetic interactions influence susceptibility to Δ9-tetrahydrocannabinol's anti-aggressive effects

Authors: *G. BRACCAGNI[1], K. M. MCFARLIN[2], M. BORTOLATO[1];
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Abstract: Substantial evidence suggests a strong link between cannabis use and pathological aggression. However, the effects of cannabis on aggression remain unclear, as they may be influenced by several factors, including the different content of tetrahydrocannabinol (THC) in cannabis, as well as potential interindividual differences. To study the relationship between cannabis use and pathological aggression, we used an animal model of this problem, based on the best-characterized gene x environment (G×E) interaction in pathological aggression, namely the exposure of MAOA hypomorphic mice to early-life stress (ES). We analyzed the expression of CB1 and CB2 receptor levels in the brains of MAOA-deficient mice exposed to early-life

stress. We observed increased CB1 receptor levels in the hypothalamus and decreased levels in the prefrontal cortex, while CB2 receptor levels remained unchanged. Building on these findings, we investigated the effects of THC at either low or high doses (0.03 mg/kg and 0.3 mg/kg, IP) in MAOA-deficient mice subjected to ES. These effects were suppressed by the CB1 antagonist AM251. We found that low doses of THC ablated aggression. Conversely, higher THC doses (0.3 mg/kg, IP) had no notable impact on aggression in ES-Neo mice, while they increased aggression in ES-WT mice. Notably, THC's effects did not cause other major behavioral alterations. In conclusion, our data suggest that the interaction of low-activity *MAOA-L* variants and ES alters the brain-regional expression of CB1 receptors, and such changes appear to predispose to the anti-aggressive effects of low-dose THC. Further studies are necessary to substantiate these neurobiological mechanisms in humans.

Disclosures: G. Braccagni: None. **K.M. McFarlin:** None. **M. Bortolato:** F. Consulting Fees (e.g., advisory boards); Asarina.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.15/J37

Topic: F.03. Stress and the Brain

Support: NSF IOS 1933264

Title: Long-term effects of Cesarean birth on the mouse vasopressin system: physiological and behavioral effects

Authors: A. CASTILLO-RUIZ[1], ***N. FORGER**[2];
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Abstract: Birth is an extraordinary event for placental mammals and occurs at a time when key developmental processes, such as neuronal cell death, are shaping the brain. We previously reported that in comparison to newborn vaginally-delivered mice, Cesarean-born mice have increased neuronal cell death in the paraventricular nucleus of the hypothalamus (PVN), and that this effect is associated with reduced numbers of vasopressin (VP) neurons in this region in adulthood. This suggests that by influencing neurodevelopment, a Cesarean birth has long-term effects on the anatomy of the VP system. Here, we investigated whether this effect amounts to meaningful changes in the *function* of the VP system. Specifically, we focused on behavioral and physiological functions regulated by VP: nest building and blood osmolality, respectively. To this end, we tested non-reproductive, vaginally- and Cesarean-born adult mice of both sexes. For the behavioral test, we measured the amount of nesting material used overnight by individual mice, and found that Cesarean-born mice had greater nest building. We also identified an effect of sex, with greater nest building in males, as previously reported. For the physiological test,

mice were acutely challenged via intraperitoneal injections (2% body weight) of hypertonic saline (900 mOsm/Kg) or vehicle (isotonic saline, 290 mOsm/Kg). Animals were euthanized 1h later, and their blood collected for the analysis of osmolality as a proxy for VP levels. As expected, osmolality was increased in hypertonically challenged mice, but the effect was modest in comparison to what has been reported for similarly-challenged rats. There was also a trend in the expected direction for the effect of birth mode, with Cesarean-born mice having slightly higher osmolality than vaginally-born mice (reflecting a diminished VP system) but this effect did not reach statistical significance. Together, our results suggest that the VP system of Cesarean-born mice may be functionally compromised. To further test this hypothesis, we are currently examining different aspects of social behavior (i.e., social preference and natural, in-cage sociality), as this has also been linked to VP. Our studies are of clinical relevance given the widespread practice of Cesarean delivery across the world.

Disclosures: A. Castillo-Ruiz: None. **N. Forger:** None.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.16/K1

Topic: F.03. Stress and the Brain

Support: NSF IOS 1933264

Title: Effects of bacterial metabolites on the neonatal mouse brain

Authors: *A. CASTILLO-RUIZ[1], H. G. STURGEON[2], N. G. FORGER[2];
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Abstract: The gut microbiota is the largest microbial population in the body, and communicates with the brain via several routes, including bacterial metabolites. Previously, we reported that in the neonatal mouse, the microbiota influences naturally occurring neuronal cell death (a key neurodevelopmental process). Here, we tested whether bacterial metabolites may modulate this effect. To this end, we provided timed-pregnant mice with a cocktail of short chain fatty acids (SCFAs; produced mainly by bacteria): sodium acetate, sodium propionate, and sodium butyrate in their drinking water. Control dams either received sodium-matched water, or untreated water to assess for effects of sodium alone. Treatments started on embryonic day (E)14 and offspring were euthanized on postnatal day (P)0 or P3. SCFAs were measured in trunk blood and brains were collected for immunohistochemical detection of cell death. We confirmed that maternal SCFA treatment increased levels of butyrate in offspring's plasma. We also found that maternal SCFA treatment reduced cell death in the hypothalamus of offspring compared to both control groups. This effect was limited to P0 and was region-dependent, i.e., observed in the paraventricular and ventromedial nuclei but not in other areas. Interestingly, the magnitude of the

effect depended on the control group used for comparisons. Next, honing in on P0 as a key time point for microbial effects, we generated another cohort of SCFA treated mothers and collected offspring's brains for gene expression analyses of blood brain barrier integrity (*Occludin*), cell proliferation/survival/growth (*Ddit4*), and SCFA transporters (*MCT1*); all previously reported to be influenced by SCFAs. Overall, we found that the control groups were significantly different from each other, with the sodium group having higher expression than the plain water group in all three genes. Interestingly, for *MCT1* the effects of SCFA treatment depended on the control used for comparison as the SCFA group was no different from the sodium group, but different from the water group. For *Occludin* and *Ddit4*, the SCFA treated group was no different from either control group. Taken together, our cell death results show that bacterial metabolites can be important signaling molecules for neurodevelopment. Our results also highlight the importance of determining appropriate controls (plain or sodium-matched water) for SCFA studies. We are currently analyzing the gut microbiota of offspring to address whether SCFA/sodium treatments may exert their brain effects in part via altering microbiota composition.

Disclosures: A. Castillo-Ruiz: None. **H.G. Sturgeon:** None. **N.G. Forger:** None.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.17/K2

Topic: F.03. Stress and the Brain

Support: AASM Bridge to Success Grant #301-BS-23.

Title: Prenatal sleep fragmentation induces maternal inflammation and elevates kynurenine pathway metabolites in the placenta and fetal brain

Authors: C. WRIGHT, M. PIROLI, C. WITT, S. WALTHER, *A. POCIVAVSEK;
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Abstract: Pregnant individuals often face stressors, including disrupted and fragmented sleep, which are associated with negative maternal and child health outcomes. These prenatal insults elevate tryptophan degradation via the kynurenine pathway and increase levels of kynurenic acid (KYNA), an endogenous astrocyte-derived antagonist of NMDA and α7nACh receptors. As both receptors are crucial to neurodevelopment, such elevations in KYNA are hypothesized to disrupt offspring health. We hypothesize that KYNA may be a molecular link between disturbed maternal sleep and poor health outcomes, as our lab has shown previously that acute sleep deprivation in pregnant rats induces fetal inflammation and elevates fetal brain KYNA (Baratta et al., 2020 *Neurobiol Stress*). Presently, we developed a novel automated sleep fragmentation (SleepFrag) protocol during the last week of pregnancy and evaluated markers of inflammation

in maternal plasma and kynurenine pathway metabolism in utero (placenta and fetal brain). Pregnant Wistar rats were subjected to SleepFrag for 18 hours/day from embryonic day (ED) 15 to 22. SleepFrag protocol significantly reduced NREM sleep (-58%, P<0.0001), reduced REM sleep (-94%, P<0.0001), and increased NREM sleep bouts (+35%, P<0.01) (N = 9-17/group). SleepFrag elevated inflammatory cytokines (IL-1β and IL-10) in maternal plasma at ED 21 but not ED 18 compared to control (N =4-9/group). SleepFrag elevated male and female placental kynurenine (P<0.01) at ED 18 and ED 21. Of note, placental KYNA was higher in control females than males (P<0.001). However, after SleepFrag, this sex difference was eliminated as the conversion of kynurenine to KYNA was reduced in the placentas of SleepFrag females (P<0.05). Placentas from SleepFrag males had elevated KYNA at ED 18 (P<0.05). Fetal brain kynurenine was elevated in SleepFrag males and females (P<0.05) at ED 18 and ED 21. Fetal brain KYNA was elevated at ED 21 (P<0.05). Taken together, we have established a translational model to study the molecular impact of sleep disturbances during pregnancy and identified elevated inflammation and kynurenine pathway metabolism in utero.

Disclosures: C. Wright: None. **M. Piroli:** None. **C. Witt:** None. **S. Walther:** None. **A. Pocivavsek:** None.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.18/K3

Topic: F.03. Stress and the Brain

Title: Maternal and Infant Oxytocin Are Linked to EEG, Stress, and Temperament in Infants of Depressed and Non-Depressed Mothers

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Abstract: Stress and depression are believed to neurologically program fetal and infant regulatory tendencies. The neurohormone oxytocin (OT) is related to the developing infant socio-emotional and mother-infant affiliative behaviors. 64 mothers provided prenatal urine samples during their third trimester (between 29- and 38-weeks' gestation) and again at 4 months postnatally. Mothers also answered questions about their depression symptoms, attachment/bonding patterns during prenatal and postnatal periods (newborn and 4 months), and infant feeding. Oxytocin was collected twice in infancy along with mother-infant behavioral interactions, EEG activity, and stress-cortisol responses at 4-months. Our analyses examined patterns of responsivity and regulation in families with and without mental health risks. Initially we noted that maternal OT was associated across time, $r = .62$, $p < .001$, while infant OT was not

significantly related and had a high degree of variability across time. In addition, prenatal maternal depression and stress were significantly positively associated, $r = .33$, $p = .01$. and prenatal depression persisted stably into infancy, $r = .31$ to $.65$, $p < .05$. Infant OT during the initial postnatal period was not associated with maternal depression yet was associated with temperamental measures of inhibitory control at 4 months postnatally, $r = .74$, $p = .015$. Infants with higher OT levels also had significantly higher anterior asymmetry scores at central sites, $r = .79$, $p = .034$, suggesting the relation between OT and left hemisphere activity and a potential shift toward greater anterior activity by 4 months. Moreover, infant OT levels were negatively correlated with cortisol levels after a stressor task, $r = -.37$, $p = .011$, and vocal reactivity, $r = -.53$, $p = .020$, suggesting relationships between OT, stress responsivity and temperament. Finally, and unexpectedly, prenatal maternal attachment measures were not able to be predicted from OT in mothers nor infants, however, regression analyses demonstrated that postnatal maternal depression measures and OT levels predicted 18% of the variance in attachment/bonding measures, with depression exhibiting stronger predictive scores than depression, $p < .05$ than for OT but $p = .15$ for OT. Links between prenatal and postnatal bio-hormonal risk, and early manifestations of temperament are important to examine in their own right; however, exploring their effects on early infant brain and temperament development illuminates the potentially enduring biological foundations of socio-emotional development in normative and at-risk families.

Disclosures: S. Gott: None. **N. Jones:** None.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: F.03. Stress and the Brain

Support: NIH Grant DK124727
NIH Grant GM060507
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Loma Linda University School of Medicine GRASP Seed Funds

Title: Sex-specific neural changes in a rat model of adolescent stress-induced binge eating

Authors: *J. SIERRA[1], P. ONTIVEROS-ANGEL[1], T. SIMON[1], V. WILLIAMS[1], A. WILLIAMS[1], F. SHARAFEDDIN[2], J. LOU[2], B. NOARBE[3], A. OBENAUS[4], J. D. FIGUEROA[1];
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Abstract: Binge-eating disorders impact more than 70 million individuals globally, disproportionately affecting females. Research highlights the crucial influence of adolescent psychosocial stress (PSS) in elevating susceptibility to both binge-eating behaviors and obesity. However, the neural substrates connecting adolescent PSS to dysregulated eating have not been fully elucidated. Limbic brain regions are regulatory centers of emotion and reward-driven behaviors that mature throughout adolescence. Consequently, these substrates are particularly prone to environmental insults. In this study, we proposed that exposure to PSS during adolescence disrupts the maturational trajectories of reward circuits implicated in binge-eating behaviors. We generated a Lewis rat model of adolescent PSS and intermittent access to an obesogenic diet to test this hypothesis. Male and female rats were divided into exposed (EXP) and unexposed (UNEXP) groups. EXP rats endured a 31-day PSS paradigm consisting of two predator exposures and social instability until adulthood. Animals underwent behavioral tests prior to the binge eating (BE) protocol. The BE paradigm comprised of 24-h access to a highly palatable Western-like high-saturated fat diet (WD, 41% kcal from fat), followed by six-day access to an ingredient-matched control diet (CD, 13% kcal from fat) for three consecutive weeks (cycles 1-3). WD consumption was measured 2.5 and 24 hours after WD (re)introduction during the light phase of the BE protocol. At the end of study, brains were prepared for ultrahigh resolution diffusion-MRI (dMRI, 17.6T) and immunofluorescence. Results demonstrate that females, regardless of exposure, ate significantly more WD than EXP males during BE cycle 1. By cycle 3, only EXP females exhibited elevated food intake compared to UNEXP females. Thus, revealing divergent influences of stress on sex and time. Furthermore, EXP females exhibited blunted acoustic startle responses compared to UNEXP controls. Finally, we used region-specific Z score values of fractional anisotropy (FA), a dMRI metric for neuron fiber density and axonal diameter, to show selective FA-related differences in hypothalamic and reward circuits of EXP rats (increase in males, decrease in females) compared to controls. Our findings indicate that unique pathways are influenced by stress and dietary exposure in both males and females. Current work is focused on evaluating neuronal activity using immunofluorescent staining of immediate early gene markers. Our model captures sex-specific differences and may offer avenues to study neuropathological targets of vulnerability and resilience to binge-eating disorders.

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Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.20/K5

Topic: F.03. Stress and the Brain

Support: NIH DA052909
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Emory National Primate Research Center (ENPRC) Base Grant OD P51OD011132

Title: Long-term effects of early life adversity on adult brain structure and dopamine and serotonin receptor systems involved in cocaine reinforcement

Authors: *J. ACEVEDO-POLO[1], E. R. SIEBERT[1], K. A. JENKINS[1], M. I. ALLEN[2], Z. KOVACS-BALINT[1], R. KIM[1], R. J. VOLL[1], L. N. CHAVAN[1], M. M. GOODMAN[1], M. A. STYNER[3], J. A. NYE[4,5], M. A. NADER[2], M. M. SANCHEZ[1];
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Abstract: Early life adversity such as child maltreatment (MALT) is associated with mental illness, including substance use disorders (SUDs), but underlying neurobiological mechanisms remain unclear. This study examined long-term effects of infant MALT on adult brain serotonin (5HT) receptors and dopamine (DA) receptors in corticolimbic regions involved in reward and emotional control, using positron emission tomography (PET) imaging, as well as structural effects using MRI. We also assessed whether neurochemical and structural alterations were associated with cocaine (COC) self-administration (SA) and dynamic changes in 5HT and DA receptors following chronic COC SA. PET receptor binding potential (BP) measured in several brain regions of adult MALT (n=13, 7M, 6F) and Control animals (n=9, 5M, 4F) prior to COC SA showed long-term effects of infant MALT on adult brain 5HT, but not DA receptors in corticolimbic circuits. Specifically, MALT animals had lower 5HT1A BP in the anterior cingulate cortex -ACC- ($F(1,18)=5.159$, $p=.036$), medial prefrontal cortex -mPFC- ($F(1,18)=6.132$, $p=.023$), and hippocampus ($F(1,18)=4.649$, $p=.045$) compared with Controls. A MALT by Sex interaction effect was detected in 5HT2A BP in the orbital frontal cortex -OFC- ($F(1,18)=5.159$, $p=.036$), with lower levels in MALT than Control males, but not in females. Volumetric differences were also detected in OFC and mPFC regions between MALT and Controls (OFC: $F(1,17)=5.794$, $p=0.028$; mPFC: $F(1,17)=8.268$, $p=0.009$). Using COC SA under a progressive-ratio (PR) schedule of reinforcement to measure COC reinforcing strength, a leftward shift in the COC dose response curve (DRC) was observed in MALT compared with Controls, suggesting higher potency of reinforcing strength of COC in MALT than Control animals. Immediately following COC SA and 5 weeks after abstinence, PET scans were repeated to examine dynamic changes in corticolimbic of 5HT and D2/D3 receptors that are being analyzed as predictors of response to pharmacological treatments targeting 5HT and DA receptors that modulate DA signaling. Preliminary findings from those pharmacological studies suggest a robust effect of a 5HT2C receptor agonist (provided by Pfizer) which blocks cocaine-induced reinstatement ($t(3)=4.18$, $p=0.025$), although with individual variability. Our findings suggest long-term neurochemical and structural effects of infant MALT on adult corticolimbic regions regulating reward processes. They also suggest specific roles of 5HT 1A, 2A and 2C receptors in early cocaine-related changes in reward circuitry of relevance to SUDs.

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Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

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Topic: F.03. Stress and the Brain

Support: NIMH RO1MH096093
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Title: Illuminating (dys)functional neuroarchitecture induced by early life adversity: Computational manganese-enhanced MRI (cMEMRI)

Authors: *T. W. USELMAN[1], R. E. JACOBS[2], E. L. BEARER[1,3];
[1]Pathology, Univ. of New Mexico Hlth. Sci. Ctr., Albuquerque, NM; [2]Zilkha Neurogenetic Inst., USC Keck Sch. of Med., Los Angeles, CA; [3]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA

Abstract: How early life adversity (ELA) affects the adult brain is a mystery. Neuroimaging has deepened our understanding of brain function and dysfunction, in part due to concurrent advances in analytical methods. Neuroimaging allows identification of brain states, i.e., widely distributed patterns of neural activity. MEMRI offers the unique opportunity to map brain states longitudinally in normally behaving animals across successive conditions. Mn(II) enters neurons of awake-behaving mice through voltage-gated calcium channels, accumulates, and is detected in the brain by MRI retrospectively. Applying computational approaches to MEMRI reveals functional architecture. Here, we apply structural equation modeling (SEM) to test for impacts of ELA and acute threat on brain state. ELA was modeled by providing mice with limited bedding from post-natal days 2-9. Adult mice (10 wk) of normally and ELA reared cohorts (n=24) were imaged by MR in 4D at 100um^3 resolution, before and 22h after $MnCl_2$ injection (0.3mmol/kg, IP). At 23h post-injection, mice experienced an acute threat (TMT, 2,3,5-Trimethyl-3-thiazoline) and were imaged again. After 8 days, a second pre-to-post-imaging sequence was performed. MR images were preprocessed using our custom pipeline - skull stripping, intensity normalization, and spatial registration. We found that covariance of inter-regional signal intensities is a meaningful measure of brain coactivations, using SEM of MEMRI intensities within a known olfactory circuit. We then developed new models based on circuitry predicted to be altered by ELA to test with SEM. We measured intensities of regions within those circuits across conditions and compared between groups. We focused on two circuits involved in emotional consequences of ELA in humans, reward seeking (mPFC, ACB, VTA, HIP and AMY) and defensiveness (IL/PL, ACA, AMY, dHIP, vHIP and LC). To verify our computational

algorithm (*lavaan*) we reproduced SEM results from a UCLA workshop. To validate our MEMRI results, we performed a series of positive and negative internal controls including models for variance only, covariance between random regions, and permutation of pairwise covariance. We considered $\chi;^2 > 0.05$, CFI > 0.9 and SRMR < 0.08 a good fit. Fit indices and regression coefficients in ELA varied dramatically from normal demonstrating a profound effect of ELA on these circuits. Results were only for two of many possible circuits, do not include interdependencies between circuits or across time, and may vary depending on anatomical segmentation. Thus, unique brain states involved in significant emotional systems are affected by ELA and further altered by subsequent threat.

Disclosures: T.W. Uselman: None. **R.E. Jacobs:** None. **E.L. Bearer:** None.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

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Title: Effects of low social status on prefrontal cortex, hippocampus and related cognitive function in adult female rhesus macaques

Authors: Z. KOVACS-BALINT[1], A. WANG[1], T. JONESTELLER[1], K. BAILEY[1], A. GRAY[1], J. ACEVEDO-POLO[1], A. GOPAKUMAR[1], R. KIM[1], R. VLASOVA[2], M. A. STYNER[2], J. RAPER[1], J. BACHEVALIER[1], M. SANCHEZ[1], ***M. C. ALVARADO**[1];
[1]Emory Univ., Atlanta, GA; [2]Univ. of North Carolina At Chapel Hill, Chapel Hill, NC

Abstract: There is a strong link between chronic psychosocial stress and cognitive impairments in human populations with low socioeconomic status, although the underlying neurobiological mechanisms remain unclear. We used a translational rhesus monkey model to examine the long-term effects of low social status/rank on adult prefrontal cortex (PFC) and hippocampus (HIPP) volumes and related cognitive function. T1- and T2-weighted structural brain MRI scans were collected in 27 adult female rhesus monkeys (13 dominant, 14 subordinate) at 2 ages (7 and 8 years, equivalent to 28-32 years in humans) to examine age-related volumetric changes of HIPP and PFC subregions important for executive function and cognitive flexibility (dorsolateral

PFC(dlPFC) -Area 46-; orbitofrontal cortex(OFC) -Area 13-; medial PFC(mPFC) -Areas 14, 25). Total intracranial volume (ICV) was included as a covariate in statistical models to control for individual differences in brain size. Executive function and cognitive flexibility were assessed with the Intra-/Extra-Dimensional (ID/ED) attention set shifting task at the later age (mean age 8.27 ± 0.9 years -about 32-36 human years-). ANOVA models were used to examine the effects of social rank (dominant, subordinate) and age, and Spearman Rank correlations were used to examine associations between variables. Our findings showed that although the total intracranial volume (ICV) was still increasing from 7 to 8 years ($F(1,24)=6.209$, $p=0.020$), certain PFC subregions already started to shrink (dlPFC: $F(1,24)=4.313$, $p=0.049$; mPFC: $F(1,24)=10.429$, $p=0.004$). Subordinate animals had larger HIPP volume than dominants ($F(1,24)=6.034$, $p=0.022$), but social rank did not affect PFC subregions volumes. In addition, our findings suggest that the subordinate group also had greater (but nonsignificant) difficulty during the ID and ED Shifts (IDS, EDS), and smaller volumes in mPFC regions at 8 years predicted higher errors in the IDS ($rho=-0.594$, $p=0.042$).Our findings suggest that aging-related shrinkage of the primate PFC starts early in adulthood across rhesus social status groups, particularly in dlPFC and mPFC, involved in executive function. Smaller volumes of mPFC at the later age were associated with greater difficulties during the IDS shifts, particularly in the low social status animals. As we continue this longitudinal study, we anticipate that the cumulative stress experienced by subordinate animals may accelerate the loss of cortical volume and cognitive decline.

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Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

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Program #/Poster #: PSTR411.23/K8

Topic: F.03. Stress and the Brain

Support: NIH grant HD091376

Title: Pubertal stress influences the developmental trajectory of the transcriptome in specific populations of hypothalamic neurons

Authors: *M. M. NICODEMUS[1], L. LUTHER[1], K. E. MORRISON[2];
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Abstract: Anxiety and mood disorders are more prevalent in women than in men. Adverse childhood experiences are a known risk for these disorders and are particularly consequential for

women when experienced during puberty, a time of dynamic hormonal change. Pregnancy, another dynamic hormonal period, also increases risk for these disorders. However, little is known about how pubertal adversity and adulthood pregnancy interact to lead to the manifestation of anxiety and mood disorders in women. We have shown that in both humans and mice, pubertal stress led to blunted stress axis regulation during the peripartum window. In mice, this included altered gene expression in the paraventricular nucleus of the hypothalamus (PVN), the key regulator of the hypothalamic-pituitary-adrenal (HPA) stress axis. In pregnant, pubertally stressed females, we found an upregulation of six immediate early genes (IEGs) in baseline, non-stimulated conditions, suggesting that the PVN of pregnant, pubertally stressed females is poised to respond differently to external stimuli. This, in addition to pharmacological studies, suggests there is a mechanistic role for altered baseline IEG expression, which could have wide-ranging effects on downstream transcription, physiology, and behavior. Pubertal stress also alters the expression of epigenetic regulators in a closely-related region of the hypothalamus, the medial preoptic area (mPOA). To further understand the cell-type specificity and developmental trajectory of our prior findings, we utilized transgenic reporter mice and in situ hybridization to examine expression of IEGs in specific PVN neurons, including corticotropin-releasing factor neurons. In one cohort, mice were exposed to chronic variable stress (CVS) from postnatal days 21-34 and were undisturbed until adulthood, during which they became pregnant or not. Brains were collected in baseline (non-stimulated) conditions during late pregnancy. In another cohort, mice were exposed to CVS but brains were collected at baseline (PN21), after 1 week of CVS (PN28), or after 2 weeks of CVS (PN35). In both cohorts, we collected the mPOA and the PVN and used RNAScope technology to examine cell type- and spatial-specific expression of key genes. These findings will provide insight into the cell type, developmental, and state (parity) specific effects of pubertal stress on the transcriptome in key hypothalamic regions. This work adds to our greater understanding of the molecular underpinnings of risk for negative outcomes following adversity during early adolescence.

Disclosures: M.M. Nicodemus: None. **L. Luther:** None. **K.E. Morrison:** None.

Poster

PSTR411

Early-Life Stress: Neural, Neurochemical, and Physiologic Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.24/K9

Topic: F.03. Stress and the Brain

Support: NIH grant HD091376

Title: Lasting epigenetic programming in the hypothalamus due to stress during puberty

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Abstract: Undergoing adverse events during the critical developmental period of puberty puts women at risk for affective disorders and stress dysregulation, and this risk is heightened if they become pregnant later in life. We previously demonstrated that stress during puberty combined with pregnancy in adulthood led to a blunted response of the hypothalamic-pituitary-adrenal (HPA) axis which is a key system involved in the stress response. Further, we found that pubertal stress altered gene expression in the brain region that is responsible for initiating the stress response - the paraventricular nucleus of the hypothalamus (PVN). Six immediate early genes (IEGs) were upregulated in the PVN of pregnant adult mice that had previously undergone stress during puberty. ATAC-sequencing data showed that pregnant, pubertally-stressed females had increased openness of chromatin, which permits an environment for increased gene expression. Further analysis implicated histone acetylation in the increased openness. Recently, we found an increase in histone acetyltransferase (HAT, enzymes that acetylate lysine tails of histones) activity in the PVN following stress. However, after a decrease of HAT in adulthood, pregnancy acts as a switch to increase HAT tone in the PVN of females. Here, we aimed to further characterize the dynamics of chromatin regulation before, during, and after pregnancy and to examine key regulators responsible for the open chromatin landscape. Beginning at postnatal day (PN) 21, mice were exposed to 14 days of chronic variable stress (CVS). Brains were collected at baseline (non-stimulated) from pubertally-stressed and control adolescent mice, adult pregnant or virgin, and postpartum mice. The PVN was isolated and prepared for either ATAC-Seq or CUT&RUN sequencing for H3K27ac. ATAC-Seq results were analyzed for the impact of pubertal stress and peripartum state on the number of open chromatin regions and transcription factor binding. CUT&RUN sequencing results were analyzed for the impact of pubertal stress and peripartum state on the H3K27ac binding regions. The results will further our understanding of the specific epigenetic modifications caused by pubertal stress and their developmental trajectory. Altogether, these findings will clearly define the epigenetic plasticity that permits the altered hypothalamic blunted HPA axis response in pubertally-stressed females during pregnancy. These studies provide novel insight into the epigenetic mechanisms underlying female-relevant risk for stress dysregulation, a central endophenotype of affective and anxiety disorders.

Disclosures: L.A.M. Luther: None. **S. Higley:** None. **K.E. Morrison:** None.

Poster

PSTR412

Blood Brain Barrier

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.01/K10

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: R01 MH121763
3R01MH121763-06S1

Title: Ahnak depletion in vascular endothelial cells protects the brain from inflammation

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Abstract: Homeostatic control of the brain-immune axis is critical for cognitive and mental health, and aberrant inflammatory responses facilitate the development of neurological or psychiatric disorders. The blood-brain barrier (BBB) plays a crucial role in limiting the entry of immune cells and immune mediators into the brain, but molecular pathways controlling this process have not been fully understood. Human Ahnak was initially identified from tumor cells and characterized as a tumor suppressor. Our previous studies have found Ahnak as a pivotal regulator of chronic stress-induced behavioral adaptation or depression-like behavior. In the brain, Ahnak is highly expressed in vascular endothelial cells which hold the barrier property in the BBB. However, the function of Ahnak in the endothelial cells remain unknown. To investigate the Ahnak function in the BBB, we have generated endothelial cell-specific Ahnak KO mice. Interestingly, endothelial cell-specific Ahnak KO mice display baseline antidepressant-like and anxiolytic behavior and stress-resilient phenotype in the chronic social defeat stress paradigm. Because chronic social defeat stress-induced social avoidance and anxiety are associated with peripheral inflammation, BBB disruption, and cerebral microbleeds, we hypothesized that endothelial Ahnak deletion might induce immune-suppressive and brain-protective mechanisms. Consistent with this idea, endothelial cell-specific translating mRNA profiling indicates that Ahnak deletion increases a group of molecules, including ligands, receptors, or adhesion molecules, that were previously studied as immune modulators in cancer microenvironment. Furthermore, endothelial Ahnak KO blocks lipopolysaccharide-induced increase of Iba1-positive cells in the brain. All these results suggest that Ahnak deletion in the vascular endothelial barrier induces active immune suppression mechanisms protecting the brain from systemic inflammation.

Disclosures: Y. Jeong: None. **B. Carabelli:** None. **C. Sung:** None. **G. Barbet:** None. **Y. Kim:** None.

Poster

PSTR412

Blood Brain Barrier

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.02/K11

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: JSPS KAKENHI Grant Number 18K19756

Title: Role of TROY in maintenance of blood-brain barrier

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Abstract: A member of the tumor necrosis factor receptor superfamily, TROY, is known to be an orphan receptor. Previous studies have demonstrated that TROY functions as co-receptor of Nogo receptor and death receptor 6 in neurons and endothelial cells of the nervous systems, respectively. Our previous studies have shown that TROY is also expressed in mature astrocytes of the cerebral cortex during postnatal and adult stages. However, the function of TROY in astrocytes remains unknown. In the present study, we first investigated the localization of TROY-ligands in the central nervous system using a chimera protein consisting of the extracellular domain of mouse TROY and the Fc portion of human immunoglobulin G_1 (TROY-Fc). Triple-immunofluorescence staining for TROY-Fc, glial fibrillary acidic protein (GFAP), and megalencephalic leukoencephalopathy with subcortical cysts 1 (Mlc1) revealed that TROY-Fc was localized in GFAP- and Mlc1-double positive perivascular astrocytes in the cerebral cortex of adult mice. These results suggest that unknown TROY ligands may be in Mlc1-positive perivascular astrocytes. To elucidate the role of TROY-ligand in vivo, we next generated transgenic (TG) mice expressing TROY-Fc, in which TROY signaling is suppressed by inhibiting binding of TROY ligands to endogenous TROY. Because Mlc1-positive perivascular astrocytes are known to be involved in the maintenance of blood-brain barrier (BBB) integrity, we investigated BBB permeability in adult TG mice using an exogenous tracer, Sulfo-NHS-biotin. Prominent extravasation of Sulfo-NHS-biotin were detected in the cerebral cortex and hippocampus of adult TG mice. In these regions of wild-type mice, however, no extravasation of Sulfo-NHS-biotin was observed. These findings suggest that BBB permeability is enhanced in TG mice. Transmission electron microscopic analysis in cerebral cortex and hippocampus revealed that flattened astrocytic endfeet enveloped the wall of vessels in wild-type mice. On the other hand, swollen astrocytic endfeet around microvessels were frequently observed in cerebral cortex and hippocampus of TG mice. Quantitative analysis showed that areas of astrocytic endfeet were significantly increased in TG mice compared with those in wild-type mice. The swelling of astrocytic endfeet is often associated with BBB disruption. These results suggest that TROY signaling in astrocytes plays an important role in the maintenance of BBB integrity and astrocytic morphology.

Disclosures: T. Hisaoka: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventor on a patent for TROY-Fc Tg mice (JP4898126). **T. Komori:** None. **E. Kuriyama:** None. **Y. Morikawa:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventor on a patent for TROY-Fc Tg mice (JP4898126).

Poster

PSTR412

Blood Brain Barrier

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.03/K12

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH R33HL159948
CZI Investigator Award

Title: Circadian dynamics in a humanized neurovascular unit

Authors: E. KIM[1], **C. DENG**[2], *J. W. MITCHELL[3,4,5], M. U. GILLETTE[4,1,2,5];
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Abstract: The neurovascular unit (NVU) forms the structural and functional link between brain and vasculature, maintains the blood-brain interface (BBI), controls cerebral blood flow, and surveils for injury. Although the neurovascular system is a dynamic homeostatic regulator of brain function, evidence for time-of-day modulation is emerging. Cerebral microbleeds (CMBs) from blood leakage across the blood -brain interface (BBI) result in millimeter-sized blood clots, which can lead to inflammation, cellular injury, and neurodegeneration. Deterioration of BBI integrity found with aging, disease, traumatic brain injury, and stroke are associated with CMBs. The occurrence of CMBs and hemorrhagic/ischemic stroke is not random, but rather clusters in early day or evening. To understand this time-of-day modulation, we integrated a clock-gene reporter (mPer1-VENUS) into human induced pluripotent stem cells (hiPSCs) using CRISPR transfection. This enables us to monitor dynamics of the NVU and to probe it in the context of the oscillatory circadian cycle that drives integrative physiology and behavior. We have improved methods for differentiating cells of the NVU. Prior works have shown that high transendothelial electrical resistance (TEER) persists in hiPSCs-derived brain endothelial-like cells (hiBECs) for at least 2 weeks, emphasizing the utility of the model for longer term studies. However, most studies evaluate iBECs within the first few days of subculture, when little is known about their proliferative state, which could influence function. We investigated whether a circadian rhythm exists in hiBECs in long-term culture after synchronization with dexamethasone (Dex), which stimulate transcriptional oscillation for the core clock components (Per1, Per2, Per3, Cry1, Cry2, Rev-Erbα, Rev-Erbβ, Dbp, Npas2, and Bmal1) in primary mesenchymal stem cells (MSCs). hiBECs at 40 days post-subculture with 10 μM retinoic acid and bFGF were fixed every four hours for 28 h after 2-h Dex treatment. Immunostaining of the clock proteins PER1 and BMAL1 and the endothelial marker, CD31, following Dex synchronization revealed a ~24 hour-circadian rhythm of both PER1 and BMAL1 expression in hiBECs at 40 days post-subculture. These hiBECs exhibit the presence of an intrinsic circadian clock that we predict enables the hNVU to anticipate rhythmic change and appropriately respond to acute alterations in brain activity and body physiology over the day-night cycle. Understanding the cell biology and physiology of the human NVU is critical to diminishing consequences of impaired neurovascular function, including cerebral bleeding and neurodegeneration.

Disclosures: E. Kim: None. **C. Deng:** None. **J.W. Mitchell:** None. **M.U. Gillette:** None.

Poster

PSTR412

Blood Brain Barrier

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.04/K13

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH R33HL159948 (MUG, HK, BH)
MCB Summer Undergraduate Research Fellowship (JOS)
MIP Ann Nardulli Graduate Student Travel Award (QTN)

Title: Circadian Clocks in Neuroendothelial Cells Regulate Daily Oscillations in Functional State at the Blood-Brain Interface

Authors: *Q. T. NGUYEN[1], A. C. WEISS[1,2], J. O. STORM[3], J. W. MITCHELL[4,2,5], H. KONG[6,2,5], B. HAN[7,2], M. U. GILLETTE[4,5,1,2];
[1]Dept. of Mol. & Integrative Physiol., [2]Beckman Inst. for Advanced Sci. & Technol., [3]Sch. of Mol. & Cell. Biol., [4]Dept. of Cell & Developmental Biol., [5]Neurosci. Grad. Program, [6]Chem. & Biomolecular Engin., [7]Dept. of Mechanical Sci. & Engin., Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract: Neuroendothelial cells of the blood-brain interface (BBI) form the inner surface of the cerebral microvasculature. This surface is sealed by tight-junctional proteins to create a diffusional barrier, which maintains brain homeostasis through restricting cerebral paracellular permeability and blood flow. Time-of-day-dependent relaxing of the BBI can cause leakage of blood fibrinogen into the brain parenchyma in early morning and early evening, which can enhance the severity of stroke. Research into mechanisms underlying these changes is limited. Our goal is to understanding these time-of-day vulnerabilities of the neurovasculature to leakage. Preliminary evidence shows that components of the BBI are under circadian regulation. We evaluated neuroendothelial cells from a mouse bearing a clock-gene reporter, *Per1-Venus*. We found the *Per1-Venus* expression of primary neuroendothelial cells oscillate over 44 h *in-vitro* synchronized by a pulse of dexamethasone. We then performed transepithelial/transendothelial electrical resistance (TEER) assays to assess barrier tightness of the circadian synchronized neuroendothelial cells. The TEER assay is non-invasive and offers the opportunity to continuously monitor living cells across multiple circadian cycles. When we monitored the integrity and permeability of the *in vitro* barrier between neuronendothelial cells for 4 days, we found that they fluctuated significantly according to time-of-day ($p < 0.001$, one-way ANOVA). We found two daily peaks in impedance ~12-h apart. Conversely, peaks in permeability to fluorescent microbeads and the blood-clotting factor fibrinogen aligned with troughs in impedance. Addition of carbachol, a pan-acetylcholine receptor agonist, shifted these impedance/permeability rhythms as well as rhythms of clock-gene expression. Because the impedance of the BBI is determined by tight junctions between neuroendothelial cells, we

investigated a key tight-junctional protein, Claudin-5. Based on immunofluorescent results, Claudin-5 expression and localization undergo circadian variations over 44 h *in vitro* ($p < 0.001$, one-way ANOVA). Our findings indicate that neuroendothelial cells possess self-sustained, autonomous circadian clocks that regulate the daily functional state of the BBI. These results have significance for periods of vulnerability to stroke and cerebral microbleeds in early morning and evening. Understanding the cell biology and physiology of these oscillations in BBI permeability is critical to diminishing consequences of impaired neurovascular function, including cerebral bleeding and neurodegeneration.

Disclosures: Q.T. Nguyen: None. **A.C. Weiss:** None. **J.O. Storm:** None. **J.W. Mitchell:** None. **H. Kong:** None. **B. Han:** None. **M.U. Gillette:** None.

Poster

PSTR412

Blood Brain Barrier

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.05/K14

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: JSPS KAKENHI Grant Number JP21K16631

Title: Penetration mechanism of albumin from blood to the hippocampal tissue

Authors: *S. HAMASAKI[1], T. MUKUDA[1], K. FUKUDA[2], N. INOUE[1], Y. KOYAMA[1], T. KAIDOH[1];
[1]Tottori Univ., Yonago, Japan; [2]Kitasato Univ., Sagamihara, Japan

Abstract: Blood proteins such as carrier proteins and hormones basically cannot penetrate the brain, including hippocampus (HIP), from systemic circulation, owing to the presence of the blood−brain barrier (BBB). However, pathological state such as ischemia and hypertension increase of BBB permeability. In addition to the pathological state, recently, we found that blood-derived protein albumin (Alb) accesses to hippocampal neurons constitutively in a physiologically normal state. In this study, we examined how circulating Alb enter the HIP in normal rats.To trace serum Alb histologically, fluorescent dye Evens blue (EB) was injected intravenously 15 min before transcardiac perfusion for fixation. EB binds to Alb in a blood immediately after injection and cannot cross the BBB. In the cryosections, the neurons taking up EB were found mainly in the dentate gyrus (DG) of the rostral HIP, suggesting that serum Alb can access to these regions. In RNA-sequencing analysis using the physiologically normal HIP, gene expression of the plasmalemma vesicle associated protein (PLVAP), composing a diaphragm of fenestrae in the vessel, was detected. Some of capillaries in the HIP showed immunoreactivity for PLVAP, suggesting a possibility of presence of the fenestrated capillaries in the HIP. To examine the microstructural basis of the blood vessels in the HIP, the capillaries in the DG of rostral HIP were examined using scanning electron microscope (SEM). As the

positive control, the fenestration (70-80 nm in diameter) of the capillaries was confirmed in the endothelial cells in the circumventricular organs such as subfornical organ and area postrema. In the DG, interestingly, small pits (approximately 70 nm in diameter) were found in the endothelia. Taken together, a possibility of presence of the fenestrated capillaries in the rostral DG is conducted even in the physiologically normal state. Serum Alb may enter to the HIP via these capillaries. These findings are meaningful to investigate novel action of systemic factors including hormones to the HIP, and HIP-related pathogenesis such as temporal lobe epilepsy triggered with Alb leakage.

Disclosures: S. Hamasaki: None. **T. Mukuda:** None. **K. Fukuda:** None. **N. Inoue:** None. **Y. Koyama:** None. **T. Kaidoh:** None.

Poster

PSTR412

Blood Brain Barrier

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.06/K15

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01NS113912

Title: Role of Brain Inflammation on Blood-brain Barrier Iron trafficking

Authors: *K. PALSA, T. HELMUTH, E. NEELY, J. R. CONNOR;
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Abstract: Brain inflammation leads to an increase in the amount of iron in brain tissue, however, studies do not address where the iron is coming from that could lead to the accumulation. Most of the brain iron uptake is mediated through the blood-brain barrier (BBB) but studies have not examined whether inflammation increases or decreases iron flux across the BBB. Herein, we investigated the role of brain inflammation on iron release and iron uptake transporters of the brain microvasculature (BMV). Our recent in vitro study discovered a novel alternate mechanism that iron transport across the BBB is mediated via the extracellular vesicles (EVs). C57BL/6 mice were injected intraperitoneally with ^{57}Fe-Tf or FTH1 in the presence or absence of GW4869, an inhibitor of sphingomyelinase 2 (nSMase2), a key regulatory enzyme generating ceramide that is necessary for EV formation. GW4869 was found to reduce the uptake of ^{57}Fe-Tf and FTH1 into the brain parenchyma compared to control mice. Furthermore, inhibition of EV formation in BMV increased the retention of ^{57}Fe in the brain microvasculature compared to the control group suggesting ^{57}Fe-Tf and FTH1 uptake into the BMV was not interrupted but release into the brain was negatively impacted. This is the first to demonstrate that EVs inhibition decreases brain iron uptake. Furthermore, we induced brain inflammation by intracerebroventricular injection of LPS. The brain inflammation increases the iron release via EVs to the brain parenchyma from BMV, which leads to decreased iron levels in BMV. Brain

inflammation degraded BMV ferroportin, an iron exporter, and released iron via CD63+EVs to the brain. Furthermore, brain inflammation dysregulated the BMV iron homeostasis and induced iron deficiency in BMV, which increased iron uptake. In summary, we discovered a novel mechanism that BMV-released EVs associated iron implied brain iron accumulation during inflammation.

Disclosures: K. Palsa: None. **T. Helmuth:** None. **E. Neely:** None. **J.R. Connor:** None.

Poster

PSTR412

Blood Brain Barrier

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.07/K16

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: The effects of several electronic cigarette additives on blood brain barrier permeability of nicotine using a blood brain barrier biomimetic chip

Authors: *X. LI[1,2], F. YU[1,2], S. HAN[1,2], H. CHEN[1,2], H. HOU[1,2];
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Abstract: When smoking, nicotine inhaled is transported by the bloodstream, crosses the blood-brain barrier (BBB) to certain brain area, induces dopamine production, thereby triggering the rewarding effect. It is unclear whether additives in e-cigarette could affect the ability of nicotine to cross BBB and thus affect the addictiveness of nicotine. Addressing this question requires high-throughput *in vitro* BBB bionic models. To this end, we constructed a biomimetic microfluidic chip to mimic BBB, and used this chip to evaluate the changes in nicotine BBB permeability in the presence of several e-cigarette additives. The chip has 24 identical units, each of which consists of an apical reservoir, a 3D cells chamber with PET membrane and a flow channel at the bottom. Immortalized human brain microvascular endothelial cells (hCMEC/D3) were inoculated in the lower chamber, and then the model was placed on a precision shaker, where the fluid flow rate was controlled by adjusting the tilt angle and swinging speed, thus simulating the *in vivo* fluid shear stress. The BBB model was characterized by measurements of trans-endothelial electrical resistance (TEER), immunofluorescence analysis of tight junction protein ZO-1, and permeation experiments with rhodamine 123 (with or without Verapamil, the inhibitor of p-glycoprotein) as well as several fluoresceins. It was confirmed that the model has barrier function, and the permeation effect was similar to that of BBB *in vivo*. In the nicotine penetration experiment, five common additives in e-cigarettes were selected and mixed with 100 μM nicotine (at the molar ratio of 1:1 for tartaric acid, benzoic acid and lactic acid; 10 μg/mL for menthol and benzaldehyde), and a pure nicotine group without additives was set as control. Each sample was prepared with cell culture medium and injected into cell chamber of the chip. After 2

hours of dynamic culture, the samples were collected for LC/MS analysis. The apparent permeability coefficients of pure nicotine was calculated to be $(2.37 \pm 0.25)\times10^{-6}$ cm/s, while the apparent permeability coefficient of nicotine in the presence of menthol, benzaldehyde, tartaric acid, benzoic acid and lactic acid were $(5.48 \pm 0.44)\times10^{-6}$ cm/s, $(6.40 \pm 1.57)\times10^{-6}$ cm/s, $(4.52 \pm 0.48)\times10^{-6}$ cm/s, $(1.78 \pm 0.19)\times10^{-6}$ cm/s and $(5.31 \pm 0.56)\times10^{-6}$ cm/s, respectively. The results indicated that all these additives except benzoic acid significantly enhance BBB permeability of nicotine. In conclusion, our study provides a high-throughput platform for detecting BBB permeability of candidate substances, and also provides evidence for the effect of additives in e-cigarettes on BBB permeability of nicotine.

Disclosures: X. Li: None. **F. Yu:** None. **S. han:** None. **H. Chen:** None. **H. Hou:** None.

Poster

PSTR412

Blood Brain Barrier

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.08/K17

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Comparative Modeling of 3-dimentional Human Blood Brain Barrier with iPSC-derived and Primary Astrocytes on Microfluidic Chip

Authors: S. JANG[1], K. XU[2], H.-Y. TAN[1], N. KUMARESAN[1], *S. LIM[3], M. L. HENDRICKSON[2], K.-D. CHOI[2];
[1]AIM Biotech, Singapore, Singapore; [2]BrainXell Inc., Madison, WI; [3]AIM Biotech Pte Ltd, Singapore, Singapore

Abstract: The blood-brain barrier (BBB) serves as a critical interface that regulates molecular exchange to neurovascular microenvironment between the circulatory system and the central nervous system (CNS). Developing precise *in vitro* models of the BBB is essential to evaluate the neurovascular interactions and disease mechanisms. The BBB mainly comprises of microvascular endothelial cells, astrocytes and pericytes which together, maintain structural and functional homeostasis. The BBB astrocytes form perivascular complexes and modulate intercellular transmission between neurovascular capillaries and neurons. In recent years, human induced pluripotent stem cell (hiPSC) technology has emerged as a promising tool to mimic diverse somatic counterparts and offers manufacturing advantages in generating target cells with consistency and availability in scale. In this study, we aim to observe the reproducibility of hiPSC-derived astrocytes comparable to human primary astrocytes for human BBB modeling on a 3D-microfluidic chip.First, astrocytes were differentiated from hiPSCs. We have confirmed protein expression of GFAP and S100β in 2-dimentional culture. Subsequently, 3-dimentional BBB cultures were established on microfluidic chips using either human primary astrocytes or iPSC-derived astrocytes. Finally, we examined the perfusability and permeability of BBB with

70 kDa Dextran.Preliminary findings indicate that hiPSC-derived astrocytes demonstrate comparable performance to primary human astrocytes in supporting the BBB integrity and function within the *in-vitro* model. Permeability assay revealed no significant differences between primary astrocyte models and hiPSC-derived astrocytes counterpart model in their ability to restrict the passage of small molecular tracers. Additionally, we validated the expression of crucial markers in astrocytes, GFAP and S100β, and the expression of the surface marker CD31 in endothelial cells within both isogenic and primary astrocyte-based BBB models on a chip.Our study underscores the potential of hiPSC-derived astrocytes as an alternative source to primary astrocytes in constructing physiologically relevant *in-vitro* BBB models. Leveraging hiPSC technology offers opportunities for standardized and scalable production of astrocytes, facilitating broader accessibility and reproducibility in neurovascular research. Our future studies will focus on modeling human BBB entirely with hiPSC derivatives as well as to elucidate disease-specific BBB alterations from patients iPSCs. These efforts would further advance our understanding of neurovascular disorders.

Disclosures: S. Jang: A. Employment/Salary (full or part-time):; AIM Biotech. **K. Xu:** A. Employment/Salary (full or part-time):; BrainXell Inc. **H. Tan:** A. Employment/Salary (full or part-time):; AIM Biotech. **N. Kumaresan:** A. Employment/Salary (full or part-time):; AIM Biotech. **S. Lim:** A. Employment/Salary (full or part-time):; AIM Biotech. **M.L. Hendrickson:** A. Employment/Salary (full or part-time):; BrainXell Inc. **K. Choi:** A. Employment/Salary (full or part-time):; BrainXell Inc..

Poster

PSTR412

Blood Brain Barrier

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.09/K18

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: RF1AG060754

Title: Exploration of Deep Brain Stimulation effects on Neurotrophic Markers in Cerebrospinal Fluid in Rhesus Macaque monkeys using an Ommaya

Authors: *K. R. PENNINGTON[1], D. T. BLAKE[2];
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Abstract: Neurotrophic biomarkers such as tissue-type plasminogen activator (tPA), plasminogen activator inhibitor-1 (PAI-1), and brain-derived neurotrophic factor (BDNF) have been identified as key players in the mechanisms of a multitude of disorders, including depression, dementia, post-traumatic stress disorder (PTSD), and many others. These are challenging to study in animal models, as they exist in cerebrospinal fluid (CSF) in pg/mL levels.

Many common laboratory animals have too little CSF to create a sample for common assays like an enzyme-linked immunosorbent assay (ELISA) test. We developed a method that allows for increased volume of CSF collection by using the nonhuman primate model and collecting CSF through an Ommaya, or ventricular drain and reservoir. The Ommaya is placed into the base of one lateral ventricle in the nonhuman primates, and may safely sample 0.25 cc (250 μL) of CSF each 30 minutes. We used this sampling to assess whether deep brain stimulation of the nucleus basalis of Meynert leads to increases in CSF levels of tPA. Stimulation was intermittent at 60 pulses per second for 20 seconds on, 40 seconds off, for one hour. Pulses were monopolar, biphasic, cathodal first, 100 μS per phase at an amplitude of 0.5 mA, and were delivered for 20 seconds in each minute. In three aged macaque monkeys, we found tPA levels of 35-90 pg/mL at baseline, which increased 2-3 fold one hour after stimulation began. Future plans include assessing other potential neurotrophic biomarkers that also exist at similar levels in CSF.

Disclosures: K.R. Pennington: None. **D.T. Blake:** None.

Poster

PSTR412

Blood Brain Barrier

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.10/K19

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH grant R21EB030173
NIH grant R01DA050159

Title: Mesoscopic imaging of neurovascular coupling in awake mice after single and repeated focused ultrasound-mediated blood-brain barrier opening

Authors: *N. FOMIN-THUNEMANN[1], E. A. MARTIN[1], P. NOWLIN[2], K. KILIÇ[1], D. BALOG[1], D. BOGATOVA[1], P. DORAN[1], J. JIANG[1], C. ANGOLANO[3], A. DEVOR[1,4], M. THUNEMANN[1], N. TODD[2];
[1]Biomed. Engin., Boston Univ., BOSTON, MA; [2]Dept. of Radiology, Brigham and Women's Hosp., Boston, MA; [3]Div. of Vascular and Endovascular Surgery, Ctr. for Vascular Biol. Research, Dept. of Surgery, Beth Israel Deaconess Med. Ctr., Boston, MA; [4]Harvard Med. Sch., Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Focused ultrasound (FUS) in conjunction with ultrasound-responsive particles, or ‘microbubbles’, is an emerging technology for non-invasive and controlled blood-brain-barrier (BBB) opening to deliver therapeutics into brain parenchyma that otherwise would not pass the BBB. Using conservative protocols, FUS-BBB opening is today being performed in clinical trials, safely and successfully, for treatment of several neurological diseases. However, there are safety concerns for repeated BBB opening that need to be addressed. These concerns include inflammatory immune responses and changes in vascular and neuronal activity. Studies

investigating secondary effects on neuronal and vascular activity have primarily been performed in slices or anesthetized animals. To investigate effects of multiple FUS treatments on neurovascular coupling in awake mice, we implanted a cranial glass window over the brain in seven mice expressing the calcium indicator jRGECO1a. From these seven mice, four received FUS-BBB opening through the window, with parameters used to induce BBB opening but no hemorrhage, and three were controls that underwent the same procedures but did not receive a FUS pulse. Using mesoscopic wide-field optical microscopy, we imaged neuronal and hemodynamic activity across the dorsal brain surface before and after single as well as repeated (once a week for 5 weeks) FUS treatments targeting the somatosensory barrel-field. We analyzed resting state (no stimulus given) as well as sensory-evoked activity in the barrel field. BBB opening was verified through leakage of fluorescent FITC-albumin from the vessels, measured with two-photon-microscopy. Our results show that resting state activity did not change after FUS treatments. A cross-correlation analysis displayed no altered relationship between calcium and hemodynamics 3 and 24 hours after the first and subsequent FUS treatments. Furthermore, the amplitude of neuronal and hemodynamic sensory-evoked responses did not change significantly after one or multiple FUS treatments. We observed a trend for stronger post-stimulus undershoots 3 hours after FUS treatment; however, the difference to control mice did not reach significant levels. Immunohistochemistry of extracted brains after 5 weeks of treatment did not show significant increases in pro-inflammatory markers compared to control mice. Overall, these results demonstrate that repeated FUS-treatments at strengths causing BBB opening but no hemorrhage do not cause severe long-lasting effects on neurovascular coupling.

Disclosures: N. Fomin-Thunemann: None. **E.A. Martin:** None. **P. Nowlin:** None. **K. Kiliç:** None. **D. Balog:** None. **D. Bogatova:** None. **P. Doran:** None. **J. Jiang:** None. **C. Angolano:** None. **A. Devor:** None. **M. Thunemann:** None. **N. Todd:** None.

Poster

PSTR412

Blood Brain Barrier

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.11/K20

Topic: H.08. Learning and Memory

Title: The cellular mechanism of brain endothelial cell in bile duct ligation mouse model

Authors: *S. AHN[1], S. CHOI[2], D. JO[3], J. SONG[4];
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Abstract: Hepatic encephalopathy (HE) is a disease accompanied by hyperammonemia and is known to cause cognitive impairment and emotional changes. In present study, we investigate the mechanisms of blood brain barrier (BBB) disruption in the HE mouse model and identify metabolic control ability change and intracellular signal regulation in astrocyte.HE mouse model was created by bile duct ligation (BDL) surgery. After BDL surgery, all mice were sacrificed after 2 weeks. When the brain tissue was taken out in all mice, BBB disruption was confirmed by immunostaining using Claudin 5, Occludin, and MMP-9 markers, a tight junction protein as a BBB marker. Also, we determined tight junction protein loss and permeability signaling pathway using Western blot analysis in BDL mouse. In addition, RT PCR was performed to confirm mRNA changes in signals related to BBB disruption. Brain cortices obtained from BDL mouse were analyzed by RNA sequences, and after that KEGG and GO pathway were analyzed to find the related signal pathways. Furthermore, related intracellular signaling changes were confirmed by various analysis. We discovered statistically significant genes in BDL mice, and cultured astrocytes under high ammonia condition, and studied gene regulation relationships using siRNA. Here, we aim to identify cell signals involved in the prevention and treatment for BBB disruption seen in HE patients.

Disclosures: S. Ahn: None. **S. Choi:** None. **D. Jo:** None. **J. Song:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.01/K21

Topic: F.07. Biological Rhythms and Sleep

Support: Female Academic Leadership Fellowship, the Wits Chancellor Fellowship Award

Title: Male blue wildebeest significantly increase activity levels during the breeding season (rut), but not at the expense of the time spent at rest

Authors: *I. B. MALUNGO;
Sch. of Anatom. Sci., Univ. of the Witwatersrand, Johannesburg, South Africa

Abstract: Rest may be viewed as a state of adaptive inactivity that increases the efficiency of activity by regulating its timing and reducing energy use when activity is not beneficial. This would mean that animals can go without rest when specific demands, such as mating, favour being awake. Sexually active male blue wildebeest (bulls) are typically territorial and it has been reported that when a wildebeest bull is protecting a harem in his territory during the mating season or rut, he neither eats nor rests. We examined the daily activity and inactivity patterns of dominant male blue wildebeest by means of actigraphy for a period of three months, which included the time of the rut. In addition, we measured faecal androgen metabolite (fAM) levels

and subcutaneous temperature, both of which have variances known to relate to the mating season in other mammalian species. During the rut, the male wildebeest experienced higher levels of activity, fAM, and a greater range of body temperatures. Despite previous qualitative reports, the male blue wildebeest did rest daily during the rut, and while the amount of rest was low, it did not appear to be substantially lower than in the period prior to the rut. The amount of time spent inactive increased substantially and significantly after the rut. The timing of daily activity and inactivity patterns did not vary substantially with the rut compared to the pre-rut and post-rut periods. Throughout the recording period, the average daily ambient temperatures decreased steadily, along with the change of season, and the subcutaneous body temperature followed this pattern, although it was not as marked. It appears that in the post-rut period a substantive increase in rest occurs, potentially allowing the male wildebeest time to recover following a period of intense activity.

Disclosures: I.B. Malungo: None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.02/K22

Topic: F.07. Biological Rhythms and Sleep

Title: Accurate Age Prediction From Sleep and EEG Using Transformer Models

Authors: *M. OGG, W. COON;
Johns Hopkins Univ. Applied Physics Lab., Laurel, MD

Abstract: Age prediction, specifically comparing one’s apparent biological age to their chronological age, is a promising biomarker for health and well-being. Using measures of the brain as input for age prediction is especially accurate and relates to numerous physical, mental and cognitive health outcomes. However, most of this work has focused on MRI-based measures which are expensive, difficult to acquire and contraindicated for many individuals. A more accessible method for quantifying the brain’s biological age, potentially even in the home or clinic, would be of great value both for clinical practitioners and for researchers. In this work we examine polysomnographic (PSG) sleep signals for age prediction. Sleep is an excellent platform for age prediction because PSG data are standardized and many public resources exist (thus, providing a large training corpus), age imparts known changes to the macro- architecture and micro-architecture of sleep, and the increase in sleep assessment tools on the consumer market means these could transition fairly quickly to widespread use. We trained a transformer-based neural network model to predict the age of individuals based on standard PSG signals involving a large training corpus (over ten thousand nights of sleep) as well as rigorous internal and external validation for our best models. Many of our models were able to predict age within 5-10

years absolute error, however accurate generalization beyond the training corpus still presents a sizable challenge. Electroencephalography (EEG) signals were critical for supporting accurate age prediction because these convey characteristic changes in sleep architecture that occur throughout the lifespan. Overall, we find that certain combinations of signals can rival MRI-based measures for age prediction at an extremely low cost, opening the door for further study that might include denser time sampling or testing in an at-home environment using consumer products.

Disclosures: M. Ogg: None. **W. Coon:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.03/K23

Topic: F.07. Biological Rhythms and Sleep

Support: NRF of Korea Grant 2018R1A6A1A03025221

Title: Lack of sleep in adolescent mice suppresses hippocampal astrocytes, resulting in impaired long-term memory until early adulthood

Authors: *J.-Y. KANG;
Dept. of Korea Med., Daejeon Univ., Seo-gu, Daejeon, Korea, Republic of

Abstract: Sleep deficiency is a rampant issue in modern society, serving as a pathogenic element contributing to learning and memory impairment, with heightened sensitivity observed in children. Clinical observations suggest that learning disabilities associated with insufficient sleep during adolescence can persist through adulthood, but experimental evidence for this is lacking. In this study, we examined the impact of early-life sleep deprivation on both short-term and long-term memory, tracking the effects sequentially into adulthood. We employed a modified multiple platform method (MMPM) mouse model to investigate these outcomes. Sleep deprivation induced over a 14-day period, beginning on postnatal day 28 (PND28) in mice, led to significant impairment in long-term memory (while short-term memory remained unaffected) at PND42. Notably, this dysfunction persisted into adulthood at PND85. The specific impairment observed in long-term memory was elucidated through histopathological alterations in hippocampal neurogenesis, as evidenced by bromodeoxyuridine (BrdU) signals, observed both at PND42 and PND85. Furthermore, the hippocampal region exhibited significantly diminished astrocyte activity, characterized by lowered levels of aquaporin 4 (AQP4), a representative molecule involved in brain clearance processes, and reduced protein expressions of brain-derived neurotrophic factor (BDNF). In conclusion, we have presented experimental evidence indicating that sleep deficiency-related impairment of long-term memory in adolescence can endure into

adulthood. The corresponding mechanisms involve alterations in hippocampal neurogenesis derived from astrocyte.

Disclosures: J. Kang: None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.04/K24

Topic: F.07. Biological Rhythms and Sleep

Support: NSF/BMBF grant 01GQ1706

Title: Cortical excitability modulates efficacy of closed-loop acoustic stimulation

Authors: *T. HOEFER[1], T. HÖSEL[1], M. MÖLLE[2], L. MARSHALL[2,3];
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Abstract: The first two authors contributed equally.
Closed-loop acoustic stimulation (CLAS) reliably enhances endogenous sleep slow oscillations (SO) but has an inconsistent effect on memory formation [1]. Transcranial direct current stimulation (tDCS) is associated with a modulation in cortical excitability. To investigate whether a different level of cortical excitability during sleep can impact CLAS efficacy on electrophysiological and behavioral responses, we combined these two non-invasive stimulation techniques in a within-subject single blinded, pseudo-randomized study on healthy humans (N=23; 17 female; 18-30 years, 21.3±2.5, mean±SD). Each participant completed two sessions of nocturnal sleep undergoing either CLAS or a combined CLAS and cathodal tDCS stimulation over the frontal cortex (CmodCLAS). Stimulations were applied during slow wave sleep. Sleep-associated memory consolidation was measured using a non-sense word paired-associate task (NSWP) and a figural paired-associate task (FPA). Post-sleep encoding was assessed using a word paired-associate task (WPA). CmodCLAS did not affect behavioral performance overall for NSWP or WPA. However, for FPA more word pairs were remembered overnight in CmodCLAS as compared to CLAS alone ($t(22)=-2.21$; $p=0.038$; N=23; student's t test). In terms of electropyhsiological data, we observed an interaction for Stimulation Condition X Electrode Topography for count, density and length of SO ($F>2.69$, $p<0.040$, rmANOVA). SO length was longer over frontal ($t(22)<-3.03$; $p<0.006$) and shorter over occipital ($t(22)>2.27$; $p<0.033$) regions. Count and density did not differ significantly at any single location. CmodCLAS furthermore increased both SO-slow spindle and SO-fast spindle temporal coupling compared to CLAS at Cz ($t(22)<-2.08$; $p=0.049$), with trends found at other electrodes ($t(22)<-1.735$; $p<0.097$). In summary, the ability of cathodal tDCS to influence the efficacy of CLAS suggests

that individual differences in cortical excitability can at least partly account for varying findings reported regarding the efficacy of CLAS. The advantage of CmodCLAS to improve sleep-associated memory consolidation requires further investigation. 1. Ngo H, Martinetz T, Born J, Mölle M (2013). Neuron 78: 545-553; Henin S, Borges H, Shankar A, et al, (2019). eNeuro 6(6): ENEURO.0306-19.2019

Disclosures: T. Hoefer: None. **T. Hösel:** None. **M. Mölle:** None. **L. Marshall:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.05/K25

Topic: F.07. Biological Rhythms and Sleep

Title: Samelisant (SUVN-G3031): Safety and Efficacy Outcome from the Phase-2 Proof-of-Concept, Double-Blind, Placebo-Controlled Study in Patients with Narcolepsy

Authors: V. GOYAL, V. BENADE, R. NIROGI, J. RAVULA, P. JAYARAJAN, S. JETTA, *A. SHINDE, V. JASTI;
Suven Life Sci. Ltd, Hyderabad, India

Abstract: Samelisant (SUVN-G3031) is a potent and selective histamine 3 receptor inverse agonist. In orexin knockout mice, samelisant produced wake-promoting and anticataplectic effects suggesting its potential therapeutic utility in the treatment of narcolepsy. Safety and tolerability studies in animals and healthy human volunteers suggested a favorable risk/benefit profile for samelisant. Samelisant was evaluated in a Phase-2 proof of concept study as a monotherapy in a double-blind randomized controlled trial for the treatment of excessive daytime sleepiness (EDS) in narcolepsy patients with and without cataplexy (NCT04072380). The study recruited subjects from about 60 sites across the USA and Canada. Subjects diagnosed with narcolepsy as per ICSD-3 criteria, aged between 18 to 65 years with an Epworth Sleepiness Scale (ESS) score of ≥12 and mean Maintenance of Wakefulness Test (MWT) time of <12 min are considered eligible for the study. A total of 190 subjects were randomized into one of three treatment arms (samelisant 2 mg, samelisant 4 mg and Placebo) in 1:1:1 ratio. Each subject received either placebo or samelisant once daily for 2 weeks. The primary efficacy endpoint was a change in ESS score from baseline to Day 14. Secondary endpoints were changes in MWT, Clinical Global Impression - Severity (CGI-S), Patient Global Impression of Change (PGI-C), and Clinical Global Impression of Change (CGI-C) scores from baseline to Day 14. Safety was monitored throughout the study by the medical monitor and by the data safety monitoring committee. Baseline characteristics from the study indicate that the median age of subjects was 32 years (range: 18-58 years) with mean BMI of 28.8 kg/m^2 (range: 18.3- 43.9 kg/m^2). Overall, 53% subjects were of narcolepsy type-1, 71% were female and 68% were Caucasian. Mean (SD)

baseline values of MWT and ESS scores are 6.0 (4.3) and 17.23 (2.8), respectively. Baseline characteristics from the study are consistent with the general narcolepsy population. Based on the analysis, the study met the primary endpoint and samelisant demonstrated a statistically significant and clinically meaningful 2.1-point reduction in EDS measured by ESS total score compared to placebo at Day 14 ($p<0.024$). Improvement based on the primary endpoint was supported by a statistically significant improvement on the secondary endpoints like CGI-S, PGI-C, and CGI-C. Samelisant was safe and well tolerated in the tested population. Samelisant could be a potential new therapy for the management of narcolepsy. Suven is planning end-of-phase-2 meeting with the FDA and a Phase-3 study for the treatment of EDS in narcolepsy is being planned.

Disclosures: V. Goyal: A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **V. Benade:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Nirogi:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **J. Ravula:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **P. Jayarajan:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **S. Jetta:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **A. Shinde:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **V. Jasti:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.06/K26

Topic: F.07. Biological Rhythms and Sleep

Support: NIH 2R15GM125073-03
NSF IOS 2042873

Title: Identification and characterization of neurons within the central complex that regulate sleep in a sex specific manner

Authors: P. SUNDARAMURTHI[1], M. REYES[2], ***D. SITARAMAN**[3];
[1]California State Univ. - East Bay, Hayward, CA; [2]Psychology, CSU- East Bay, Hayward, CA; [3]California State Univ. East Bay, hayward, CA

Abstract: Sleep is a fundamental behavioral state that is important for survival of many animal species. Distinct populations of neurons have been identified in multiple organisms such as mice, worms, and flies that either increase (sleep-promoting) or decrease (wake-promoting) the animals' propensity to sleep. Interactions between these neurons is critical for the sleep-wake behavior, but the precise mechanisms of communication between these identified sleep-regulating neurons is poorly understood. The fruit fly Drosophila melanogaster has emerged as

an excellent model to study the genetic, neuronal and circuit basis of sleep regulation. Previous research shows that a central region of the fly brain called the Mushroom body and Central Complex (CX) plays a key role in integrating environmental sensory information with past experiences and the insect's internal states to influence sleep and arousal. To identify the role of Central Complex in the context of sleep regulation, we conducted an unbiased screen where individual cell types within CX were activated using temperature-sensitive neuronal regulators, and sleep and wakefulness were measured using activity monitoring. Among the 85 cell types screened we found 12 classes of neurons that either increase sleep (sleep promoting) or increase wakefulness (wake promoting). One of these classes of neurons are called PFGs and were found to induce sleep when activated in female but not male flies. Neuronal silencing and gene specific knockdown of neurochemicals and connectomic analysis points to several upstream and downstream neuronal pathways by which PFGs regulate sleep. In addition to sleep, we are also investigating how environmental factors like food impact PFGs and the CX network. We will present these data and hypotheses related to how these neurons regulate sleep and arousal within the CX network.

Disclosures: P. Sundaramurthi: None. **M. Reyes:** None. **D. Sitaraman:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.07/K27

Topic: F.07. Biological Rhythms and Sleep

Support: UAM-I 14407025

Title: Blockade of hippocampal M_1 muscarinic Acetylcholine receptors impairs memory in rats with chronic REM sleep restriction

Authors: R. MARTINEZ-GONZALEZ, ***A. JIMENEZ-ANGUIANO**;
UAM-Iztapalapa, Mexico City, Mexico

Abstract: Sleep-wake cycle is involved in several neurophysiological processes, such as learning and memory. Sleep and memory are regulated by Acetylcholine (ACh), in particular by muscarinic Acetylcholine receptors (mAChR). However, it is still unknown the selective participation of M_1 mAChR during the Chronic REM Sleep Restriction (CREMSR). Therefore the objective of this study was to evaluate the effect of M_1 mAChR in the chronic REM sleep restriction (CREMSR) on episodic memory, as well as during the recovery period of CREMSR with the Novel Object Recognition test (NOR test). 40 male Wistar rats (230-250 g) were anesthetized and implanted with a cannula in the dorsal hippocampus (P=3.8, L=2.2, V=3.2). After one week the animals were randomly placed in the following groups (n=10): 1. Control, 2. CREMSR x 21 days, 3. CREMSR x 21 days + antagonist M_1 mAChR-Pirenzepine (PIRENZ-

0.35 ug, in 0.2 ul of saline). 4. CREMSR x 21 days + PIRENZ-0.7 ug, in 0.2 ul of saline). Animals were CREMSR x 18 h during 21 days using the multi-platform technique. At the beginning of the experiment, at 11 days and 21 days of the CREMSR, and after 21 days during the recovery period the animals were evaluated using the NOR test. The results obtained showed that sleep deficit and PIRENZ produced a decrease in the execution of NOR test. The CREMSR x 21 days was the condition that caused the greatest alterations affecting performance in the NOR test. From these results we suggest that differential sleep loss and the blocking of M_1 mAChR have an impact in the correct learning and produces degradation in declarative memory.

Disclosures: R. Martinez-Gonzalez: None. **A. Jimenez-Anguiano:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.08/K28

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant R01DA056193

Title: Sleep disturbances are associated with mesolimbic dopamine dysfunction and cue-induced drug seeking during cocaine abstinence

Authors: *S. R. COHEN[1], I. P. ALONSO[2], V. M. MIGOVICH[1], R. A. ESPAÑA[3]; [1]Neurobio. & Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Neurobio. & Anat., Drexel Univ., Philadelphia, PA; [3]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Individuals who struggle with cocaine use disorder display sleep dysregulation which is often marked by persistent reductions in rapid eye movement (REM) sleep and increased REM sleep fragmentation that persist throughout abstinence. We recently demonstrated that intermittent access (IntA) to cocaine, but not short access (ShA) to cocaine increases dopamine uptake rate and dopamine transporter (DAT) sensitivity to cocaine following a period of abstinence. Further, we and others have shown that mesolimbic dopamine transmission fluctuates across sleep/wake behavior, and that sleep/wake state influences the effects of cocaine. In these studies, we examined to what extent sleep impairments observed during abstinence from IntA to cocaine contribute to increased cue-induced cocaine seeking and changes in dopamine transmission. We monitored sleep/wake behavior prior to and following IntA or ShA cocaine self-administration throughout a 7- or 28-day abstinence period to examine sleep disruptions using EEG/EMG recordings and dopamine adaptations using ex vivo FSCV. To determine if restoring sleep following IntA to cocaine is sufficient to reduce cocaine seeking and aberrant dopamine adaptations, we implemented a behavioral sleep restoration procedure to normalize sleep architecture. During sleep restoration periods, rats were kept awake in their active period (dark phase) to consolidate sleep during their inactive period (light phase), thereby improving

sleep quality. Cue-induced cocaine seeking was measured on the first and last day of abstinence, and dopamine transmission was measured 24 hours after the final seeking test. Preliminary results suggest disrupted sleep architecture following IntA, but not ShA, and is accompanied by aberrant increases in both cocaine seeking and dopamine transmission. Further, sleep restoration prevented these aberrant cocaine-associated effects. Together, these preliminary findings suggest that manipulation of sleep may serve as a novel therapeutic to prevent alterations in dopamine transmission that contribute to drug seeking.

Disclosures: S.R. Cohen: None. **I.P. Alonso:** None. **V.M. Migovich:** None. **R.A. España:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.09/K29

Topic: F.07. Biological Rhythms and Sleep

Support: from VIEP-BUAP 2022-2023 to CA in Neuroendocrinología (BUAP-CA-288)
CONACYT No. 926368. to AFR

Title: Effects of posttraumatic stress disorder on sleep-wake cycle at two critical developmental stages in two yawning sublines that differs in their anxiety response.

Authors: *A. FIERRO-ROJAS[1], C. CORTES[2], J. R. EGUIBAR, Sr.[3];
[1]Inst. of Physiol., Benemérita Univ. Autónoma De Puebla, Puebla, Mexico; [2]Inst. of Physiol., B. Univ. Autonoma de Puebla, Puebla, Mexico; [3]Behavioral Neurophysiol., Benemerita Univ. Autonoma De Puebla, Puebla, Mexico

Abstract: Posttraumatic stress disorder (PTSD) can be modeled in rats when there is an association between one neutral conditioned stimulus (CS) and one aversive unconditioned stimulus (US), inducing a freezing response. Subjects that are more susceptible to anxiety-like behaviors exhibit higher freezing behavior on subsequent re-exposures and might develop disruptions on their sleep-wake cycle due to PTSD. We have selectively bred two sublines from Sprague-Dawley rats that differs in their spontaneous yawning frequency. The high-yawning (HY) rats have a mean of 20 yawns/h, whereas low-yawning (LY) rats have just 2 yawns/h. HY rats have shown resilient behavior and LY rats have shown an anxious trait when evaluated in different anxiogenic tests. The aim of this study was to assess the effect of a fear conditioning protocol on sleep-wake cycle in HY and LY rats. We used six male rats of each HY and LY at 35-40 and 55-60 days of age for early and late adolescence groups, respectively. Subjects were maintained in standard conditions with free access to purified water and food pellets. All rats were exposed to a single training session of 25 foot shocks of 0.5 mA (US). Shocks were

delivered on the last two seconds of a 10 second exposure to light and sound cues with an interval of one minute. At 90 days of age, a context and cue with different context re-exposition happened on separate days. Then, all subjects were implanted for electroencephalographic (EEG), electromyographic (EMG) and electrooculographic (EOG) recordings to characterize the sleep-wake stages. LY rats of early adolescence presented higher freezing response during cue re-exposure with respect to HY rats (P=0.05). However, HY and LY rats of late adolescence group did not differ on their freezing response on the following re-exposures. Additionally, LY rats showed a significant reduction in the total sleep time with respect to HY rats of both age groups (P=0.05). In conclusion, HY and LY rats are a model of resilience and anxiety respectively. LY rats is an anxiety model due to their different responses and are an adequate model for studying the behavioral mechanisms of PTSD.

Disclosures: A. Fierro-Rojas: None. **C. Cortes:** None. **J.R. Eguibar:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.10/K30

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant R01AG078134 to G.V. and G.A.M.
Department of Anesthesiology, University of Michigan Medical School, Ann Arbor, USA.

Title: Sleep Disruption in a Mouse Model of Surgery Differs as a Function of Sex and Genotype

Authors: *A. JADIDIAN, V. S. HAMBRECHT-WIEDBUSCH, L. FLORAN-GARDUNO, H. FAYAD, G. A. MASHOUR, G. VANINI;
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Abstract: Despite the high incidence of perioperative sleep disturbances and ample clinical evidence that links sleep disruption with poorer patient outcomes, the neural mechanisms by which surgery impairs postoperative sleep remain unknown. The preoptic area of the hypothalamus is a critical node in the brain network regulating sleep onset and sleep homeostasis. To date, no study has investigated whether preoptic GABAergic and glutamatergic neurons that promote sleep and wakefulness play a causal role in postoperative sleep disruption. The present study uses the skin/muscle incision and retraction model (a rodent model of surgery) to characterize the effect of surgery on postoperative sleep-wake architecture in Vglut2-Cre (Slc17a6$^{tm2(cre)Low/}$J) and Vgat-Cre (Slc32a1$^{tm2(cre)Lowl/}$J) mice without chemogenetic intervention. Mice were implanted with electrodes to record the electroencephalogram and electromyogram. After recovery and habituation, mice underwent a 24-hour baseline sleep recording, surgery (including postoperative analgesia for 24 hours with meloxicam), and a second 24-hour sleep

recording that started immediately after the recovery from surgery. Total time spent in wakefulness, quiet wakefulness (wake with no movements), non-rapid eye movement (NREM) sleep, rapid eye movement (REM) sleep, and the frequency of brief arousals from NREM sleep were analyzed over the first 3 hours in each of the two recording periods. In Vglut2-cre mice (n=13), surgery reduced the time in REM sleep (mean % of recording time ± SEM: 4.59 ± 0.64 versus 1.23 ± 0.28, p=0.0003), and increased brief arousals (% time: 3.42 ± 0.56 versus 5.04 ± 0.74, p=0.03) and the time in quiet wakefulness (4.37 ± 0.82 versus 10.02 ± 2.52, p=0.02). REM sleep was reduced in female (n=8, p=0.004) but not in male mice (n=5, p=0.07). In Vgat-cre mice (n=16), surgery reduced the total time spent in wakefulness (82.19 ± 5.10 versus 67.98 ± 5.17, p=0.002), increased brief arousals (% time: 1.84 ± 0.61 versus 4.89 ± 0.92, p<0.0001) and the time in NREM sleep (14.65 ± 4.22 versus 26.13 ± 4.15, p=0.003). Changes in wakefulness, brief arousals, and NREM sleep were observed in male (n=11; p=0.0002, 0.0003 and 0.0001) but not female (n=5; p=0.83, 0.09, and 0.99) mice. Our preliminary results suggest that, in this model, the impact of surgery on postoperative sleep appears to differ as a function of genotype and sex. Ongoing experiments are increasing the sample size and will use chemogenetics to stimulate preoptic GABAergic (sleep-promoting) and inhibit glutamatergic (wake-promoting) neurons to assess whether this can mitigate the negative impact of surgery on sleep-wake architecture.

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Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.11/K31

Topic: F.07. Biological Rhythms and Sleep

Title: Camus: Optimizing Mouse Home Cage Behavior Monitoring using Video

Authors: *T. MEHTA[1], N. ANGULO[2], S. STATEN-LUSTY[2], A.-Z. LU[2], R. PALKAR[2], V. IM[2], E. Y. KIMCHI[2];
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Abstract: Mice have rich home cage and circadian behavior that is relevant to neuropsychiatric diseases, suggesting the importance of continuous home cage monitoring. However, lab mice are typically housed in compact home cages with opaque lids, making video monitoring challenging. We therefore developed Camus: A camera based open-source system for home-cage recordings of Mus musculus mice, to enable continuous, cost-effective monitoring within standard lab cages. Our goal with Camus was to develop an open-source system that could be used to

measure 24 hour locomotor activity and circadian patterns, feeding and drinking behavior, and nesting behavior, within standard lab animal housing. We developed this system by integrating commercial off-the-shelf hardware such as a USB camera with 3D printed designs to optimize infrared illumination and unobstructed food and water sources. All design files have been released as modifiable, open source files, along with Python scripts to facilitate data collection and analysis and a downloadable computer vision model (DeepLabCut). We additionally tested whether mouse weight and nest building were similar in Camus compared to standard lab housing.The Camus system could record independently from up to 20 home cages using a single desktop computer, at an incremental cost of $300/cage. Using a resolution of 640x360 pixels and 30 frames per second, we captured over 99% of potential frames. Automated analysis revealed anticipated circadian activity, with increased activity in dark periods and decreased activity in light periods. Mice were also more likely to eat and drink during dark periods. Weights and nest building were validated as similar to mice in standard home cages. Detailed analysis revealed the dynamics of nest building.Camus is a cost-effective, open source system to record home cage activity continuously from mice in standard laboratory housing. We have released instructions and documentation for the system. The system can potentially be modified as needed for other lab cage sizes or extended to other behaviors which may benefit from continuous and/or parallel monitoring of independent subjects. Given the ubiquity of lab mice, efficient monitoring of continuous home cage behavior may facilitate research into various neuropsychiatric diseases

Disclosures: T. Mehta: A. Employment/Salary (full or part-time):; Northwestern University. **N. Angulo:** None. **S. Staten-Lusty:** None. **A. Lu:** None. **R. Palkar:** None. **V. Im:** None. **E.Y. Kimchi:** A. Employment/Salary (full or part-time):; Northwestern University.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

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Topic: F.07. Biological Rhythms and Sleep

Support: Alzheimer's Association AARG-NTF-21-852299
NIH R35 GM140854-01

Title: Implication of Chronotherapy in mouse models of Alzheimer’s Disease

Authors: *Y. SHI[1], W. MCROBERTS[1], S. ROZEN[1], J. SWINT[1], G. S. BLOOM[2], A. D. GULER[1];
[1]Univ. of Virginia, Charlottesville, VA; [2]Dept. of Biol., Univ. of Virginia, Charlottesville, VA

Abstract: Sleep disturbances are commonly observed in early-stage Alzheimer's Disease (AD) patients and correlate with their cognitive performance over time. Sleep in mammals is governed by the central circadian system, which responds to environmental cues like light and aligns with

daily routines such as eating. In this study, we developed a regimen with a shortened light phase, time-restricted feeding, and exercise (LiFE) to strengthen the circadian system and enhance sleep quality in mice. When applied to 5xFAD and 5xFAD;PS19 AD, this approach enhanced cognitive performance in Morris water maze and novel object recognition tests. The LiFE treatment did not reduce amyloid plaques or tau phosphorylation. Our study shows that, independent of AD histopathological changes, LiFE chronotherapy enhances cognition, with the potential to improve the care for AD patients. We are currently exploring the neural mechanisms and biological processes impacted by the LiFE treatment.

Disclosures: Y. Shi: None. **W. McRoberts:** None. **S. Rozen:** None. **J. Swint:** None. **G.S. Bloom:** None. **A.D. Guler:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

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Topic: F.07. Biological Rhythms and Sleep

Support: SHAPE (Spectrum Health Academy of Professional Educators) grant, Corewell Health Grand Rapids
CHM (College of Human Medicine) Research Enhancement, Michigan State University

Title: Seasonal affective disorder, stress and burnout in teaching faculty, residents, and fellows

Authors: *A. C. MOODY[1], N. HABIB[2], H. M. HOFFMANN[3];
[1]Michigan State Univ. Col. of Human Med., Grand Rapids, MI; [2]Michigan State Univ. Col. of Human Med., Orchard Lake, MI; [3]Animal Sci., Michigan State Univ., East Lansing, MI

Abstract: The field of healthcare has a long history of difficult work hours and sleep schedules. Teaching faculty, residents, and fellows work 12-hour days, 12-hour nights, and/or 24-hour shifts. The constant change in sleep schedules leads to frequent sleep-wake cycle disturbances. This disruption is exacerbated in the winter due to limited sunlight. Because of these long workdays, burnout is very common. Burnout is self-reported symptoms of emotional exhaustion, depersonalization, and low sense of personal accomplishments. Physician burnout is linked to decreased patient safety and care quality. Few studies have looked at the role of seasonal affective disorder (SAD) on burnout. SAD is a seasonal pattern of recurrent episodes of depression that occurs in the fall and winter. The change in sunlight causes a disruption in circadian rhythm. Irregular rhythms can lead to declines in mental and physical health, with many studies showing difficulty with memory consolidation, body healing, and metabolic regulation. The purpose of our study is to assess the incidence of seasonal affective disorder and its effects on stress and burnout in residents, teaching faculty, and fellows. The general design of

the study includes administering an anonymous, longitudinal survey sent to multiple teaching hospitals. Participants will answer questions about their sleep habits, work hours, and a PHQ-9 (patient health questionnaire). The survey will be sent twice; the first was already sent in the winter and the second will be sent this summer. The first survey received 448 responses from 15 different states. The second survey is scheduled to be sent out this summer. Preliminary data showed that 19% of participants reported a formal diagnosis of depression. 2.2% reported a formal diagnosis of SAD and 20% reported a self-diagnosis. Once the second survey is complete, the PHQ-9 scores can be compared to determine depression status in the different seasons. Because this study is longitudinal, the sequence of events can be established, allowing better insight into cause-and-effect relationships. Significant results will show a correlational relationship between different seasons and healthcare workers mental health. We hope that to shine a light on the prevalence of SAD in healthcare workers. Future studies could focus on strategies that could promote a healthier and safer work/educational environment. Intervention examples include, decreasing number of night shifts allowed in a row, implementing light therapy in resident/physician lounges, etc. By treating and reducing SAD rates, burnout rates may improve, and ultimately patient care and safety will improve.

Disclosures: A.C. Moody: None. **N. Habib:** None. **H.M. Hoffmann:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.14/K34

Topic: F.07. Biological Rhythms and Sleep

Title: Sleep and Cognitive Performance Among Military Service Members with Mild Traumatic Brain Injuries

Authors: *H. RIZEQ[1], E. ESPEJO[1], W. ZHENG[1], C. DAQUINO[1], M. ETTENHOFER[2], S. GIMBEL[2], L. HUNGERFORD[2], P. SESSOMS[1];
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Abstract: Individuals with mild traumatic brain injury (mTBI) frequently experience sleep disturbances and cognitive dysfunction, the interplay of which is complicated, yet critically understudied in the context of military operation in the field. To explore the interaction between these elements, participants performed a version of the Bethesda Eye & Attention Measure (BEAM) task within an immersive virtual reality environment that simulated a military patrol mission. Participants were required to focus on a central cue, respond to color-coded arrows, and execute decisions to shoot at an avatar. In this go/no-go task, performance metrics included response times for misdirectional (RT-MisDir) and directional cues (RT-Dir), and the percentage of correct shots made (%Shots). Total Sleep Time (TST) was assessed using the ReadiBand®

sleep tracker (Fatigue Science, Vancouver, BC) and calculated based on the average total sleep per night during the duration of the study (8-12 weeks). Preliminary analyses were conducted using Pearson's correlations between BEAM task indices and TST in 26 active-duty military members (10 chronic mTBI, 10 subacute mTBI, and 6 controls). TST and RT-Dir were moderately correlated among all participants (r=-0.34). This was driven by a large effect in subacute TBI participants (r = -0.62) and in chronic mTBI participants (r = -0.59), and a small effect in control (r=0.10). In addition, TST and RT-MisDir were moderately correlated among all participants (r=-0.32), driven by a large effect in chronic mTBI participants (r = -0.65), a moderate effect in subacute mTBI participants (r = -0.36), and a small effect in control (r = -0.16). Lastly, the correlation between TST and %Shots was small for the full sample (r= 0.14) but appeared to vary greatly across TBI subgroups. This correlation was large in subacute mTBI participants (r = 0.69), and large among chronic TBI participants though in the opposite direction (r = -0.52), while uncorrelated among control (r = 0.00). Further analyses will be conducted in the full sample, including data from more than 60 additional participants, while controlling for potential confounding variables, such as posttraumatic stress disorder symptoms. The findings could shed light into the complex interplay between sleep and cognitive functioning, and reveal potential differences in this interplay between the subacute and chronic phases of TBI.

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Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

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Program #/Poster #: PSTR413.15/L1

Topic: F.07. Biological Rhythms and Sleep

Support: NSF: 1736026
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NIH: 2R25NS080687

Title: The effects of gut microbes on the development of circadian rhythm

Authors: *Y. B. KORU[1], K. BEER[2], A. RUGGIERI[1], J. RODRIGUEZ CORDERO[1], M. A. GIANNONI-GUZMÁN[3], Y. ORTIZ-ALVARADO[4], M. DÖKE[5], M. ANDERSON[3], C. ANDUJAR[1], E. COURTNEY[6], A. STRUBBE[1], E. J. AVILES-RIOS[1], E. CINTRÓN[1], A. MONTES-MERCADO[1], A. GHEZZI[1], T. GIRAY[1], J. L. AGOSTO[1];
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Abstract: Circadian rhythms are essential biological processes that regulate behaviors crucial for colony survival, such as age-dependent division of labor. In a honey bee colony, nurse bees are responsible for caring for brood, and newly emerged bees lack circadian rhythms, whereas forager bees, which collect pollen, nectar, and water, exhibit a daily circadian rhythm to optimize the foraging time. These rhythms are regulated by one of the neuropeptides called Pigment-Dispersing Factors (PDF). Previous studies show that the number of PDF neurons increases with age. While the importance of gut microbes on brain development, function, and behavior has been apparent, its role in the development of circadian rhythm is understudied. We hypothesize that manipulating gut microbes affects the development of circadian rhythm in honey bees. To test this hypothesis, initially, we measured activity using Locomotor Activity Monitors (LAMs). First, we administered antibiotic to reduce the gut microbiota and measured the activity by utilizing Locomotor Activity Monitors (LAMs). As the second manipulation, the newly-emerged bees were placed in a cage with nurse bees for two hours to increase their gut microbiota. Lastly, the late-stage pupae were manually removed and allowed to eclose in the LAMs, minimizing bacterial loads. Our results show that the honey bees with reduced gut microbiota resulted in delayed onset of circadian rhythm. Additionally, we conducted an RNA-Seq assay on brain tissue to understand the effects of antibiotics on genes related to circadian circuit maturation, identifying 16 differentially expressed genes. Finally, we did an immunohistochemistry assay using antibiotic and brood cap manipulations. We found that gut microbiota affects the number of PDF neurons. In other words, the honey bees with reduced gut microbiota have fewer PDF neurons. These findings show the significance of gut microbes on the ontogeny of the circadian rhythm.

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Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.16/L2

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant U54GM133807

Title: Assessing the Effects of the "Night-Float Rotation" in Physician Residents: Impact of Shiftwork on Sleep/Wake cycles and circadian rhythms

Authors: *J. R. MARRERO[1], A. C. SEGARRA[2], J. L. AGOSTO[3];
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Abstract: Introduction: Sleep disruption through residency programs is a significant risk factor for medical trainees' exhaustion and can lead to burnout. This leads to an increased risk of medical errors, development of anxiety and health complications, both physiological and emotional. Individual coaching intervention with physicians in-training revealed that a sizeable portion of residents struggle with sleep difficulties during the night-float rotation. We propose that differences in the synchrony of the residents' circadian oscillators and their work shift may be associated with sleep disturbances. Methods: This is a longitudinal cohort study following sixteen medical residents. Participants were followed for 3 consecutive rotation blocks covering a diurnal pre-float rotation, the night float rotation and a post-float diurnal rotation. Participants also completed a series of questionnaires after each rotation block. A dermal sensor was used to evaluate the oscillations in core body temperature to determine circadian oscillation. A wrist sensor monitored sleep and activity of the participants to assess the degree of synchrony between parameters measured. Saliva samples were collected to measure cortisol levels, to analyze stress and explore circadian fluctuations. Analysis: Demographic information was collected for each participant to measure the outcome variation according to hazard exposures and other habits. We have taken repeated measurements on the same participants at various points in time to increase statistical power. Due to the small sample size, the importance of study's results is not based solely on statistical significance, but instead on clinical significance. We used specialized circadian analysis software to study the oscillation of measurements, assess the shift in phases and periodicity along the night float rotation, and explore possible causal relationships between parameters measured. Conclusions: Evaluation of temperature data shows a disruption in the oscillation of temperature including shifts in phase and period during the night float. Preliminary sleep analysis shows differences in sleep duration, onset, and timing.

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Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.17/L3

Topic: F.07. Biological Rhythms and Sleep

Title: Chronic fatigue syndrome related to post Covid -19 condition

Authors: *O. ZAMBAL[1], B. LKHAGVASUREN[4], B. UURIINTUYA[2], B. DANSRAN[2], B. BULGANTUYA[2], U. ANGARAG[2], N. JAMIYANDORJ[2], T. DELGERSAIKHAN[2], O. GANBAATAR[2], E. TUMURBAATAR[3];

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Abstract: Introduction: Chronic Fatigue Syndrome (CFS) is characterized by persistent physical and mental fatigue. The post-COVID-19 condition patients refer physical fatigue and cognitive impairment sequelae. The chronic, debilitating disease known as myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is characterized by extreme, non-relieving exhaustion that does not go away with rest. Other typical symptoms include multifocal pain, disturbed sleep patterns, and cognitive dysfunction. **Methods:** The sample included total 546 individuals. Fatigue, sleep quality, anxiety and depressive symptoms, the frequency and severity of different symptoms, olfactory function and a wide range of cognitive domains were evaluated. **Results:** Chronic Fatigue Syndromes are characterized by excessive physical fatigue, sleep problems and myalgia. There were statistically significant differences in education level with the post-covid group having more years of enough education. No significant differences were found in age. Statistically significant differences were also found in the proportion of women between groups 66.7% of CFS. **Keywords**: post COVID-19, CFS, chronic fatigue syndrome

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Poster

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Sleep and Behavior: Biological Rhythm and Sleep

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Topic: F.07. Biological Rhythms and Sleep

Support: NINDS Intramural Grant: ZIA NS003027-17

Title: Living in the dream: exploring the relationship between lucid insight in dreams and sleep prefrontal cortex functional correlations.

Authors: *D. GREENE, H. HALIVNI, J. DE ZWART, H. MANDELKOW, N. YANG, P. VAN GELDEREN, J. H. DUYN, D. PICCHIONI;
Advanced MRI Section, Lab. of Functional & Mol. Imaging, Natl. Inst. of Neurolog. Disorders & Stroke, Natl. Inst. of Hlth., Bethesda, MD

Abstract: Limited research exists on high spatial resolution imaging during dreaming, so the neurological correlates of lucidity, or conscious awareness, in dreams remain unknown. We used functional magnetic resonance imaging (fMRI) to elucidate if functional correlations of the

anterior medial prefrontal cortex (amPFC), an important area for the inhibition of impulsive thoughts and behaviors, were related to lucid insight during dreams. The frontal cortex has also been related to control experienced during dreams in non-REM and REM sleep. We hypothesized that amPFC functional correlation strength would be positively related to lucid insight across the brain. Data were collected during an all-night simultaneous EEG-fMRI study (N = 12; M_{age}=24, SD = 3.5; 66.7% female). Participants slept for two consecutive nights in a 3 Tesla MRI scanner and were awoken throughout the night with auditory tones. Upon arousal, they were administered the Lucidity and Consciousness Scale (L&C; Voss 2013 *Conscious Cogn*). This analysis used data from the L&C's insight subscale, 6 questions scored 0-5 each, with higher scores indicating greater insight into the fact that one was dreaming. We only used data from the second night, and arousals missing insight data were removed (n = 39 dream reports from 10 participants). The mean composite insight score was 13.5 ± 8.02. Skewness was -0.09 (skewed left) and kurtosis was 1.56 (platykurtic). FMRI data were preprocessed with a tailored version of the "afni_proc" pipeline from the AFNI software. We correlated amPFC activity in the four minutes prior to the arousal with that of the rest of the brain. We took the absolute value of these correlations and completed a Fisher's Z transformation. A linear mixed effects model was used to correlate the transformed absolute values with insight scores. Resulting whole-brain statistical maps were thresholded at a two-sided p-value of 0.05, and multiple testing correction with a minimum cluster size of 1159 voxels was applied. There were no significant clusters after these corrections, refuting our hypothesis of an appreciable positive relationship between amPFC functional correlation strength and lucid insight. This null finding indicates that other subregions of the prefrontal cortex and/or other regions of the brain may be more relevant to the neurological mechanisms of lucid insight. The finding also suggests that the inhibition necessary for rational consciousness during adulthood may have different underlying mechanisms in waking consciousness versus lucid dreaming. Future work will include alternative statistical thresholding methods and assessment of other brain areas as they relate to lucid insight.

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Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

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Topic: F.07. Biological Rhythms and Sleep

Support: NIH/NIGMS 5P20GM109098-08
NSF 2242771
NIH/NIGMS 5T32GM132494
NASA West Virginia Space Grant Consortium, Grant # 80NSSC20M0055

Title: The homeostatic response to sleep deprivation in three mouse models of autism spectrum disorder

Authors: *B. RODRIGUEZ[1], B. MENARCHEK[1], M. BRIDI[2];
[1]West Virginia Univ., Morgantown, WV; [2]Neurosci., West Virginia Univ., Morgantown, WV

Abstract: Sleep is an important physiological state that is known to be involved in memory consolidation, neuronal plasticity, and cognitive function. In individuals with neurodevelopmental conditions such as autism spectrum disorder (ASD) there is a high prevalence of sleep disturbances that are correlated with symptom severity. However, sleep architecture is remarkably normal under baseline conditions in many mouse lines used to model ASD. We hypothesized that sleep phenotypes will emerge in these mouse lines when they are challenged with sleep deprivation (SD). In wildtype (WT) mice, acute SD is followed by a homeostatic sleep rebound, consisting of increased in delta (0.5-4Hz) power during non-rapid eye movement (NREM) sleep and increased total sleep time. In the current study, BTBR, *fmr1*-KO, and *cntnap2*-KO mouse strains that model ASD, as well as their respective WT controls, were implanted with electroencephalogram (EEG)/electromyogram (EMG) electrodes, and then allowed a week of recovery before being put through our SD paradigm. Following three days of baseline recording in their home cage, mice were sleep deprived for six hours starting at lights on using gentle handling. Trained experimenters gently nudged the mouse with a ruler or swab or tapped on their enclosure when signs of sleep were observed. At the end of SD, the mice were allowed recovery sleep for 24hrs. We used Sirena Sleep Pro semi-automated software to score arousal states as NREM, REM, or Wake in 4-second epochs. There was no difference in NREM delta power between *fmr1*-KO, BTBR or *cntnap2* KO mice and their respective WT counterparts during recovery sleep. All genotypes had an intact homeostatic response following SD such that delta power increased above baseline during recovery sleep and diminished over the next six hours. Interestingly, during recovery sleep, BTBR mice spent significantly less time asleep than their WT counterpart, and this was not observed in the *fmr1* or *cntnap2*genotypes. Lastly, latency to enter the first sleep bout following the end of SD, which has been associated with an insomnia-like phenotype, was not different between ASD-related lines and their WT counterparts. These findings demonstrate that the homeostatic response to SD is largely intact in the ASD-related mouse lines studied, although BTBR mice show a minor deficit.

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Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

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Topic: F.07. Biological Rhythms and Sleep

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NIH 1F31NS132434
SRSF Small Research Grant
Wake Up Narcolepsy Research Grant
Gilmore Award for Sleep and Research Education

Title: Norepinephrine release in the vlPAG-LPT suppresses cataplexy

Authors: *B. A. TOTH[1,2], V. C. ORTIZ[3], C. R. BURGESS[1,2,3];
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Abstract: REM sleep expression is carefully regulated by a network of brainstem nuclei, including the ventrolateral periaqueductal grey and adjacent lateral pontine tegmentum (vlPAG-LPT). Disorders of REM sleep are debilitating for those afflicted and may involve improper regulation of the vlPAG-LPT. For example, patients with the sleep disorder narcolepsy experience cataplexy - the inappropriate intrusion of REM sleep muscle atonia during wakefulness, often in response to strong emotions. The vlPAG-LPT is known to regulate REM sleep through inhibition of the downstream REM-promoting regions like the sublaterodorsal nucleus, however, little is known about how afferent regions modulate vlPAG-LPT activity to influence REM sleep. To address this, we used retrograde tracing to identify upstream neuronal populations that project to the vlPAG-LPT and found that it receives input from norepinephrine neurons in the locus coeruleus (LC-NE). While LC-NE neurons have an established role in promoting arousal, recent evidence also demonstrates a role for LC-NE in sleep - particularly in regulating the timing of entrances into REM sleep. Furthermore, NE receptor pharmacology reduces cataplexy, but the precise release dynamics and functional role of LC-NE has not been determined. In the present study, we used in vivo fiber photometry of NE release and circuit-specific optogenetics to study the release and functional role of NE in the vlPAG-LPT during sleep in orexin knockout mice, a murine model of narcolepsy. First, we show that NE release in the vlPAG-LPT abruptly halts at the onset of cataplexy and is similarly decreased during REM sleep. We then show that tonic stimulation of LC-NE projections to the vlPAG-LPT suppresses entrances into cataplexy. These findings suggest that NE release functionally modulates the REM sleep ‘flip-flop’ switch and advances our understanding of the neurobiology of both REM sleep and cataplexy.

Disclosures: B.A. Toth: None. **V.C. Ortiz:** None. **C.R. Burgess:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.21/L7

Topic: F.07. Biological Rhythms and Sleep

Support: NS122589
RDL R03

Title: The role of neurotensin-expressing neurons of the extended ventrolateral preoptic nucleus in REM sleep regulation

Authors: *I. BOCCALARO[1], E. RILLOSI[2], S. NARDONE[3], Y. WU[4], P. FULLER[5], R. DE LUCA[6], E. ARRIGONI[7];
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Abstract: The ventrolateral preoptic nucleus (VLPO) plays a crucial role in sleep regulation, but understanding its core circuits has proven challenging. Research over the past two decades has highlighted the necessity of galanin-expressing neurons within the ventrolateral preoptic nucleus (VLPOGal) for sleep. These neurons are divided into two distinct clusters within the VLPO: the core (cVLPO) and the extended (eVLPO). Lesion and anatomical studies have elucidated that cVLPOGal neurons are crucial for non-rapid eye movement sleep (NREMs), while eVLPOGal neurons are essential for rapid-eye movement sleep (REMs). However, significant gaps persist in our understanding on how eVLPOGal neurons drive REMs, primarily due to the challenging proximity of cVLPO and eVLPO, with no selective targeting markers available until now. Recently, we identified a key marker gene for eVLPOGal neurons, the neuropeptide neurotensin (eVLPONts), enabling selective manipulation of these neurons from neighboring cVLPOGal neurons. Our findings revealed that inhibiting eVLPONts neurons through chemogenetics decreases REMs, and activating eVLPONts neurons using optogenetics enhances REMs. Moreover, we aim to investigate whether the REM-promoting activity of VLPONts neurons is restricted to normal physiological conditions or persists even in stressful situations. Our observations indicate that mice exposed to innate stressors (cage exchange with other male or female bedding) have difficulty entering REMs, preferring NREMs to maintain higher responsiveness to environmental stimuli. Furthermore, we aim to determine if we can restore normal REM sleep pattern by manipulating VLPONts neurons and alleviate stress-related sleep disturbances. Additionally, our conditional anterograde tracing shows that eVLPONts neurons projects to areas like the ventrolateral periaqueductal gray (vlPAG) and the locus coerulus (LC), known to inhibit REMs. These findings suggest that eVLPONts neurons facilitate REM sleep by inhibiting GABAergic neurons in the vlPAG and monoaminergic neurons in the LC, thus disinhibiting REM-generating neurons in the pontine reticular formation. Despite these advancements, further research is needed to fully understand the cellular and synaptic mechanisms underlying the role of eVLPOGal neurons in REMs regulation in both physiological and stressful conditions.

Disclosures: I. Boccalaro: None. **E. Rillosi:** None. **S. Nardone:** None. **Y. Wu:** None. **P. Fuller:** None. **R. De Luca:** None. **E. Arrigoni:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.22/Web Only

Topic: F.07. Biological Rhythms and Sleep

Support: UNAM-PAPIIT: IN221324

Title: Power spectral density during sleep spindles in men and women with obstructive sleep apnea

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Abstract: Sleep disruptions involve changes in brain function and alter sleep architecture. Obstructive sleep apnea (OSA) is a sleep disorder characterized by multiple episodes of apnea-hypopnea that occurs when the upper respiratory tract becomes blocked, resulting in pauses in airflow that lead to brief awakenings and sleep interruptions. This in turn has consequences in sleep microstructure and cognitive functioning. Thus, it is important to study every component during sleep to understand the pathophysiological mechanisms that could be influencing the symptoms in patients with OSA. Sleep spindles (SS) are bursts of brain electrical activity generated in the thalamus and propagate to the neocortex. SS characterized stage 2 of non-REM sleep (Non-Rapid Eye Movement) and they are related to memory consolidation and sleep maintenance, aspects that are degraded in patients with OSA. Furthermore, a higher prevalence of OSA has been reported in men, as well as more pronounced symptoms than women. Therefore, this study aims to compare the characteristics of sleep spindles in men and women with two levels of severity of obstructive sleep apnea. Polysomnography was performed on 37 patients with untreated OSA (19 men and 18 women). SS were automatically detected to analyze their duration and power spectral density from 4-second segments using the Fast Fourier Transform. We found shorter SS in women than in men in moderate apnea, while in severe apnea, the opposite was observed men presented shorter SS compared to women. Regarding spectral power density, there was a decrease in the sigma frequency bands (9-16 Hz) in men compared to women. These results suggest that the characteristics of SS in patients with OSA may vary according to sex and the severity of apnea, highlighting the importance of considering these differences when evaluating the effects of OSA on sleep and cognitive functions.

Disclosures: F. Mendez Ueda: None. **Z. Munoz-Torres:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.23/L8

Topic: F.07. Biological Rhythms and Sleep

Title: Infection-related changes in sleep architecture during the COVID-19 pandemic

Authors: *D. M. WEINERT[1], H. D. BENNEFELD[2];
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Abstract: The long-term health consequences of SARS-CoV-2 infection include impairment of physical, mental, and psychological health, as well as an impact on daily functioning and quality of life.
The symptoms that may occur following a coronavirus infection include fatigue, muscle weakness or muscle pain, persistent cough, concentration and memory problems, and a risk of developing high blood pressure, sleep disorders, depressive symptoms, and anxiety. Disturbances of the REM sleep phase may lead to frequent awakenings during the night and daytime sleepiness.
The free reference website Worldometer launched its coronavirus tracker on January 29, 2020, and ceased providing updates on April 13, 2024. This long-term course of a test subject with two mild SARS-CoV-2 infections and four mRNA-based vaccinations involves the evaluation of internal and neurological parameters over a 40-month period by a self-tracking system.
The data set encompasses a period between December 30, 2020, and April 30, 2024. During this period, there were two instances of Covid-19 infections, in 2021 (Ct = 34.42) and in 2022 (Ct = 27.09), both of which occurred on March 10. The period was divided into three phases: a pre-Covid phase (90 days), a post-Covid-1 phase (364 days), and a post-Covid-2 phase (784 days). On the day before infection, point-of-care rapid tests were negative. The data set included information on activity levels, internal metrics (daily steps, body temperature, weight/body mass index, blood pressure, heart rate, VO2max, pulse wave velocity), and sleep patterns (REM, deep and light sleep, total/restorative sleep). The data was subjected to statistical analysis to identify any deviations across the individual phases.
No statistically significant changes were observed in the parameters of blood pressure and sleep architecture in the pre-Covid phase. Similarly, no significant deviations were found in the post-Covid-1 phase regarding blood pressure parameters. In contrast, there was a decrease in the proportion of time spent in light sleep ($P = 0.004$), accompanied by increases in the proportion of time spent in restorative sleep phases ($P = 0.011$) and a significant increase of the heart rate during sleep ($P = 0.0001$). Conversely, there were statistically significant increases in both systolic ($p < 0.0001$) and diastolic blood pressure values ($p = 0.015$) in the post-Covid-2 phase. Furthermore, a notable reduction in the duration of the REM phase ($p = 0.0001$), the DEEP phase ($p = 0.023$) and the restorative phase ($p = 0.011$) was observed. The results demonstrate a long-lasting impact on blood pressure and sleep data even in mild cases, without the need for inpatient hospitalization.

Disclosures: D.M. Weinert: None. **H.D. Bennefeld:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.24/L9

Topic: F.07. Biological Rhythms and Sleep

Title: Whole-brain functional imaging of sleep and wake in zebrafish

Authors: *J. MO[1], P. LUU[1], T. V. TRUONG[2], A. NADTOCHIY[3], M. ZANON[1], Z. DU[4], A. ANDREEV[5], R. WONG[6], D. PROBER[7], G. J. GOODHILL[8];
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Abstract: Sleep occupies a third of our lives, yet the mechanisms governing its regulation remain poorly understood. Despite substantial progress in the discovery and understanding of specific sleep-promoting and wake-promoting neuronal and molecular pathways, we still lack an integrated understanding of how these mechanisms work together in the intact brain to regulate sleep and wake as whole-brain behavioral states. Toward this goal, we are recording the activity of virtually every neuron in the larval zebrafish brain during sleep and wake states, and in response to perturbations that induce these states, then applying mathematical analysis and modeling to uncover fundamental principles that underlie sleep.
Here we report our initial progress in optimizing a custom-developed two-photon-excitation selective plane illumination microscopy (2p-SPIM) platform to record the whole-brain functional activity in zebrafish larvae. We use two-photon excitation, at the infrared wavelength of 920 nm, which is invisible to the zebrafish, to avoid the sleep-perturbing visual stimulation from visible wavelengths typically used in one-photon excitation. Our 2p-SPIM platform achieves 1 Hz volumetric rate, over a volume of ~ 480 x 870 x 230 (XYZ) µm3, with corresponding voxel size of 1.6 x 1.6 x 3.8 µm3, with sufficient signal-to-noise ratio that allows motion-correction, single-cell segmentation, and signal extraction using standard publicly-available computational tools. Concurrently with the fluorescent brain signals, we also record the heart beating and tail movement of head-fixed, tail-freed zebrafish, enabling readout of the animal's behavioral state. With our platform, we can record whole-brain functional activity continuously over multiple hours, as the animal undergoes natural and induced sleep/wake transitions. This system paves the way for future studies that will yield insights toward a more complete understanding of the neuronal mechanisms that underlie sleep.

Disclosures: J. Mo: None. **P. Luu:** None. **T.V. Truong:** None. **A. Nadtochiy:** None. **M. Zanon:** None. **Z. Du:** None. **A. Andreev:** None. **R. Wong:** None. **D. Prober:** None. **G.J. Goodhill:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.25/L10

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant AA028175-01
VA Merit Award I01BX002661
VA Merit Award I01BX006240-0

Title: A novel role of rostromedial tegmental nucleus in mediating the effects of fentanyl on sleep-wakefulness

Authors: *R. SHARMA[1], A. CHISCHOLM[2], M. PARIKH[3], M. M. THAKKAR[4];
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Abstract: Fentanyl is an analgesic and addictive substance that contribute to opioid use disorders (OUD), a chronic brain disease characterized by compulsive opioid use and harmful consequences. Although the critical association between fentanyl addiction and insomnia/sleep disruptions is known, the precise mechanisms through which fentanyl affects sleep-wakefulness have not been investigated. The GABAergic rostromedial tegmental nucleus (RMTg), also referred to as GABA brake for midbrain dopaminergic systems, the region that is known to be a strong sleep promoter and projects to ventral tegmental area (VTA) along with other major wake-promoting centers including dorsal raphe nucleus. Thus, we propose that chemogenetic activation of RMTg will attenuate the wake-promoting effects of fentanyl. To test this hypothesis, male C57BL/6J mice were infused with excitatory Designer Receptor Exclusively Activated by Designer Drug (DREADD; Gq), bilaterally in the RMTg (anteroposterior = −3.8 mm, mediolateral = 0.5 mm, and dorsoventral = 4.0 mm), along with implantation of sleep recording electrodes. The first set of experiments examined the effect of RMTg activation on spontaneous S-W during the dark period. The second set of experiments were designed to evaluate the effect of chemogenetic activation of RMTg on the wake-promoting effects of fentanyl (1.2 mg/kg, i.p.). Initial results suggest that mice administered with fentanyl (1.2 mg/kg, i.p.) suppressed both NREM and REM sleep while promoting euphoria-like symptoms and long-lasting insomnia. Ongoing experiments are examining the effects of chemogenetic activation of RMTg on the wake-promoting effects of fentanyl.

Disclosures: R. Sharma: None. **A. Chischolm:** None. **M. Parikh:** None. **M.M. Thakkar:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.26/L11

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant AA028175-01
VA Merit Award I01BX002661
VA Merit Award I01BX006240-0

Title: Serotonergic mechanism in the dorsal raphe nucleus mediates fentanyl-induced sleep promotion

Authors: R. SHARMA[1], A. CHISCHOLM[2], M. PARIKH[3], ***M. THAKKAR**[4];
[2]Depart of Neurol., [1]Univ. of Missouri, Columbia, MO; [3]Univ. of Missouri, Columbia, Columbia, MO; [4]HSTMV Hospital/University of Missouri, Columbia, MO

Abstract: Fentanyl, an ultra-potent synthetic opioid, was implicated in 71% of drug overdose deaths in 2021. While the lower doses alleviate pain and promote wakefulness, higher doses of fentanyl can lead to increased sedation and respiratory depression, contributing significantly to opioid overdose fatalities. Fentanyl's impact on sleep-wakefulness (S-W) has led to its application in various contexts such as sedation during surgeries, dental procedures, and wildlife immobilization. It's also used as an incapacitating agent in antiterrorism operations and as medication for sleep issues in chronic fentanyl users. When combined with alcohol, fentanyl's sedative effects are intensified, worsening central nervous system (CNS) and respiratory depression, which can be fatal. Despite extensive research on fentanyl's effects on S-W, the neuroanatomical mechanisms underlying its sleep-promoting effects remain elusive. The dorsal raphe nucleus (DRN), a serotonergic center, is known to play a crucial role in sleep regulation. Serotonin, released by DRN neurons, has a complex role in sleep, initially promoting wakefulness before inducing non-rapid eye movement (NREM) sleep. The relationship between fentanyl and serotonin has gained attention due to cases of "serotonin syndrome" observed after opioid use. We hypothesized that DRN serotonergic inhibition by local infusion of 8-OH-DPAT will prevent fentanyl-induced sleep promotion. To test this hypothesis, male C57BL/6J mice, instrumented with sleep recording electrodes and guide cannula above the DRN, were utilized in this study. Starting 6h after light onset, animals were administered with 8-OH-DPAT (a serotonergic antagonist; 2 µg/500 nl) in the DRN followed by an intraperitoneal injection of fentanyl (1.2 mg/Kg). S-W was examined for the next 12h (Last 6h of light period and first 6h of dark period). Our results suggest that systemic administration of fentanyl significantly increased

the wakefulness during the light period which was followed by a significant increase in NREM and REM sleep during the dark period. Local serotonergic inhibition in the DRN significantly reduced this effect as evidenced by a significant reduction in fentanyl-induced wakefulness during the light period and sleep-rebound during the dark period. Our findings suggest that serotonergic mechanism in the DRN plays a vital role in mediating the fentanyl-induced sleep promotion after an initial wake-promotion.

Disclosures: R. Sharma: None. **A. Chischolm:** None. **M. Parikh:** None. **M. Thakkar:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.27/L12

Topic: F.07. Biological Rhythms and Sleep

Support: NIH R01AG072513
NIH T32NS115667

Title: Elucidating Sleep and Running Dynamics from Continuous, Longitudinal Video Actigraphy in Freely Behaving Mice

Authors: *K. K. NAYLOR[1,2], C. J. BOUCHER[3,2,1,4], T. M. RIVERA[3,2,1,4], B. J. GLUCKMAN[3,2,1,5,6];
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Abstract: Sleep patterns naturally evolve over the lifespan of an animal. Disruption of sleep - regardless of cause - has been identified as a risk factor for neurological diseases and is potentially implicated in the mechanisms underlying disease progression. Prospective observational studies have shown that physical inactivity is one of the most common preventable risk factors for neurological disease. It is generally accepted that exercise has positive benefit on sleep-wake regulation, but less is known about how other factors (i.e., sex, age, sleep quality, activity) mediate this complex system. Sleep and exercise interact through a series of complex, bidirectionally linked processes that affect multiple downstream physiologic pathways. Here, we observe the interactions between sleep and exercise in freely behaving animals using a custom designed cage and acquisition system. Mice are provided access to a home cage with or without access to a functional, self-propelled running wheel. Animals are continuously monitored in their home cages for their lifespan. Using this system, we quantify measures of sleep architecture and general exercise, patterns that link exercise to sleep and how those measures evolve over the course of aging. Here we display example analysis of the temporal dynamics associated with

sleep and/or exercise behavior, how these values fluctuate daily, and how these values shift over long-term aging.

Disclosures: K.K. Naylor: None. **C.J. Boucher:** None. **T.M. Rivera:** None. **B.J. Gluckman:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.28/L13

Topic: F.07. Biological Rhythms and Sleep

Support: NIH RO1AG072513
NIH T32NS115667

Title: Noninvasive Sleep Characterization from Longitudinal Murine Continuous Video Actigraphy

Authors: *C. J. BOUCHER[1,2,3,4], K. K. NAYLOR[1,2,3], T. M. RIVERA[1,2,3,4], B. J. GLUCKMAN[1,2,3,4,5];
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Abstract: The baseline architecture and quality of sleep change during normal aging. In the brain, changes in vasodilation and extracellular space fraction are associated with sleep and have been implicated in metabolic waste clearance from brain. Disruptions to sleep and sleep architecture have been shown to be comorbid with various neurological diseases. Exercise is thought to modulate sleep architecture in a way that improves sleep quality and is correlated to ameliorating symptoms associated with neurological disease. However, it is unknown how and when the bidirectional interactions between sleep and exercise instigate changes in the brain. Established protocols for recording and classifying sleep are invasive and/or require removal of the animal from their home cage. These methods typically do not allow for continuous recording across aging and perturb both sleep patterns and behavior. Here we have implemented a robust, noninvasive video-based system for continuous, long-term recording of behavior and activity in mice from their home cage. These recordings allow us to investigate sleep behavior in mice with or without access to a functional running wheel. Motion is detected and quantified from changes in the pixel intensity across consecutive frames in real time, and sleep states are determined based on minimal rhythmic oscillations detected from the animal's breathing. We present data to

characterize our video-based sleep scoring method across the lifespan of animals, from which we can compare patterns across age and between free wheel and locked wheel groups.

Disclosures: C.J. Boucher: None. **K.K. Naylor:** None. **T.M. Rivera:** None. **B.J. Gluckman:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.29/L14

Topic: F.07. Biological Rhythms and Sleep

Support: R01AG072513
T32NS115667

Title: Scalable, Longitudinal Monitoring Systems for Real-Time EEG and Behavioral Data Acquisition in Freely Behaving Mice

Authors: *T. M. RIVERA[1,2,3,4], K. K. NAYLOR[1,2,3], C. J. BOUCHER[1,2,3,4], B. J. GLUCKMAN[1,2,3,4,5];
[1]Ctr. for Neural Engin., [2]Cross Disciplinary Neural Engin. Training Program, [3]Engin. Sci. and Mechanics, [4]Grad. Program in Neurosci., [5]Biomed. Engin., Pennsylvania State Univ., University Park, PA

Abstract: Non-invasive methods for monitoring freely behaving animals are crucial yet challenging to implement at a large scale, and many existing systems either restrict natural behaviors or necessitate frequent, manual interventions. In our previous work, we addressed this limitation by developing an industrial scale animal housing and recording unit capable of continuously recording from a cohort of 250 individually housed animals. The custom-built homes cages are equipped with running wheels (either free or locked) and recording systems. Each cage has a single board computer and camera recording the animal's movement within it. A subset of these cages also integrates a custom acquisition system for recording chronic electrophysiology. This setup allows for longitudinal, high-fidelity EEG recording and robust, long-term behavioral monitoring within the home cage environment. Our comprehensive setup facilitates detailed sleep scoring and simultaneous brain activity measurements during natural behaviors (e.g. running) thereby allowing advanced understanding of brain state and behavior and their evolving relationship over the course of aging or development of neurological diseases. This system is deployed to investigate sleep architecture and exercise dynamics over aging, and it showcases our system's capability to monitor mice longitudinally for a broad spectrum of additional neurological studies.

Disclosures: T.M. Rivera: None. **K.K. Naylor:** None. **C.J. Boucher:** None. **B.J. Gluckman:** None.

Poster

PSTR413

Sleep and Behavior: Biological Rhythm and Sleep

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.30/L15

Topic: F.01. Neuroethology

Title: Light wavelength effect on Danionella locomotion

Authors: *S. S. FERZIGER, P. ZMARZ, A. LEMIRE, C. SATOU;
Janelia Res. Campus, Ashburn, VA

Abstract: Whole-brain recordings in fish have led to the identification of circuit models underlying several behavioral strategies. These recordings are most often made in larval animals whose behavioral repertoire tends to be limited. Promisingly, whole-brain recordings have recently become possible in the small teleost, *Danionella*, whose small brain permits whole-brain recordings in adult fish. However, the behavioral repertoire of *Danionella* has not been systematically explored, including its circadian rhythm. To explore *Danionella* behavioral repertoire, we developed long term fish behavior recording system and we monitored groups of fish for several days across their light/dark cycle using cameras and near-IR back illumination. Since *Danionella*'s visual spectrum is unknown, we tested 850nm, 950nm, and 1050nm back illumination and quantified the effect of back illumination wavelength on the circadian rhythm of the fish. Across all wavelengths, *Danionella* displayed a significant decrease in locomotion during the dark cycle with this effect being most pronounced at 1050nm. These results help to optimize experimental setups for long-term recordings in *Danionella* by minimizing disruption to the fish circadian rhythm.

Disclosures: S.S. Ferziger: None. **P. Zmarz:** None. **A. Lemire:** None. **C. Satou:** None.

Poster

PSTR414

Neural Circuits of Reward and Appetitive Learning and Memory

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR414.01/L16

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R01DA057084

Title: Dopamine projections to the basolateral amygdala mediate reward prediction for learning and decision making

Authors: *K. I. RAMIREZ-ARMENTA[1], C. FENG[1], J. E. ANDRADE[1], A. SIAS[1], K. M. WASSUM[2];
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Abstract: Our ability to predict upcoming and available rewarding events is critical to both learning and decision making. These predictions are enabled by learned associations between cues and the specific rewards they signal. Such cue-reward memories form an internal model of environmental relationships that allow us to use external cues to predict which rewards will be available to both make adaptive decisions and learn when our expectations have been violated. But we know very little of how the brain allows us to use cue-reward memories to generate reward predictions. Midbrain dopamine neurons may be involved. This system has canonically been implicated in signaling reward prediction errors for learning. Our recent data extend this to reveal that ventral tegmental area dopamine projections to the basolateral amygdala (VTA_{DA} BLA) drive model-based learning, linking cues to the specific rewards they predict. We have also found that, with learning, reward-predictive cues come to trigger dopamine release in the BLA. Thus, we reasoned that this cue-evoked VTA_{DA}BLA activity might mediate reward predictions. To evaluate this, we optogenetically inhibited cue-evoked VTA_{DA}BLA activity during tests of both decision making and learning. Inhibition of cue-evoked VTA_{DA}BLA activity attenuated the ability to use the cue to guide decision making during Pavlovian-to-instrumental transfer and to adapt cue responses following selective devaluation of the predicted reward. Thus, VTA_{DA}BLA activity mediates the use of cue-reward memories to inform the reward predictions needed for adaptive decision making. Inhibition of cue-evoked VTA_{DA}BLA activity also promotes learning of a new cue-reward association that would otherwise be blocked by a previously learned association. Thus, cue-evoked VTA_{DA}BLA activity conveys the reward predictions that attenuate new learning when predictions are not violated. These results reshape our view of dopamine function, indicating that VTA_{DA} neurons, via projections to the BLA, not only contribute to cue-reward learning, but also mediate the cue-evoked reward prediction necessary for learning and adaptive decision making.

Disclosures: K.I. Ramirez-Armenta: None. **C. Feng:** None. **J.E. Andrade:** None. **A. Sias:** None. **K.M. Wassum:** None.

Poster

PSTR414

Neural Circuits of Reward and Appetitive Learning and Memory

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR414.02/L17

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R21DA059233
R01DA057084

Title: Contribution of dopamine projections to the amygdala in action-outcome and habit learning

Authors: *A. K. CRAWLEY[1], K. NETT[1], A. SHAMOONY[1], J. GIOVANNIELLO[1], K. M. WASSUM[2];
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Abstract: When making decisions, we often rely on an internal model of our actions and their consequences to consider our options and choose the one that is currently most beneficial. Encoded action-outcome memories support such thoughtful, flexible goal-directed decisions. Goal-directed behavior is, however, cognitively taxing. Therefore, humans and other animals have another strategy for more routine behaviors, habits. Habits are formed with repeated practice and enable behavior to be more automatically executed based on past success, rather than forethought of consequences. We balance goal-directed decisions and habits to allow behavior to be adaptive when needed, but efficient when appropriate. Despite importance to understanding adaptive and maladaptive decision making, much is unknown about the neuronal circuitry that supports action-outcome learning and habit formation. The midbrain dopamine system may be involved. Dopamine neurons increase firing in response to unexpected rewards. This signal has been canonically been interpreted as the prediction error term in the model-free temporal difference reinforcement learning algorithms that support habit formation. Resent research, however, indicates that dopamine is also involved in model-based learning. Dopamine may regulate action-outcome and habit learning through projections to subregions of the amygdala. Based on the known function of the basolateral amygdala (BLA) in goal-directed behavior and the central amygdala (CeA) in habit, our hypothesis is that ventral tegmental area (VTA) dopamine projections to the BLA mediate action-outcome learning for goal-directed behavioral control and VTA dopamine projections to the CeA mediate habit formation. To address this, we are using fiber photometry recording of dopamine sensors to characterize dopamine release in the BLA and CeA during instrumental lever press - food reward conditioning on schedules of reinforcement that either promote action-outcome learning and goal-directed behavior or habit formation. For a functional assessment, we are optogenetically inhibiting VTA dopamine projections to CeA or BLA at the time of reward during learning and evaluating the extent of action-outcome v. habit learning with the outcome-specific devaluation test.

Disclosures: A.K. Crawley: None. **K. Nett:** None. **A. Shamoony:** None. **J. Giovanniello:** None. **K.M. Wassum:** None.

Poster

PSTR414

Neural Circuits of Reward and Appetitive Learning and Memory

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR414.03/L18

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant DA046679

Title: Convergent and divergent function of dorsomedial striatum D1 and A2A neurons in action-outcome and habit learning

Authors: *M. MALVAEZ[1], J. GIOVANNIELLO[2], N. PAREDES[3], A. M. WIKENHEISER[2], K. M. WASSUM[2];
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Abstract: Optimal behavior relies on a balance between two distinct strategies. During goal-directed decision making the relationship between actions and their consequences is considered to enable adaptive choices. Habits allow routine tasks to be conducted more automatically, without forethought of their consequences. The balance between these systems allows behavior to be adaptive when needed and efficient behavior when appropriate. But disruption of this balance can lead to symptoms characteristic of several psychiatric and neurological diseases. Goal-directed behavior relies on the dorsomedial striatum (DMS). Little is known about the contribution of the two major striatal projection neurons (SPNs) subtypes: D1 and A2A type neurons. Using cell-type specific cellular resolution microendoscopic calcium imaging and chemogenetic manipulation coupled with instrumental lever press-food reward conditioning and outcome-specific devaluation tests, we characterized the function of these two DMS neuron subtypes in goal-directed learning. DMS D1 neurons are active throughout instrumental learning, especially around action initiation. The D1 ensemble that represents action initiation is stable from early goal-directed learning through the transition to habit. Correspondingly, DMS D1 neurons are required for goal-directed learning and the expression of goal-directed behavior. DMS D1 neuron activity is also sufficient to promote goal-directed behavioral control even after overtraining, which would normally enable habit. DMS A2A neurons are also active during instrumental learning around action initiation. However, the A2A ensemble representing action initiation shifts as behavioral control transitions from goal-directed to habitual. Accordingly, DMS A2A neurons are necessary for early goal-directed learning, but are insufficient to promote goal-directed behavior after overtraining. Thus, DMS D1 and A2A neurons have coordinated function to promote the action-outcome learning that supports flexible goal-directed behavioral control early in learning. But only D1 neurons maintain a representation of goal-directed actions as habits form, whereas A2A neurons lose such a representation and instead come to represent habitual behavior.

Disclosures: M. Malvaez: None. **J. Giovanniello:** None. **N. Paredes:** None. **A.M. Wikenheiser:** None. **K.M. Wassum:** None.

Poster

PSTR414

Neural Circuits of Reward and Appetitive Learning and Memory

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Program #/Poster #: PSTR414.04/L19

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH R01DA057084

Title: Cue-evoked dopamine release in the nucleus accumbens core encodes reward predictions for strategy selection

Authors: *N. GRIFFIN[1], M. MALVAEZ[2], A. SUAREZ[2], S. B. OSTLUND[3], K. M. WASSUM[2];
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Abstract: Environmental cues that signal rewarding events are a major source of motivation. To ensure adaptive motivation, our responses to reward cues are regulated by the reward predictions they generate. While it is adaptive to exert effort engaging in exploratory reward seeking when rewards are predicted to be sparsely available, this urge to explore should be suppressed in favor of waiting when reward delivery is expected soon. We asked how dopamine release in the nucleus accumbens core (NAc) is related to the influence of cue-evoked reward predictions over motivational strategy. We used a Pavlovian-to-instrumental transfer task in which cues signaling reward with low probability (30%) invigorate instrumental lever pressing (reward seeking), whereas cues signaling reward with high probability (90%) suppress seeking in favor of waiting at the reward-delivery port for the imminently expected reward. Using fiber photometry dopamine sensor recordings, we found that high reward probability cues elicit more dopamine release in the NAc than those signaling lower reward probability. Thus, cue-evoked NAc dopamine is shaped by reward probability prediction. Optogenetic inhibition of cue-evoked NAc dopamine increased reward seeking in response to the highly predictive cue, similar to the response typically elicited by a weakly predictive cue. Optogenetic stimulation to augment the cue-evoked NAc dopamine to the low probability cue increased waiting at the reward-delivery location, similar to motivational response typically elicited by a high probability cue. Thus, NAc dopamine conveys reward probability prediction to control motivational strategy.

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Poster

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: This work was supported by the DICBR of the NIAAA [ZIA AA000455 to AJK].

Title: Chemogenetic manipulations of medial septum glutamate neurons alters nucleus accumbens dopamine dynamics during reward-seeking behaviors

Authors: *A. KESNER[1], S. RAMOS-MACIEL[2], N. WESTCOTT[3], M. MARKS[4], C. DARDEN[5];
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Abstract: The septum was first region discovered by Olds and colleagues to support electrical intracranial self-stimulation in the rat. Further studies in the 1970s showed humans will similarly press a button to earn intra-septal electrical stimulation. Despite these landmark studies, further interest in the septum, in particular the medial septum (MS), as a locus for reward related behaviors remained limited. We previously found that mice will lever press to earn optogenetic stimulation of the MS, and in particular MS glutamate neurons (MS-GLUn), and MS-GLUn in turn project to the VTA to influence dopamine (DA) release in the nucleus accumbens (NAc) (Kesner et al., 2021). Little else is known about the role of MS-GLU neurons during natural reward-seeking behaviors. To address this knowledge gap, we recorded MS-GLUn population activity using GCaMP7F and fiber photometry techniques while mice performed various operant and Pavlovian reward-seeking behaviors. We found that MS-GLUn indeed respond differentially to reward-related stimuli (e.g., active vs inactive lever presses, reward consumption, and reward predictive cues). We next modulated MS-GLUn activity using a chemogenetic approach (Gi and Gq DREADDs, or mCherry control), and found that enhancing MS-GLUn excitability increased the rate that mice incorporated new information to obtain goals, i.e., strategy switching. Since we know optogenetic stimulation of MS-GLUn can increase NAc-DA, we next hypothesized that the effect on strategy switching behavior from chemogenetic modulation of MS-GLUn may be driven by resultant changes in NAc-DA during these tasks. We recorded NAc-DA via fiber photometry of dLight1.3b during the same operant and Pavlovian strategy switching behaviors while MS-GLUn were chemogenetically manipulated. We observed differences in NAc-DA during these tasks dependent on chemogenetic modulation of MS-GLUn that appears to correspond to NAc-DA responses to new reward related cues/stimuli. These findings are an important step in understanding the role of this understudied population of neurons in an understudied brain region related to reward and motivational processes, and could lead to novel therapeutic interventions for treating psychiatric disorders related to maladaptation in motivated behaviors.

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Poster

PSTR414

Neural Circuits of Reward and Appetitive Learning and Memory

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Program #/Poster #: PSTR414.06/L21

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R01MH104450

Title: Nucleus accumbens ensembles encode social reward

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Abstract: Autism Spectrum Disorder (ASD) is a prevalent neurodevelopmental condition affecting approximately 1% of the world population. Characterized by impaired social interactions, the precise etiology of ASD remains elusive, but emerging research suggests deficits in the mesostriatal pathway may contribute to deficits in social reward processing. In order to better understand the role of the mesostriatal pathway in social reward processing, I performed *in vivo* microscopy coupled with viral delivery of genetically encoded fluorescent sensors to record nucleus accumbens (NAc) neuronal ensemble activity during an operant task in which mice were trained to press a lever in order to gain access to a social partner mouse. I found distinct populations of neurons that were more active or less active during the social reward task at discrete time points. Understanding the neurobiological mechanisms involved in social reward may pave the way for novel medications and treatments for ASD.

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Poster

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Program #/Poster #: PSTR414.07/L22

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant P50 MH096889

Title: Sex Differences in the Role of a CRH/GABA Expressing Basolateral Amygdala-Nucleus Accumbens Projection in Motivated Behaviors after Early Life Adversity

Authors: *L. TANIGUCHI[1], C. M. GOODPASTER[2], M. T. BIRNIE[3], G. DE CARVALHO[1], A. CHIANG[1], A. PAUL[1], Y. CHEN[3], L. Y. CHEN[1], L. A. DENARDO[2], T. Z. BARAM[1,3]; [1]Anat. and Neurobio., UC Irvine, Irvine, CA; [2]Physiol., UCLA, Los Angeles, CA; [3]Pediatrics, UC Irvine, Irvine, CA

Abstract: Rationale: Early life adversity (ELA), such as childhood neglect, poverty, or abuse, is associated with an increased risk for mood disorders such as depression and drug use disorders. In our limited bedding and nesting mouse model, ELA induces anhedonia-like behaviors in adult male mice while increasing motivation for rewards such as palatable food and sex cues in adult females. While disruptions in reward circuits are involved in anhedonia, it is unclear how and if ELA affects the developing reward circuitry. We identified a novel corticotropin-releasing hormone (CRH) expressing GABA-ergic projection connecting the basolateral amygdala (BLA) to the nucleus accumbens (NAc) and have observed that it mediates reward-seeking deficits in adult males following ELA. The pathway's function in augmenting reward behavior following ELA in female mice is unknown. Here, we investigate the function of this projection in female mice and the hypothesis that there are fundamental differences in the projection to explain sex or ELA-dependent differences in its function. Methods: CRH-Cre mice raised in either control or ELA conditions were injected bilaterally into the BLA with an excitatory or inhibitory Cre-dependent DREADD, followed by infusion of clozapine N-oxide (CNO) or vehicle into the medial shell NAc preceding reward behavioral tasks. Separately, mice were injected bilaterally into the BLA with a Cre-dependent channelrhodopsin. We mapped BLA CRH projections using brain clearing to determine differences in NAc innervation quantity and location using brain clearing. We verified that the projection is GABA-ergic using immunostaining and electrophysiology. Results: Excitation or inhibition of the projection did not influence reward behaviors in either control or ELA female mice, suggesting that the pathway is not involved in mediating reward behavior in females. Brain clearing data suggests sex and ELA-specific quantification differences of projection innervations across different axes of the NAc. Experiments are ongoing to verify the projection's neurotransmitter identity and target cells in females. Experiments using optogenetics to elucidate the projection's function in females are ongoing. Conclusions: We characterized a novel CRH/GABA BLA-NAc pathway mediating the effects of ELA on reward circuits in male mice and are striving to determine if the function, neuroanatomy, and cell-type specificity are the same in females. Investigating this projection will give us a deeper understanding of intrinsic sex differences in the organization of the reward circuitry and the effects of ELA on reward behaviors that underlie many mood disorders.

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Poster

PSTR414

Neural Circuits of Reward and Appetitive Learning and Memory

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: NSERC RTI
NSERC Discovery Grant

Title: Simultaneous multi-region optogenetic stimulation with single-cell resolution calcium imaging to probe integration of glutamatergic afferents in nucleus accumbens

Authors: *S. WU[1], E. IYER[1], P. VITARO[3], R. C. BAGOT[2,4];
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Abstract: The nucleus accumbens (NAc) is a key region implicated in reward processing and goal-directed behavior. The NAc integrates various glutamatergic inputs from brain regions, including the medial prefrontal cortex (mPFC) and ventral hippocampus (vHIP). mPFC and vHIP inputs to NAc have been specifically associated with differential adaptation to chronic stress and reward-related behaviors. In vivo electrophysiology studies in anesthetized animals suggest complex gating interactions between mPFC-NAc and vHIP-NAc afferents with non-linear integration of afferent signals. Ex vivo electrophysiology studies find a high degree of convergence of glutamatergic inputs on individual NAc medium spiny neurons (MSNs). However, how individual MSNs integrate distinct glutamatergic afferents to support behavior has not been directly examined. Here, using the recently developed Chromatone multi-region miniscope (Bruker), we combine projection-specific optogenetic stimulation with single-photon single-cell resolution calcium imaging in NAc in freely behaving mice. By independently stimulating mPFC and vHIP inputs to NAc using fully automated triggering, and recording post-synaptic activity at single-cell resolution with synchronized behavioral monitoring, we seek to identify novel motifs of behaviorally significant neural integration.

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Poster

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: Fulbright-Anid Scholarship 56160012
NIMH Grant R01MH068073

Title: Effect of chemo-genetic inhibition of basolateral amygdala neurons projecting to the nucleus accumbens on autoshaped anticipation to differentially informative cues

Authors: *J. I. MALLEA[1,3], M. A. BUTTON[3], T. SOHRABI[3,4], I. TELLEZ[5,6], E. H. SIMPSON[3,2], P. D. BALSAM[5,2];
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Abstract: When experiencing the occurrence of distinctive cues preceding the delivery of a reward, animals, including humans, eventually display differential behavior; approaching and interacting with the expected location of the reward (i.e., Goal-Tracking; GT). They can also direct this behavior towards the cue informative of the reward (i.e., Sign-Tracking; ST). A strong predominance of sign-tracking behavior is associated with a variety of maladaptive behaviors including higher susceptibility to drug abuse, risky decision-making, lower behavioral-flexibility, reduced impulse control, and attentional deficits. Sign-tracking is affected by uncertainty with respect to the delivery of reward as well, with increased levels of ST towards more uncertain cues (e.g., partial reinforcement). Multiple brain regions are involved in the emergence and development of ST behavior, including the basolateral amygdala (BLA) and the nucleus accumbens (NAcc). These two regions are critical for the and expression of ST; and importantly, population activity in the BLA is sensitive to changes in predictability of the stimuli. Given this, the present experiments aimed to explore the role of the BLA, on the acquisition and expression of sign and goal tracking in the context of a within-subjects 2-cue probabilistic learning paradigm. For this, we injected a virus expressing the Designer Receptor Exclusively Activated by Designer Drug (DREADD) receptor, hM4D(Gi) in the BLA of c57 mice. Neural activity in the BLA was chemo-genetically inhibited during a Pavlovian learning task by intraperitoneal injection of the DREADD ligand, JHU37160, 30 minutes before behavioral sessions. During the task, the presentation of 2 distinctive cues (right and left lever) were followed by reward in either 25 or 100% of trials, but with the same mean delay to reward (1 reward every 24s). Under these conditions, animals that received a control virus showed rates of ST and GT behavior directly and inversely related to the probability of reward (respectively). Critically, subjects that experienced the task while BLA was inhibited, showed no differences in the rate of ST across cues, while still showing different rates of GT behavior to each cue, similar to subjects in the control group. In a second experiment, we extended these results by selectively targeting BLA neurons that project to the NAcc. This was achieved by injecting a cre-expressing retrograde virus in the NAcc, and a cre-dependent hM4D(Gi) virus in BLA. Overall, the results suggest that ST behavior is driven by the information signaled by cues, and that BLA neurons are necessary for information to drive this behavioral phenotype during learning.

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Poster

PSTR414

Neural Circuits of Reward and Appetitive Learning and Memory

Location: MCP Hall A

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Program #/Poster #: PSTR414.10/L25

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Neuronal signatures of Pavlovian to Instrumental Transfer within basolateral amygdala circuits

Authors: *C. RIFFAULT[1], Y. BITTERMAN[2], A. LUTHI[3], J. COURTIN[4];
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Abstract: Reward-predictive Pavlovian cues can selectively invigorate instrumental behaviors, a phenomenon known as specific Pavlovian to Instrumental Transfer (sPIT). Although accumulating evidence pinpoints the basolateral amygdala (BLA) as a key brain structure supporting sPIT, its encoding principles within BLA circuits remain unknown. Here, we tracked the activity of the same BLA neurons across the different days of a behavioral assay, including Pavlovian learning, instrumental learning, and the final sPIT. We found that the neuronal ensembles activated at the time of Pavlovian-conditioned responses and instrumental actions during learning were overlapping and were reactivated during sPIT. This neuronal representation that generalizes across Pavlovian and instrumental behaviors was outcome-specific and correlated positively with sPIT performance. Interestingly, neuronal ensembles activated at the time of Pavlovian cues during learning were not overlapping with the other ensembles and were reactivated during sPIT. These ensembles emerged mainly at the onset of the cues and were characterized by poor outcome specificity and no correlation with sPIT performance. These findings highlighted two distinct neuronal signatures within BLA circuits that can explain the sPIT phenomenon.

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Poster

PSTR414

Neural Circuits of Reward and Appetitive Learning and Memory

Location: MCP Hall A

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Topic: G.02. Reward and Appetitive Learning and Memory

Title: The Role of Central Amygdala CRF Neurons in Recruiting Reward Circuitry

Authors: *R. THAKRAR[1], K. EMERY[2], K. C. BERRIDGE[3];
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Abstract: Corticotropin releasing factor (CRF) is a stress-associated peptide generally thought to generate aversive states like anxiety and distress. However, recent evidence suggests that optogenetic activation of CRF neurons in the nucleus accumbens (NAc) and central amygdala (CeA) can generate positive incentive motivation and increase the desire for a reward without producing distress or negative affective states. Activation of CeA and NAc CRF neurons also recruits and activates reward systems, including the VP, VTA, and LH. However, it is unclear if CRF receptor activation is required for fos recruitment of reward systems since optogenetic activation of CeA CRF neurons also likely causes co-release of other neurotransmitters such as GABA and somatostatin. Utilizing fos counts as a measure of neuronal activity, we administered an intraventricular CRF antagonist in *Crh*-Cre rats prior to CeA laser optogenetic stimulation and examined local and distant fos recruitment to determine whether the incentive effects of the CeA CRF neuronal activation are specifically due to activation of CRF signaling mechanisms. Given that the circuitry underlying this recruitment is unknown and that CRF neurons in the CeA tend to be GABAergic interneurons, we also sought to determine whether CRF microinjections within the CeA are capable of generating incentive motivation in a conditioned place preference paradigm. These findings will help elucidate the role of CeA CRF neurons in generating positive incentive motivation and activating reward systems in the brains.

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Poster

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant DA051662
NIH Grant DA045913

Title: Optogenetic stimulation or inhibition of dopamine neurons alters behavior and event encoding in the nucleus accumbens during Pavlovian conditioning

Authors: *E. W. HERRING, K. LEAR, E. MCLAUGHLIN, S. Y. ZENG, T. SYAMALA, P. A. KUPELIAN, E. PATEL, S. E. MORRISON;
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Abstract: If a Pavlovian cue predicts reward in a different location, some animals will preferentially approach and interact with the cue – a behavior known as sign tracking – while

others will approach the site of reward delivery, a behavior known as goal tracking. Acquisition of sign tracking, but not goal tracking, depends on changes in dopamine release in the nucleus accumbens (NAc). We have previously demonstrated that reward-evoked activity in the NAc reflects what we know to be different patterns of dopamine release in sign trackers vs. goal trackers. However, it remains unclear whether there is a causal relationships among dopamine release, NAc neural activity, and sign tracking. Therefore, we used male and female TH (tyrosine hydroxylase)-Cre rats to express an excitatory or inhibitory opsin specifically in dopamine neurons of the ventral tegmental area (VTA). In order to target the dopaminergic reward prediction error signal, we stimulated or inhibited dopamine neurons at the time of reward delivery for the first several sessions of Pavlovian conditioning.We found that inhibition of VTA dopamine neurons completely abolished the acquisition of sign tracking, but not goal tracking. Surprisingly, stimulation of dopamine neurons did not speed up or strengthen the acquisition of sign tracking, implying that there is a form of "gain control" in the mesolimbic dopamine system; however, removal of stimulation disrupted further acquisition of sign tracking (but not goal tracking) for several sessions. At the same time, we recorded from individual neurons in the NAc during either stimulation or inhibition of VTA dopamine neurons. We found that, in a subset of neurons, stimulation of dopamine neurons enhanced both excitatory and inhibitory responses to reward delivery, while inhibition primarily decreased the magnitude of excitatory reward responses. Meanwhile, inhibition at the time of reward prevented neural responses to the cue from increasing over the course of acquisition. Overall, these findings support the idea that sign tracking, but not goal tracking, depends upon dopaminergic reward signaling, which in turn promotes excitatory responses to both cue and reward in the NAc.

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Poster

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant DA051662
NIH Grant DA045913

Title: Distinct profiles of nucleus accumbens activity in adolescent vs. adult rats during Pavlovian conditioning

Authors: T. SYAMALA[1], S. Y. ZENG[2], E. HERRING[2], *S. MORRISON[2], P. A. KUPELIAN[3]; [1]Neurosci., Univ. of Pittsbugh, Pittsburgh, PA; [2]Neurosci., [3]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: When a cue is paired with reward in a different location, some animals will approach and interact with the site of reward during cue presentation - a behavior called goal tracking - whereas others will approach and interact with the cue itself: a behavior called sign tracking. Sign tracking is thought to reflect a tendency to transfer incentive salience from a reward to a predictive cue. Across species, adolescence is a time of heightened sensitivity to rewards, along with enhanced impulsivity, exploration, and risk-taking. Surprisingly, we and others have shown that adolescents are actually less likely to perform sign tracking behavior, and more likely to perform goal tracking behavior, than adult animals. This is consistent with some findings indicating that adolescents are also less likely to perform cued habitual actions. However, the neural basis for this difference is unknown.
To address this question, we recorded from individual neurons in the nucleus accumbens (NAc) of adolescent male rats (P35-42) during the acquisition and expression of sign tracking and/or goal tracking behavior. In a subset of these subjects, we recorded from the same individuals again after they attained adulthood. We found that adolescent behavior and NAc signaling differs from adults in several ways. Consistent with previous findings, we identified more goal trackers and fewer sign trackers among adolescents; and even among adolescents identified as sign trackers, sign tracking was more vigorous in adulthood. Unexpectedly, NAc neural responses to both cues and rewards were lower in adolescents, relative to adults, at the start of training, but increased rapidly over the course of acquisition. After training, cue responses were similar in the two age groups, but neural responses to reward were significantly higher in adolescents than in adults, including within individual subjects. In fact, the change in reward-evoked activity from adolescence to adulthood resembled the difference in reward response magnitude between adult goal trackers and sign trackers. These findings may provide part of a neural mechanism for adolescents' enhanced reward sensitivity, and their tendency to perform goal-tracking rather than sign-tracking behavior.

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Poster

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant DA14241
NIH Grant DA036151

Title: Effect of chronic nicotine on VTA cholinergic and GABAergic signaling during progressive ratio responding for a palatable reward

Authors: *K. KIM[1,2], M. SARKOZY[1], M. PICCIOTTO[1];
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Abstract: Tobacco smoking remains the leading cause of preventable death in the United States. Nicotine is the main psychoactive component in tobacco that lends its abuse potential. Vaping, or e-cigarette use, is rising in popularity and most smoking cessation treatments consist of nicotine replacement products. The question remains as to how long-term nicotine exposure affects reward-seeking behavior. Nicotine acts in the ventral tegmental area (VTA), a midbrain structure that mediates the rewarding and motivational effects of addictive drugs. Within the VTA, expression of nicotinic acetylcholine (ACh) receptors on both dopamine and GABA neurons is essential for nicotine reinforcement. Furthermore, cholinergic signaling via nicotinic and muscarinic ACh receptors in the VTA has been shown to be necessary for appetitive learning. In the current study, we investigated the role of VTA cholinergic signaling and GABAergic activity during progressive ratio (PR) responding for a palatable reward, and how chronic nicotine exposure affects the activity of these circuits and motivation for reward. We used dual-sensor fiber photometry to record intracellular calcium levels in VTA GABA neurons using the red genetically-encoded calcium indicator RCaMP, while also recording extracellular ACh levels in VTA using the genetically-encoded fluorescent GRAB ACh sensor during reward-motivated behaviors such as Pavlovian conditioning, conditioned reinforcement tests, and PR. The activity of VTA GABA neurons increased in response to consumption of rewards and delivery of a conditioned stimulus (CS) as it gained incentive value. Similarly, extracellular ACh levels in VTA also increased in response to delivery of rewards and CS. We then exposed mice to nicotine chronically through their drinking water and trained them on PR for a palatable reward. Mice were trained on operant responding via increasing fixed-ratio (FR) schedules of reinforcement, then tested on PR. There were significant sex differences in the number of active nose pokes made on the FR5 schedule, as well as on the first two sessions of PR. On the third PR session, mice exposed to chronic nicotine reached a higher breakpoint compared to controls. This effect of nicotine was largely driven by the male nicotine-treated mice. Finally, we recorded changes in VTA GABA neuron activity and extracellular ACh levels in VTA using fiber photometry in mice treated with nicotine during operant training and PR paradigms. Our results support the idea that both cholinergic and GABAergic signaling in VTA are involved in reward-seeking behaviors, and that nicotine exposure enhances motivation to work for palatable rewards.

Disclosures: K. Kim: None. **M. Sarkozy:** None. **M. Picciotto:** None.

Poster

PSTR414

Neural Circuits of Reward and Appetitive Learning and Memory

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR414.15/L30

Topic: G.02. Reward and Appetitive Learning and Memory

Support: K99DA060209
R01DA022340

Title: A Pallido-Midbrain Endocannabinoid Circuit for the Encoding of Threat-Predictive Cues

Authors: *M. Á. LUJÁN[1], R. YOUNG-MORRISON[2], J. F. CHEER[3];
[1]Neurobio., Univ. of Maryland, Sch. of Med., Baltimore, MD; [2]Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Anat. and Neurobio., Univ. of Maryland Sch. of Med., Columbia, MD

Abstract: Proactive behavioral control over threat-predictive stimuli allows individuals to avoid aversive events and maximize environmental fitting by diminishing generic threat responses (i.e., freezing). This learning process depends on phasic dopamine signals in the mesolimbic system that encode the association between threat-predictive stimuli and aversive outcomes. In this study, we investigate how the endocannabinoid (eCB) system modulates dopamine signaling and active avoidance learning in mice. We use viral-genetic tools to selectively delete the enzyme diacylglycerol lipase alpha (DGLa) in ventral tegmental area (VTA) dopamine neurons, which impairs the synthesis of the eCB 2-arachidonoylglycerol (2-AG). We also use fiber photometry to measure phasic dopamine release (GrabDA) in the nucleus accumbens (NAc) and eCB release (GrabeCB$_{2.0}$) in the VTA during active avoidance training. We reveal that midbrain dopamine neurons require the mobilization of 2-AG to sculpt NAc dopamine release events predictive of active avoidance learning. DGLa deletion in VTA dopamine neurons impairs active avoidance performance, but not escape behavior. We also find that VTA dopamine neurons release 2-AG in response to foot-shock presentations. To identify the presynaptic target of this 2-AG retrograde signal, we delete cannabinoid receptor type 1 (CB1R) in ventral pallidum (VP) terminals that project to the VTA. We show that this manipulation mimics the effects of DGLa deletion on NAc dopamine signals and active avoidance learning. These results reveal a novel $VTA_{TH+} \rightarrow VP_{CB1+}$ 2-AG message sculpting NAc dopamine release events and therefore orchestrating active avoidance learning. This mechanism could have implications for developing new treatments for anxiety disorders based on enhancing proactive coping strategies.

Disclosures: M.Á. Luján: None. **R. Young-Morrison:** None. **J.F. Cheer:** None.

Poster

PSTR414

Neural Circuits of Reward and Appetitive Learning and Memory

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR414.16/L31

Topic: G.02. Reward and Appetitive Learning and Memory

Support: CONAHCyT
DGAPA-PAPIIT-UNAM
ProductosMedix

Title: Top-down circuitry from the anterior insular cortex to VTA dopamine neurons modulates drug-related reward-memory

Authors: *E. ORTIZ[1,2], J. LUIS ISLAS[3], F. TECUAPETLA[4], R. GUTIERREZ[5], F. BERMUDEZ-RATTONI[6];
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Abstract: The insular cortex (IC) has been linked to the processing of interoceptive and exteroceptive signals associated with addictive behavior. However, whether the IC modulates the acquisition of drug-related affective states by direct top-down connectivity with ventral tegmental area (VTA) dopamine neurons is unknown. We found that photostimulation of VTA terminals of the anterior insular cortex (aIC) induces rewarding contextual memory, modulates VTA activity, and triggers dopamine release within the VTA. Employing neuronal recordings and neurochemical and transsynaptic tagging techniques, we disclose the functional top-down organization tagging the aIC pre-synaptic neuronal bodies and identifying VTA recipient neurons. Furthermore, systemic administration of amphetamine altered the VTA excitability of neurons modulated by the aIC projection, where photoactivation enhances, whereas photoinhibition impairs, a contextual rewarding behavior. Our study reveals a key circuit involved in developing and retaining drug reward-related contextual memory, providing insight into the neurobiological basis of addictive behavior, and helping develop therapeutic addiction strategies.

Disclosures: E. Ortiz: None. **J. Luis Islas:** None. **F. Tecuapetla:** None. **R. Gutierrez:** None. **F. Bermudez-Rattoni:** None.

Poster

PSTR414

Neural Circuits of Reward and Appetitive Learning and Memory

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR414.17/L32

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH R01 DA046457

Title: Sex differences in afferents to dorsal striatum in the rat

Authors: *A. N. MILLER[1], S. HANDEL[1], M. PALADINO[1], R. J. SMITH[1,2];
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Abstract: The dorsal striatum is implicated in cognitive and motor functions and can be divided into two main subregions, the dorsomedial striatum (DMS) and dorsolateral striatum (DLS), which are critical for goal-directed and habitual responding, respectively. Previous work in our lab characterized and compared the brain structures that project to DMS or DLS in male rats. Here, we aimed to examine potential sex differences by characterizing and comparing DMS and DLS inputs in female rats. We injected retrograde tracers (cholera toxin β-subunit or Fluorogold) into DMS or DLS of female Sprague Dawley rats and then qualitatively characterized retrograde labeling in different brain regions as light, medium, or heavy based on cell density. We found that for both males and females, DMS and DLS received heavy input from cortex and thalamus, and light to medium input from amygdala and brainstem structures. DMS received input from medial prefrontal cortex, whereas DLS received input from lateral areas in frontal cortex, as well as motor and sensory cortices. For DMS, males and females showed similarity in afferent structures and density. However, for DLS, females showed greater ipsilateral and contralateral input from primary somatosensory cortex and insular cortex (dysgranular and granular). These results show sex differences in DLS connectivity, which may indicate sex differences in DLS function and habit learning.

Disclosures: A.N. Miller: None. **S. Handel:** None. **M. Paladino:** None. **R.J. Smith:** None.

Poster

PSTR415

Motivation: Compulsive Behaviors, Impulse Control, and Addiction

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR415.01/L33

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA-IRP/NIH

Title: Incubation of discriminative stimulus-controlled heroin seeking in rats

Authors: *H. E. BONBREST[1], D. PHAM[2], M. B. BRENNER[3], V. A. LENNON[4], O. R. DRAKE[4], S. J. WEBER[2], O. BERKO[2], Y. GERA[5], J. M. BOSSERT[6], Y. SHAHAM[7], B. T. HOPE[8], R. MADANGOPAL[9];
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Abstract: Background: We previously reported that rats' response to cocaine-associated discriminative stimuli (DSs) progressively increases (or incubates) during the first 60 abstinence days and persists up to 300 days (Madangopal, *eLife*, 2019). Here we tested if incubation of DS-

controlled drug seeking generalizes to heroin.
Methods: We first trained rats to self-administer heroin using a continuous access procedure (2 x 3 h/d; 0.025 mg/kg/infusion; no drug-paired CSs). Next, we used our trial-based procedure to train rats to continue heroin self-administration during DS+ trials (heroin available), but not DS- trials (heroin unavailable) in the same session (2 x 3 h/d; 30 each DS+/DS- trials). In Experiment 1, we repeatedly tested rats for DS-controlled heroin seeking (3 h; 30 each DS+/DS- trials; extinction conditions) after 1, 21, 60, 120, 200, 300 and 385 abstinence days. In Experiment 2, we tested contributions of DS+/DS- to heroin seeking after 21 abstinence days (3 h; 15 each *no-DSs*, *DS+*, *DS-*, and *both-DSs* trials; extinction conditions).
Results: In Experiment 1, DS-controlled relapse to heroin seeking was higher after 21 and 60 days of abstinence than after 1 day and persisted for up to 385 days. In Experiment 2, responding on day 21 was low during 'DS-' trials, intermediate during 'no-DSs', and 'both-DSs' trials, and maximal during 'DS+' trials.
Conclusions: Incubation of DS-controlled drug seeking generalizes to heroin. Additionally, DS+ and DS- independently control expression and suppression of heroin seeking during abstinence. We are investigating DS-specific neuronal ensembles that contribute to this new form of incubation of heroin seeking.

Disclosures: H.E. Bonbrest: None. **D. Pham:** None. **M.B. Brenner:** None. **V.A. Lennon:** None. **O.R. Drake:** None. **S.J. Weber:** None. **O. Berko:** None. **Y. Gera:** None. **J.M. Bossert:** None. **Y. Shaham:** None. **B.T. Hope:** None. **R. Madangopal:** None.

Poster

PSTR415

Motivation: Compulsive Behaviors, Impulse Control, and Addiction

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR415.02/L34

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA IRP / NIH

Title: Brain-wide activation patterns of persistent discriminative stimulus-controlled cocaine seeking in rats

Authors: *O. BERKO[1], Y. GERA[2], D. PHAM[1], M. BRENNER[1], V. A. LENNON[3], O. R. DRAKE[1], S. J. WEBER[4], L. E. KOMER[1], H. BONBREST[5], L. A. RAMSEY[1], J. M. BOSSERT[6], F. RUBIO[7], Y. SHAHAM[8], B. T. HOPE[9], R. MADANGOPAL[10];
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NIH/NIDA, Baltimore, MD; [10]Neuronal Ensembles in Addiction Section, NIH, NIDA IRP, Baltimore, MD

Abstract: Background: Cues previously associated with drug-taking can precipitate relapse long after the last drug-taking experience. Discriminative stimuli (DSs) signal drug availability (DS+) or unavailability (DS-), and guide drug seeking-behavior. We previously reported that DS-controlled cocaine seeking progressively increases (or incubates) during abstinence and persists up to 300 days (Madangopal, *eLife*, 2019). Here we used the activity marker Fos to quantify brain-wide activation patterns following DS-controlled cocaine seeking during early and late abstinence.
Methods: We used our trial-based procedure to train rats to self-administer cocaine (0.75 mg/kg/infusion) during DS+ trials (cocaine available) and suppress responding during DS- trials (cocaine unavailable) during the same session (2 x 3 h/d; 30 each DS+/DS- trials). Next, we tested rats for cocaine seeking after 1 or 21 days of abstinence (1.5 h; 15 each DS+/DS- trials; extinction conditions) and extracted brains either immediately after the test (day 1 or day 21 test groups) or directly from the homecage (no-test group).
Results: We observed reliable DS-controlled cocaine self-administration during training and time-dependent DS-controlled relapse to cocaine seeking during abstinence. Fos-based activity mapping identified multiple cortical, striatal, and thalamic brain regions that showed (1) increased activation following DS-controlled relapse (test vs. no-test) and (2) time-dependent increase in activation during abstinence (day 1 test vs. day 21 test).
Conclusions: Incubation of DS-controlled cocaine seeking is associated with increased neuronal activity in multiple brain regions. We are currently using a dual-memory labeling strategy to identify brain regions that contain separate DS+ and DS- ensembles that support DS-controlled cocaine seeking.

Disclosures: O. Berko: None. **Y. Gera:** None. **D. Pham:** None. **M. Brenner:** None. **V.A. Lennon:** None. **O.R. Drake:** None. **S.J. Weber:** None. **L.E. Komer:** None. **H. Bonbrest:** None. **L.A. Ramsey:** None. **J.M. Bossert:** None. **F. Rubio:** None. **Y. Shaham:** None. **B.T. Hope:** None. **R. Madangopal:** None.

Poster

PSTR415

Motivation: Compulsive Behaviors, Impulse Control, and Addiction

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR415.03/L35

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA IRP
HHMI Visiting Project Grant

Title: In vivo labeling and molecular characterization of cocaine memory-specific active neurons using the photo-convertible calcium integrator CaMPARI2

Authors: *R. MADANGOPAL[1], K. E. SAVELL[1], O. R. DRAKE[2], M. BRENNER[3], D. PHAM[3], P. SARAVANAN[3], O. NURUDEEN[4], S. J. WEBER[3], V. A. LENNON[3], Y. GERA[1], O. BERKO[3], L. E. KOMER[3], F. RUBIO[3], A. LEMIRE[5], K. SCHAEFER[5], E. R. SCHREITER[6], V. MENON[7], B. T. HOPE[8];
[1]NIH, NIDA IRP, Baltimore, MD; [2]Natl. Inst. On Drug Abuse, Baltimore, MD; [3]NIDA IRP / NIH, Baltimore, MD; [4]NIDA IRP / NIH, BALTIMORE, MD; [5]HHMI Janelia Res. Campus, Ashburn, VA; [6]Janelia Res. Campus, HHMI, Ashburn, VA; [7]Neurol., Columbia Univ., New York, NY; [8]NIH IRP / NIDA, Baltimore, MD

Abstract: Background: In abstinent drug users, drug-associated cues can provoke craving and precipitate relapse long after cessation of drug use. These maladaptive drug-cue associations are encoded in sparse patterns of strongly activated neurons (ensembles) typically identified using immediate early gene based labeling approaches. However, these approaches lack temporal specificity needed to study short behavioral events (e.g., the first minute of cocaine seeking). To address this gap, we used the green-to-red photo-convertible calcium-based activity marker CaMPARI2 to permanently label active neurons *in vivo* during behavior and used single-nucleus RNA sequencing (snRNAseq) for unbiased transcriptomic profiling of CaMPARI-labeled neurons.
Methods: We expressed CaMPARI2 in infralimbic cortex (IL) of male and female Sprague-Dawley rats, implanted optical fibers for photoconversion and inserted jugular catheters for cocaine self-administration. Following self-administration training and 21 abstinence days, we used CaMPARI2-photoconversion to permanently label IL cocaine-memory ensembles during a 1-min cocaine-seeking test. We collected brains either immediately after test (0-min group) or waited 10 minutes for experience-induced gene expression (10-min group). We isolated red (active) and green (inactive) CaMPARI2-labeled nuclei and performed snRNAseq.
Results: We observed reliable cocaine self-administration during training and robust cocaine seeking during the 1-min test on abstinence day 21. CaMPARI2-snRNAseq revealed distinct clusters of glutamatergic and GABAergic IL neurons that subclustered into expected layer and subtypes. Furthermore, IEGs were selectively induced in red neurons from 10-min, but not 0-min group.
Discussion: We will identify unique molecular alterations within IL cocaine-memory ensembles and investigate their distribution across IL cell types. Molecular and cell-type characterization of drug-memory ensembles could identify new targets to selectively weaken persistent drug memories and prevent relapse.

Disclosures: R. Madangopal: None. **K.E. Savell:** None. **O.R. Drake:** None. **M. Brenner:** None. **D. Pham:** None. **P. Saravanan:** None. **O. Nurudeen:** None. **S.J. Weber:** None. **V.A. Lennon:** None. **Y. Gera:** None. **O. Berko:** None. **L.E. Komer:** None. **F. Rubio:** None. **A. Lemire:** None. **K. Schaefer:** None. **E.R. Schreiter:** None. **V. Menon:** None. **B.T. Hope:** None.

Poster

PSTR415

Motivation: Compulsive Behaviors, Impulse Control, and Addiction

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR415.04/L36

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant ZIA DA000467 (BH)
NIH Grant K99 DA059608 (KS)
NIH CCB
NIH SIP

Title: Cell-type specific transcriptional signatures of cocaine relapse in the rat medial prefrontal cortex

Authors: *K. E. SAVELL, R. MADANGOPAL, O. R. DRAKE, D. PHAM, M. BRENNER, D. THOMPSON, A. R. HOLMES, M. KANE, K. WOODS, R. PALAGANAS, P. SARAVANAN, B. T. HOPE;
NIH, NIDA IRP, Baltimore, MD

Abstract: Environmental stimuli previously associated with drug-taking can provoke drug-seeking and precipitate relapse long after cessation of drug use. Maladaptive cue-drug associations are hypothesized to be encoded within specific patterns of neurons activated by the drug-related cues, called neuronal ensembles. Previous region-wide pharmacological inactivation, and ensemble-specific ablation studies identified a critical role for the medial prefrontal cortex (mPFC) in cocaine seeking and cue-induced reinstatement. However, how mPFC ensembles encode this association at the molecular level is unknown. To answer this question, we recently developed Multiple**X**ed **Po**pulation **S**election and **E**nrichment single nucleus RNA-**seq**uencing pipeline (XPoSE-seq) to perform targeted snRNA-seq on cocaine relapse ensembles, which provides an unbiased screen of ensemble-specific cell types and transcriptional changes during cocaine relapse at the individual animal level.
We trained male and female Fos-mRFP rats to self-administer cocaine (FR1 reinforcement schedule, 0.75 mg/kg/inf. cocaine paired with a 3.5-s light cue) during twice daily 3 h sessions. Following 10 days of training and 21 days of abstinence, we tested rats for cocaine seeking (30 min under extinction conditions) and collected brains 3 h after test (peak Fos-driven mRFP expression). We observed reliable cocaine self-administration during training and robust cue-induced cocaine seeking following abstinence. We applied the XPoSE-seq pipeline to identify which cell types comprise the cocaine relapse ensemble and characterize the cell-type specific transcriptional signatures of cocaine relapse. Our analysis revealed distinct clusters corresponding to known cell types in the mPFC (excitatory and inhibitory neurons) that further subcluster into expected layer and interneuron sub-types within mPFC. Using this unbiased approach, ongoing analysis is aimed at characterizing cell-type and ensemble-specific transcriptional signatures that contribute to drug-seeking behaviors. We will employ transcriptional modulators in future experiments to assess causal roles for these cocaine memory-specific genes in relapse and determine relevant circuits.

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Poster

PSTR415

Motivation: Compulsive Behaviors, Impulse Control, and Addiction

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR415.05/L37

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA IRP

Title: Using single synaptoneurosome flow cytometry to identify ribosomal S6 protein as a synaptic activity marker following food and cocaine seeking in rats

Authors: *E. UKPONG[1], Y. GERA[1], D. PHAM[1], D. OLIVARES[1], C. DUNN[2], R. MADANGOPAL[1], F. RUBIO[1], B. T. HOPE[1];
[1]Behavioral Neurosci. Res. Br., NIH, NIDA IRP, Baltimore, MD; [2]Flow Cytometry Unit, NIH, NIA IRP, Baltimore, MD

Abstract: Different stimuli activate sparsely patterns of neurons called neuronal ensembles to mediate highly specific behaviors and memories. These learned behaviors and memories are thought be encoded more specifically within a small subset of selectively activated synapses on these ensembles. However, we do not have a marker to identify these subset of activated synapses that together can be called synaptic ensembles. To identify synaptic activity marker candidates, we developed a flow cytometry of synaptoneurosomes (FCS) procedure to identify protein alterations within individual synapses after behavioral activation. AAV expressing ChR2-eYFP was injected into mPFC 8 weeks prior to stimulation to identify alterations specifically within mPFC-NAc synapses. For initial methods development, we injected cocaine into rats in a novel context. We then isolated synaptoneurosomes (containing both sealed presynaptic and postsynaptic sacs) from the NAc at 0, 5, 10, 30, 60 min timepoints. Synaptoneurosomes were labeled with antibodies against different candidate synaptic acivity markers and analyzed one at a time via FCS. Ribosomal S6-positive synaptoneurosomes were reliably increased 5-60 min following injections. We then used FCS to identify activated synapses following cue-induced cocaine or food seeking. We trained all rats for 10 days (2 x 3 h session a day) to lever press for cocaine infusions or food pellets paired with a 3.5-s light cue. After 3-4 weeks abstinence, rats were exposed for 30 min to either the cocaine- or food-paired light cue. S6-positive synaptoneurosomes were increased after 30 min food seeking but not after 60 min cocaine seeking (30 min cocaine seeking+30 min home cage). We hypothesize that S6 protein acts as a synaptic activity marker due to activity-induced translocation of S6 protein (and

associated ribosomes) from the base of the spine to the spine head, which then returns to the base when synaptic activity ceases.

Disclosures: E. Ukpong: None. **Y. Gera:** None. **D. Pham:** None. **D. Olivares:** None. **C. Dunn:** None. **R. Madangopal:** None. **F. Rubio:** None. **B.T. Hope:** None.

Poster

PSTR415

Motivation: Compulsive Behaviors, Impulse Control, and Addiction

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR415.06/M1

Topic: G.09. Drugs of Abuse and Addiction

Support: DA042029
DA041563

Title: Dorsal striatal dopamine and glutamate release dynamics as a common mechanism for cognitive inflexibility and addiction

Authors: *D. BORTZ, M. M. TORREGROSSA;
Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The presence of general cognitive inflexibility, as measured by cognitive batteries, is a trait marker that can predict the development and severity of substance abuse across species. Although general cognitive inflexibility is conceptually distinct from the inflexibility that drives compulsive drug seeking, these data suggest that the two are related. We hypothesize that a common neurochemical mechanism could contribute to both. Recent work from our lab has found that the development of habit-like, inflexible cocaine seeking behavior is associated with a progressive decrease in neural activity in the dorsal medial striatum (DMS) and increase in dopamine (DA) release in the dorsal lateral striatum (DLS). We hypothesized that cognitive flexibility and flexibility-associated neurochemical signatures in the DMS and DLS would be both predictive of inflexible cocaine seeking and taking and biased by addiction-like patterns of cocaine intake. To test this we first measured DA and glutamate (glut) release in DMS and DLS (fiber photometry) of male and female rats during 10 days of discrimination learning and a subsequent cognitive flexibility test that measures the ability to switch discrimination strategies (strategy switching). We found that poor performance on the strategy switching test was associated with reduced DMS glut release and increased DLS DA release during early portions of the strategy switch, as compared to rats who performed well. This was primarily seen following correct and incorrect choices during the test. All rats were then trained to self-administer cocaine on an FR1 schedule. We found that the rats that performed poorly in the strategy switching test took more cocaine and were more punishment resistant compared to those who performed well, in support of our hypothesis. We then trained a separate set of male and female rats to self-administer cocaine or saline on an FR1 schedule. We measured DA and glut

release in DMS and DLS during the same cognitive behaviors as above during or after cocaine self-administration training. We found that experience with cocaine impaired performance on the strategy switching test compared to saline rats. This impaired performance was associated with reduced DMS glut and elevated DLS DA release during early portions of the strategy switching test, similar to the poor switchers from the prior experiment. These data support our hypothesis and suggest that dynamic fluctuations in DMS and DLS DA and glut release could be targeted to improve cognitive flexibility and reduce compulsive drug seeking.

Disclosures: D. Bortz: None. **M.M. Torregrossa:** None.

Poster

PSTR415

Motivation: Compulsive Behaviors, Impulse Control, and Addiction

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR415.07/M2

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA-NIH DA055068
Mercer Startup Funds
Mercer Seed Grant

Title: The involvement of the noradrenergic system in re-exposure to food seeking after a period of forced abstinence in the absence mPFC Fos neuronal ensembles.

Authors: A. CAROLAND-WILLIAMS[1], L. CALLAN[2], U. MODI[1], C. ARANT[3], C. ARANT[3], A. PATEL[3], H. PATEL[3], J. BELFLOWER[1], J. T. BELFLOWER[1], R. RUDD[2], *A. GHEIDI[4]; [1]Sch. of Med., Mercer Univ., Macon, GA; [2]Biomed. Sci., Mercer Univ., Macon, GA; [3]Mercer Univ., Macon, GA; [4]Biomed. Sci., Mercer Univ. Sch. of Med. (MUSM), Macon, GA

Abstract: Both norepinephrine and medial prefrontal cortex (mPFC) Fos neuronal ensembles have been linked to the resumption of drug-seeking behaviors after a period of abstinence. However, their roles in the return to palatable food seeking following abstinence have been less explored. This study required male and female *Fos-LacZ* transgenic rats to self-administer sugar across ten sessions. *Fos-LacZ* transgenic rats also express the bacterial enzyme β-galactosidase in strongly active neurons. When the prodrug Daun02 is infused into the mPFC of these animals, it is converted to daunorubicin by β-galactosidase, leading to apoptosis of only Fos-expressing neurons. On the last day of sugar self-administration, animals were injected with 50 mg/kg/i.p. of the adrenergic neurotoxin DSP-4 or saline. Rats were then placed into their home cages (forced abstinence) for 10 days. On day 11, they were given a single exposure to the self-administration chambers, and their mPFC was infused with Daun02 (0.2 µg/0.5ul/hemisphere) 90 minutes later before returning to their home cages for 3 more days. On the second exposure, the discrete stimulus was omitted to increase the difficulty in recalling the self-administration memory. Lastly, vaginal lavage from the females was taken to determine their estrus cycle. Preliminary

results showed that rats increased their lever presses over days, and control animals could remember the active lever from the inactive one on both re-exposure days. Interestingly, the DSP-4 group could not distinguish the active from the inactive on re-exposure day 1. We are running more animals to increase sample size, and quantifying Fos with immunofluorescence in the mPFC.

Disclosures: A. Caroland-Williams: None. **L. Callan:** None. **U. Modi:** None. **C. Arant:** None. **C. Arant:** None. **A. Patel:** None. **H. Patel:** None. **J. Belflower:** None. **J.T. Belflower:** None. **R. Rudd:** None. **A. Gheidi:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NIDA Drug Supply Program, NIH.

Poster

PSTR415

Motivation: Compulsive Behaviors, Impulse Control, and Addiction

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR415.08/M3

Topic: G.09. Drugs of Abuse and Addiction

Support: DA031695

Title: Epigenetic manipulation of Anterior Insular Cortex alters neural signals and impulsivity

Authors: *S. PERRY[1], M. R. ROESCH[1], D. R. SARUBIN[2], A. T. BROCKETT[3,4], X. LI[5]; [1]Psychology, Univ. of Maryland, College Park, MD; [2]Psychology, Univ. of Maryland, Maryland, MD; [3]Dept. of Biol. Sci., Univ. of New Hampshire, Durham, NH; [4]Psychology, University of Maryland, Maryland, MD; [5]Dept. of Psychology, Univ. of Maryland, College Park, MD

Abstract: The correct balance between impulsivity and inhibition is a critical feature of self-control and is disrupted in aging, addiction, and numerous other neuropsychiatric illnesses. Both the insula and epigenetic enzymes called histone deacetylases (HDACs) are thought to be dysregulated in many of these disorders, and HDAC inhibitors have been shown to be therapeutic. At the level of a single neuron firing, it is unknown how the insula contributes to impulsivity or how activity is impacted by HDAC manipulation in behaving animals. Here we focus on HDAC5, a class IIa HDAC that shuttles between the nucleus and cytoplasm in response to intracellular signaling. These epigenetic enzymes deacetylate histones resulting in reduced gene transcription rates. Here, we asked how overexpression of HDAC5 in the anterior insula impacts impulse control and related neural correlates in rats performing a STOP task that pits frequent prepotent responding (i.e., GO trials) against infrequent response inhibition and redirection of behavior (i.e., STOP-change trials). We found that HDAC5 overexpression increased and decreased directional neural executive control signals in the anterior insula, which corresponded to increased speed of prepotent responding and reduced ability to inhibit behavior (i.e., increased impulsivity). Lastly, we found that firing in the insula was modulated by trial history, but this feature was not impacted by HDAC5 overexpression. This work implicates that

increased HDAC5 activity in the insula increases impulsivity, suggesting that manipulation of HDAC5 in the insula may provide a potential therapeutic target to improve executive control, as well as other cognitive, emotional, or social factors that might be factored into models of impulsivity.

Disclosures: S. Perry: None. **M.R. Roesch:** None. **D.R. Sarubin:** None. **A.T. Brockett:** None. **X. Li:** None.

Poster

PSTR415

Motivation: Compulsive Behaviors, Impulse Control, and Addiction

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR415.09/M4

Topic: G.09. Drugs of Abuse and Addiction

Support: MRC Grant MR/W019647/1

Title: Relationship between spatial navigation and instrumental memory systems in adaptive and compulsive coping behaviours

Authors: *J. S. INNES, D. J. BELIN;
Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Different types of learning and memory have long been known to be subserved by dissociable neural systems. Animals can learn to navigate towards goals by one of at least two strategies: a dorsolateral striatum-dependent, egocentric response strategy, and a hippocampus-dependent, allocentric place strategy. It has been suggested that place and response navigation reflect the two-fold associative nature of instrumental behaviour: goal-directed actions and habits. Indeed, response navigation/habits and place navigation/goal-directed actions share some of the same predictive factors and are subserved by overlapping neural systems. It follows that the individual tendency to rely on response vs place navigation, often considered a trait, should predict the tendency to rely on habits vs goal-directed actions over instrumental behaviour. As the loss of control over habits is implicated in the development of compulsivity, the individual tendency to employ response navigation and/or instrumental habits may also predict the development of compulsive behaviours, such as those that stem from a loss of control over coping behaviours, operationalised as schedule-induced hyperdipsia in rats. To test these hypotheses, we trained 72 Sprague Dawley rats (36/sex) in a T maze task solvable by place and response navigation. To reveal the underlying strategy, animals received a post-training test trial in which the maze was rotated 180°: place and response strategies predict opposite turns at the choice point. Rats were then trained in a free operant food task, after which the reinforcer was devalued by pre-feeding to satiety: the resistance of responding to devaluation indexes its habitual nature. 24 rats (12/sex) subsequently underwent schedule-induced polydipsia for water (SIPw) and alcohol (SIPa) in order to identify animals that engage in maladaptive, compulsive

coping behaviours. At the end of the experiment, the compulsive nature of SIPa was assayed by adulterating the alcohol with quinine. We found that free operant responding was significantly *more goal-directed*, i.e. *less resistant* to devaluation, in rats identified as response vs place learners. This effect was confined to females. Neither navigation strategy nor the devaluation-resistance of free operant responding predicted the development of SIPw/SIPa, nor the compulsive nature of the coping response. These findings suggest a counterintuitive, sex-dependent relationship between response navigation, habitual instrumental behaviour, and compulsivity. We are currently characterising the psychological and neural factors underlying animals' reliance on one navigation strategy over another.

Disclosures: J.S. Innes: None. **D.J. Belin:** None.

Poster

PSTR415

Motivation: Compulsive Behaviors, Impulse Control, and Addiction

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR415.10/M5

Topic: G.09. Drugs of Abuse and Addiction

Support: Deutsche Forschungsgemeinschaft (DFG) - Project number 402170461

Title: Alcohol dependence leads to a generalized shift to habitual tendencies mediated by the posterior dorsomedial striatum

Authors: F. GIANNONE[1], M. MEINHARDT[3], R. SPANAGEL[4], W. H. SOMMER[5], *A. HANSSON[2];
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Abstract: Alcohol Use Disorder is a psychiatric condition that affects over 250 million people globally, characterized by the inability to control alcohol consumption. It has been hypothesized that the transition to the later stages of AUD, when loss of control is most severe, occurs through an interstriatal shift in neuronal activity from the posterior dorsomedial striatum (pDMS) to the anterior dorsolateral striatum (aDLS), leading to habitual behavior. Here, we investigate this phenomenon by assessing habitual behavior of rats rendered alcohol-dependent via chronic intermittent alcohol vapor exposure, on vastly different behavioral paradigms: operant conditioning, spatial navigation, and motor skill learning.Our results demonstrate that dependent animals show less flexible and more habit-prone behavior in all behavior paradigms, indicating a generalized habitization. We then evaluate the role of the pDMS via a chemogenetic approach using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). DREADD-mediated inhibition of pDMS in naïve animals led to habit-prone behavior, while goal-directed control is re-established by pDMS activation in dependent rats. Finally, we analyze the

molecular underpinnings using a single-nuclei RNA sequencing approach. Thus, in pDMS of dependent rats most differentially expressed genes were found in dopamine D1 and D2 neuronal populations.In conclusion, we provide evidence for generalized habitual tendencies in alcohol dependence, with pDMS being critically involved in mediating this behavior, potentially due to D1- and D2-specific neuroadaptations.

Disclosures: F. Giannone: None. **M. Meinhardt:** None. **R. Spanagel:** None. **W.H. Sommer:** None. **A. Hansson:** None.

Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.01/M6

Topic: G.04. Emotion

Support: R01 Grant MNS138199A

Title: Neural activity in emotion-relevant nodes is time-locked to changes in emotional experience

Authors: *A. LIN[1], P. HULLETT[1], Q. GREICIUS[1], M. BIJANZADEH[1], J. HAN[2], E. F. CHANG[1], V. STURM[2];
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Abstract: The neural underpinnings of emotional experience are not well understood. Using intracranial electroencephalography, we collected time-locked measures of neural activity and emotional experience in eleven participants as they watched emotion-eliciting video clips (75 video clips ± 39). During each trial, participants used a dial to provide continuous ratings of the intensity of their emotional experience. Using a Likert-type scale, they also provided post-trial intensity ratings of the primary emotion that they felt. Results indicated that the continuous ratings of emotional experience intensity (as measured by the area under the curve for the dial ratings) correlated with post-trial ratings, $r_s(624)=.47$, $p<0.001$. To investigate the neural correlates of emotional experience, we computed the derivative of the dial ratings to identify the timepoint in each trial that reflected the participants' maximum change in emotional experience intensity. In the seconds that preceded and followed this timepoint, neural activity in emotion-relevant notes (anterior cingulate cortex, insula, orbitofrontal cortex, amygdala) differed from baseline levels, with some nodes showing increased activity and others showing decreased activity. These results provide a unique window into the neural basis of emotional experience and suggest that neural activity in emotion-relevant nodes is time-locked with the subjective feelings that arise during emotions.

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Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.02/M7

Topic: G.04. Emotion

Support: Marie Sklodowska-Curie Action 795994

Title: How to improve the test-retest reliability of the emotion regulation network using fMRI

Authors: *S. BERBOTH[1], C. MORAWETZ[2];
[1]Dept. of Psychiatry and Neurosciences, Charité Universitätsmedizin Berlin, Berlin, Germany;
[2]Dept. of Psychology, Univ. of Innsbruck, Innsbruck, Austria

Abstract: Introduction Given the important role of emotion regulation (ER) in affective disorders, it becomes a growing priority to identify neurobiological biomarkers of disease risk and treatment response. However, with this shift of research focus on the measurement of individual characteristics regarding emotion regulation and the underlying brain functions, one key challenge is to ensure the reliability of the measure. Extending previous findings on the reliability of task-based functional magnetic resonance imaging (fMRI) activity within the ER network (Berboth et al., 2021), we now discuss methodological considerations on how to enhance test-retest reliability in emotion regulation research by focusing on the task design, more specifically the emotional intensity of stimuli.
Methods 25 healthy participants (21 female, mean age = 22.8 ± 3.3 yrs) performed a well-established ER task using high- and low-intensity stimuli during three scanning sessions separated by one week. We acquired four runs/session and 80 trials (40 high- and 40 low-intensity)/session using the CMRR multiband EPI sequence (TR=1.4s; TE=23ms; 78 slices; voxel size=1.5x1.5x1.2mm^3; 371 whole-brain images per run) at ultra-high field (7 Tesla). We conducted region-wise reliability analyses by computing Intercorrelation Coefficients (ICCs) for previously defined Regions of Interest (ROIs) that are implicated in the emotion regulation network for high- and low-intensity trials, respectively. In addition, we performed whole-brain, voxel-wise reliability analyses by computing ICC maps.
Results Region-wise and voxel-wise test-retest reliability of fMRI activity within the ER network across the three sessions depended on the respective ROI. We found higher ICCs for cortical regions compared to subcortical regions. Further, test-retest reliability depended on the contrast, the number of trials and stimulus intensity. Regarding stimulus intensity, test-retest reliability was enhanced for the analyses of high- compared to low-intensity trials.
Conclusions The present findings show that methodological considerations, including the task

design, can enhance the test-retest reliability of neural activity within the ER network. Our results provide first steps toward improving the reliability of fMRI measures, which is a prerequisite for the validity and predictive utility of ER research focusing on individual differences.
Berboth, S., Windischberger, C., Kohn, N., & Morawetz, C. (2021). Test-retest reliability of emotion regulation networks using fMRI at ultra-high magnetic field. *NeuroImage, 232*(February), 117917.

Disclosures: S. Berboth: None. **C. Morawetz:** None.

Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.03/M8

Topic: G.04. Emotion

Support: NSF IRES NSF-1854158
Way-Klingler Science Fellowship, Marquette University
NCATS UL1TR001436
TL1TR001437

Title: Divergent associations of alexithymia facets with resting-state EEG alpha power

Authors: *M. C. POLKING[1], E. R. PAITEL[2], C. B. D. OTTEMAN[1], E. M. O'REILLY[1], J. A. CRUZ[1], J. M. RUGZIE[1], K. A. NIELSON[1];
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Abstract: Alexithymia, a trait associated with dysregulated emotion processing, is described in terms of three facets: difficulty identifying feelings (DIF), difficulty describing feelings (DDF), and externally oriented thinking (EOT). In general, high alexithymia is associated with poorer performance on a variety of cognitive tasks, and these individual facets have been differentially associated with deficits in discrete domains of cognitive processing. Specifically, DIF is associated with poorer cognitive control and inhibition, while EOT has been associated with poorer memory. Extremely limited investigation of resting-state neural activity using EEG suggests an influence of alexithymia on oscillatory brain activity. Specifically, one study showed that high alexithymia was associated with lower alpha power in the posterior cingulate cortex (PCC), suggesting disrupted default-mode activity at rest. However, unique contributions of the facets of alexithymia to differences in resting-state power have not yet been characterized. Thus, we investigated the relationship between individual alexithymia facets as evaluated by the Toronto Alexithymia Scale (TAS-20) and eyes-closed resting-state EEG alpha power at posterior (parietal through occipital) electrode sites in a sample of young adults (*n*=33, 25 female,

M_{age}=20.06). Results demonstrated that individual facet relationships with alpha power differ both in direction and by spatial topography. Greater DIF was associated with lower alpha power at parietal and parietal-occipital electrodes (*r*s -0.353 to -0.380, *p*s<0.05), while greater EOT was associated with greater alpha power at a left hemisphere occipital electrode (*r*=0.395, *p*<0.05) and a general trend toward greater alpha power at other occipital sites. These separate, and divergent, relationships indicate that individual facets of alexithymia may be implicated in different neural processes that contribute to alexithymic tendencies and corresponding cognitive deficits. More specifically, greater EOT may be associated with baseline neural processing differences in visual sensory (i.e. occipital) cortical areas, whereas DIF may be associated with baseline activity differences in more parietal regions implicated in the default-mode network, such as the precuneus, posterior cingulate, and inferior parietal cortex. These differential contributions of DIF and EOT to resting-state alpha power suggest that the discrete functional deficits observed with each facet may be associated with unique differences in baseline neural activity.

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Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.04/M9

Topic: G.04. Emotion

Title: Emotional responses to advertisements, as measured by increased frontal EEG asymmetry, modulate implicit attention and brand salience

Authors: *M. E. CANO[1], J. WILLKE[1], K. L. ANDERSON[1], E. TEMPLE[2], K. KASINATHAN[1], A. Y. SHESTYUK[1];
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Abstract: Consumer brand perceptions (i.e., brand associations, salience) are key for building consumer preferences and loyalty to brands and products. They are formed over time and are often implicit in nature (Sharp, 2010), which can be difficult to measure and even more difficult to change. While marketers strive to create ads that can reinforce or change consumer brand perceptions, it is difficult to assess whether ads are successful and what makes some ads more likely to increase brand salience. We sought to determine how exposure to an ad can influence the salience of a target brand. To assess brand salience, we modified the dot-probe paradigm, previously shown to measure implicit attention (MacLeod et al, 1986). First, participants performed the dot-probe task in which brand logos were used as the stimuli. Then, an ad for one

of the target brands was presented. The dot-probe task was then repeated to capture any changes in the response to the advertised brand. By employing the task in a pre-to-post design, this approach controls for perceptual aspects of the logo stimuli and preexisting implicit brand salience. Thirty ads were tested online monadically (n = 400 per ad). We found that exposure to an advertisement increased attentional salience of the target brand relative to competitors (t = 9.1, $p < .001$). To determine how ad exposure influences implicit attention to the target brand, we measured participant responses to the ads using electroencephalography (EEG) in a separate set of studies. Each ad was seen by a unique group of participants (n = 32) while their EEG signals were recorded using an Emotiv Flex headset (32 electrodes, sampled at 256 Hz). We hypothesized that emotional or attentional responses to the ads may modulate how much ads can shift implicit attentional salience of the target brand. We computed the frontal alpha asymmetry as an index of emotion (approach) and the decrease in global alpha power as an index of attention (Shestyuk et al, 2019). We then correlated these EEG metrics with the outcomes of the pre-to-post dot-probe task for each ad. Results demonstrated that ads with a greater increase in implicit attentional salience of the target brand elicited the strongest frontal asymmetry (r = 0.57, $p < 0.005$), while no relationship existed between the global alpha power decrease and implicit brand salience (r = -0.07, $p > 0.71$). This suggests that it is the emotional impact of the ads (rather than attention to them) that is important to increase implicit salience of the advertised brand.

Disclosures: M.E. Cano: A. Employment/Salary (full or part-time):; NielsenIQ. **J. Willke:** A. Employment/Salary (full or part-time):; NielsenIQ. **K.L. Anderson:** A. Employment/Salary (full or part-time):; NielsenIQ. **E. Temple:** A. Employment/Salary (full or part-time):; NielsenIQ. **K. Kasinathan:** A. Employment/Salary (full or part-time):; NielsenIQ. **A.Y. Shestyuk:** A. Employment/Salary (full or part-time):; NielsenIQ.

Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.05/M10

Topic: G.04. Emotion

Title: Heart rate variability moderates the link between trait catastrophizing and EEG microstate B activity

Authors: A. GANAPATHI, *B. MARTINS-KLEIN, PHD, Z. CHEN, B. WINCHELL; USC, Los Angeles, CA

Abstract: High heart rate variability (HRV) is considered a biomarker of adaptive self-regulation across the lifespan. Indeed, low HRV is associated with greater psychopathology and difficulties with emotion regulation. EEG microstates are brief (80-120 ms) quasi-stable

distributions of cortical activity that also predict psychopathology. Of the four prototypical microstate that have been widely observed in previous resting EEG (rsEEG) studies, occurrence of microstate B has been found to predict mood and anxiety disorders. However, the relationship between these two physiological markers of psychopathology and emotion regulation abilities is unclear. In this study, we analyzed rsEEG, HRV, and emotion regulation using the Leipzig Study for Mind-Body-Emotion Interactions (LEMON) dataset. We predicted that maladaptive emotion regulation strategy use would be positively associated with microstate B, but negatively associated with HRV. Additionally, given that impaired interoception underscores a wide range of psychopathology and emotion dysregulation, we anticipated that the coupling between the maladaptive emotion regulation and microstate B would be higher for individuals with high HRV. The dataset included $n = 96$ adult right-handed participants with no history of psychiatric illness or current substance use were analyzed in this study. Multivariate multiple regression models were run in R with microstate B temporal parameters, HRV tracked via log-transformed RMSSD, and trait cognitive emotion regulation use as tracked by the Cognitive Emotion Regulation Questionnaire (CERQ). No significant relationships between HRV and microstate B or emotion regulation strategies were observed; however, we found a significant positive relationship between catastrophizing and microstate B duration ($t = 3.657$, $p < 0.005$), coverage ($t = 3.937$, $p < 0.005$), and GEV ($t = 4.272$, $p < 0.005$). These relationships were blunted, however, in individuals with lower HRV across all three parameters ($t = 2.824$, $p < 0.05$; $t = 2.250$, $p < 0.05$; $t = 2.101$, $p < 0.005$). Although we did not find significant patterns for adaptive or other maladaptive strategies, this suggests that biobehavioral coupling may be a useful index of emotion regulation. Greater connection between self-regulation strategy use and neurophysiological regulation may be an adaptive association. Future work should examine this relationship in clinical populations to further elucidate the relationship between biobehavioral regulation and psychopathology.

Disclosures: A. Ganapathi: None. **B. Martins-Klein, PhD:** None. **Z. Chen:** None. **B. Winchell:** None.

Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.06/M11

Topic: G.04. Emotion

Support: NIH R01 DC004290

Title: Characteristics of single-unit neuronal activity elicited by intracranial stimulation in the human brain

Authors: H. OYA[1], *P. M. KASKAN[2], J. I. BERGER[3], M. A. HOWARD III[4];
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Abstract: The amygdala has extensive connections with the anterior cingulate (ACC), medial and orbital frontal cortices, and the insula; areas routinely implicated in associative learning, encoding valence, arousal, attention, and hedonic value. We have developed intracranial stimulation protocols and stimulation isolation methods in human neurosurgical participants to manipulate interactions between the amygdala and cortex during behavioral tasks involving eye tracking. To characterize temporal dynamics of spiking following intracranial stimulation of the amygdala, and compare with sites of intracranial cortical stimulation, we have analyzed recordings from as many as 96 microwires of Behnke-Fried assemblies, while delivering single-pulse bi-phasic stimulation across adjacent macro-contacts during rest in 16 participants. Constant current bipolar square wave pulses (6 mA; pulse-width 200 uSec) were delivered 60 times with an ISI of 1.99 sec. Microwire data was sampled at 32 kHz, filtered (0.1 - 8,000 Hz), and re-referenced with median waveforms from all 8 microwires. Epochs containing stimulation artifacts (-3 to 3ms) were replaced with artifact-free pre-stimulus data. To remove long-lasting slow fluctuations caused by stimulation, averaged evoked potentials were removed from single trial data. Line-noise was removed, and spatial whitening was applied through zero-phase component analysis. Spike detection and sorting were performed; only units that showed SnR >6, inter-spike-interval violation < 3% and firing rate >2Hz were selected. The significance of stimulation induced spiking was assessed with a Zenith of timelocked anomalies test. Three patterns of single-unit spiking were identified in the OFC, insula, and ACC following intracranial stimulation of the amygdala: 1) prolonged suppression; 2) phasic activation; and 3) suppression followed by increased spiking activity. Baseline spike rates in the amygdala were usually <10 Hz. Bi-phasic responses typically exhibited an initial suppression followed by increases in spiking. Increased spiking with late onset latencies (~= 100 ms) was also apparent, suggesting some spikes were triggered by indirect/multi-synaptic connections. Ongoing analyses suggest cortical stimulation also elicited single-unit activity in other cortical areas and the amygdala. The reliable identification of spikes elicited by intracranial stimulation is necessary to determine how experimentally activated neurons might influence ongoing behaviors. By surveying areas activated, we can also identify areas that are not affected by stimulation and therefore unlikely to influence behavior.

Disclosures: H. Oya: None. **P.M. Kaskan:** None. **J.I. Berger:** None. **M.A. Howard:** None.

Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.07/M12

Topic: G.04. Emotion

Support: NIH grant MH125615

Title: Neural Responses to Natural Versus AI-generated Affective Images

Authors: *Y. CHEN;
Univ. of Florida, Gainesville, FL

Abstract: The International Affective Picture System (IAPS) contains 1,182 well-characterized photographs depicting natural scenes varying in affective content. These pictures are used extensively in affective neuroscience to investigate the neural correlates of emotional processing. Recently, in an effort to augment this dataset, we have begun to generate synthetic emotional images by combining IAPS pictures and diffusion-based AI models. The goal of this study is to compare the neural responses to IAPS pictures and matching AI-generated images. The stimulus set consisted of 60 IAPS pictures (20 pleasant, 20 neutral, 20 unpleasant) and 60 matching AI-generated images (20 pleasant, 20 neutral, 20 unpleasant). In a recording session, a total of 30 IAPS pictures and 30 matching AI-generated images were presented in random order, where each image was displayed for 3 seconds with neighboring images being separated by an interval of 2.8 to 3.5 seconds. Each experiment consisted of 10 recording sessions. The fMRI data was recorded on a 3T Siemens Prisma scanner. Pupil responses to image presentation were monitored using an MRI-compatible eyetracker. Our preliminary analysis of the fMRI data (N=7) showed that IAPS pictures and matching AI-generated images evoked similar neural responses in the visual cortex. In particular, MVPA (Multivariate Pattern Analysis) classifiers built to decode emotional categories from neural responses to IAPS pictures can be used to decode emotional categories from neural responses to AI-generated images and vice versa. Efforts to confirm these findings are underway by recruiting additional participants. Analysis is also being expanded to include the comparison of such measures as functional connectivity and pupillometry.

Disclosures: Y. chen: None.

Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.08/M13

Topic: G.04. Emotion

Support: R21MH124674

Title: Effects of human amygdala stimulation on perception of valence

Authors: C. CASTELBLANCO RIVEROS[1], Z. LEEDS[2], Y. SONG[3], ***K. BUJARSKI**[4]; [1]Dartmouth Col., Hanover, NH; [2]Dartmouth-Hitchcock, Lebanon, NH; [3]Neurol., Dartmouth-Hitchcock Med. Ctr., Lebanon, NH; [4]Dartmouth Hitchcock Med. Ctr., Lebanon, NH

Abstract: Neuromodulation, a method used for the long-term alteration of neural circuits by targeted electrical brain stimulation (EBS), is a promising tool for regulating cognitive and pathological circuits. The human amygdala is an ideal location for neuromodulation as extensive evidence implicates its involvement in a wide range of cognitive functions, including affective valence and arousal. Dysfunctional processing within the amygdala, which is a common mechanism for different psychiatric conditions, could be treated using EBS. This study examines whether EBS of the basolateral nuclei of the human amygdalae can modulate emotion perception. We obtained stereo-electroencephalography (SEEG) recordings from nine refractory focal epilepsy patients without psychiatric diagnosis as they observed emotionally salient pictures and rated them on valence and arousal scales. Pictures were obtained from the OASIS dataset and balanced across valence (negative and positive) and arousal (low and high) categories. We used a randomized block design where the second half of the images in each block were presented with EBS at 200 Hz (200us, biphasic pulses for 3 seconds); the maximum safe EBS intensity was determined patient-wise to avoid triggering after-discharges or seizures. During non-EBS trials, we found intracranial event-related potentials (iERPs) at ~300 and ~800 ms in several regions of the valence network, including the amygdala and insular cortex. Power spectral analysis (log-transformed) revealed oscillatory periodic components between 65 and 75 Hz during the first 1000ms of image viewing in the amygdala. Behaviorally, valence (negative, $p = 0.62$; positive, $p = 0.75$) and arousal (low, $p = 0.78$; high, $p = 0.18$) ratings for images presented with and without EBS didn't differ statistically. Nonetheless, patients with EBS amplitude greater than 0.8 mA tended to report lower mean valence ($p = 0.267$, $t = -1.197$, $\beta = -0.266$) and arousal ($p = 0.333$, $t = -1.037$, $\beta = -0.353$) ratings based on a linear mixed-effects model. These findings suggest that EBS in the amygdala may modulate emotion perception amplitude-dependently. Future studies may systematically investigate EBS amplitude and frequency across the valence network to enhance behavioral effects.

Disclosures: C. Castelblanco Riveros: None. **Z. Leeds:** None. **Y. Song:** None. **K. Bujarski:** None.

Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.09/M14

Topic: G.04. Emotion

Support: UROP from University of Minnesota

Title: Relationship between sexual and emotional infidelity is moderated by both sex and sexual orientation

Authors: *R. LLOYD;
Univ. of Minnesota, Duluth, Duluth, MN

Abstract: Evolutionary psychology posits that the relative emotional response to sexual and emotional infidelity should be moderated by the sex of the individual. Because of paternity uncertainty, males should be more distressed by sexual infidelity on the part of his partner. Because males have historically controlled material resources, upon which a female partner and her offspring are dependent, females should be more distressed over her partner becoming emotionally involved in another female, causing him to leave and take with him his resources. The majority of the literature have found these inverse relationships, using both survey and electrophysiological data. Because brain morphology in gay men resembles that of heterosexual females (corpus callosum) and high levels of testosterone in utero masculinizes the brains and behavior of experimental animals, and the behavior of human females (high levels of lesbianism, bisexuality, and traditionally masculine occupations). We predict that homosexual men and women should have emotional responses similar to that of opposite sex heterosexuals. Survey research has been inconsistent on this point.
We have conducted two studies recapitulating the findings of others with respect to heterosexual participants. The electrodermal response in heterosexual men was higher to suggestions of sexual infidelity in their partner, or theoretical partner, relative to suggestions of emotional infidelity. The opposite was found in heterosexual females. Same vs. opposite sex of researcher-participant dyads has been found to affect electrophysiological responses to emotional stimuli. A male researcher was employed in the first experiment, while a female researcher was employed in the second, with consistent results. The second experiment involved cohorts of male and female homosexuals, as well as heterosexual participants. In this experiment, homosexuals had the opposite ratio of responses as their heterosexual counterparts.

Disclosures: R. Lloyd: None.

Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.10/M15

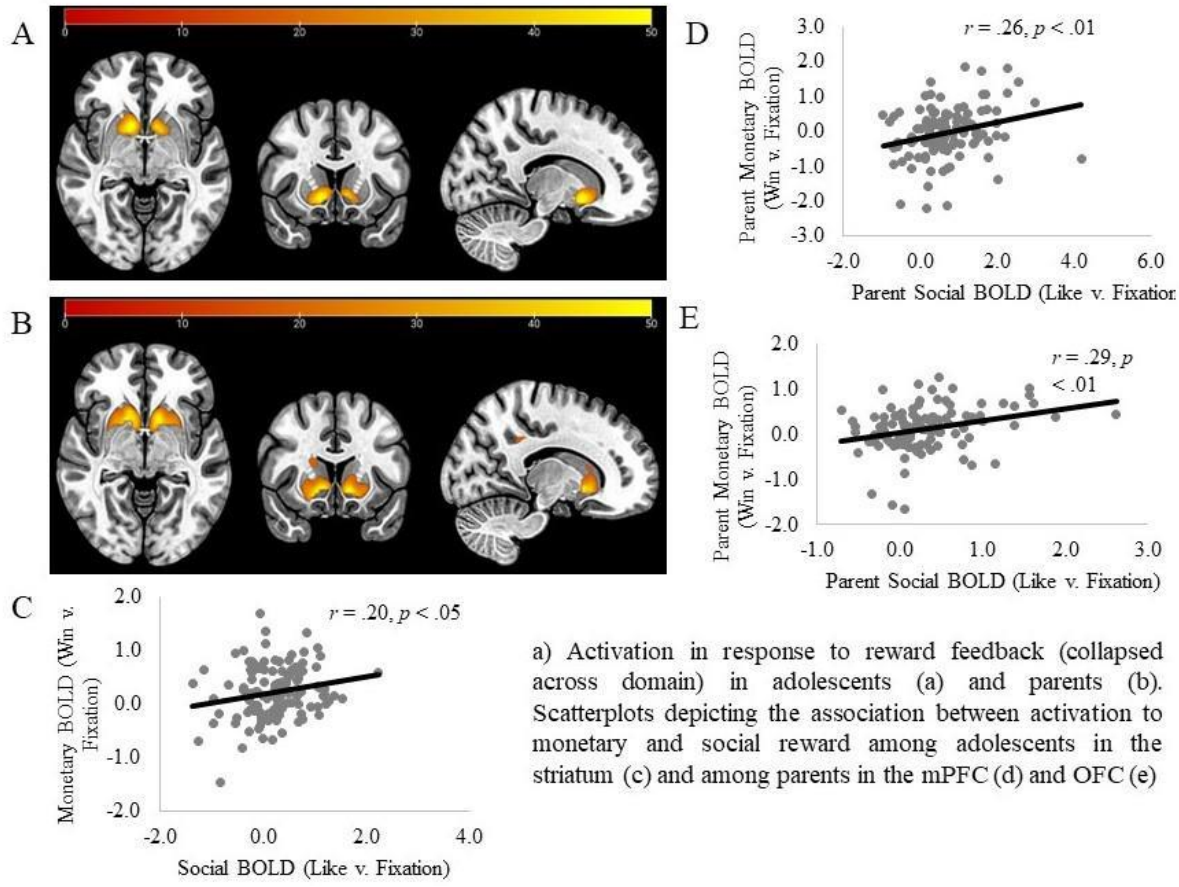
Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIMH Grant R01MH097767

Title: Neural Response to Monetary and Social Rewards in Adolescent Girls and their Parents

Authors: *R. A. FERRY;
Stony Brook Univ., Stony Brook, NY

Abstract: Functional magnetic resonance imaging (fMRI) studies have indicated that the mesocorticolimbic dopamine system is heavily involved in all stages of reward processing. However, most research has been conducted using monetary rewards and it is unclear to what extent other types of rewards, such as social rewards, evoke similar or different neural activation. There have also been few investigations into differences or similarities between reward processing in parents and offspring. The present study examined fMRI neural activation in response to monetary and social reward in a sample 14-22-year-old adolescent girls (N = 145) and a biological parent (N = 124) and compared activation across adolescent-parent dyads (N = 82). Across all participants, both monetary and social reward elicited bilateral striatal activation, which did not differ between reward types or between adolescents and their parents. Neural activation in response to the different reward types were positively correlated in the striatum among adolescents and in the medial prefrontal cortex and orbitofrontal cortex among parents. Overall, the present study suggests that both monetary and social reward elicit striatal activation regardless of age and provides evidence that neural mechanisms underlying reward processing may converge differentially among youth and adults.



a) Activation in response to reward feedback (collapsed across domain) in adolescents (a) and parents (b). Scatterplots depicting the association between activation to monetary and social reward among adolescents in the striatum (c) and among parents in the mPFC (d) and OFC (e)

Disclosures: R.A. Ferry: None.

Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.11/M16

Topic: G.04. Emotion

Support: NIH R01 EY032162

Title: The Affects of Empathy on Emotion Representation

Authors: *B. CUI, P. BEX;
Northeastern Univ., Boston, MA

Abstract: Emotion recognition and communication are critical for everyday life and may be disrupted in affective disorders. Standard affective research stimuli utilizes actors and prototypical “Ekman-like” face stimuli, however, this approach lacks variability and assumes that typical and atypical humans perceive emotion similarly. We develop an assumption-free approach using a morphable face model and genetic algorithms to create individualized representations of affect expression. 53 undergraduate participants completed the Empathy Quotient (EQ) questionnaire and generated individualized faces that manifest each of 13 target affects (Amusement, Anger, Awe, Contempt, Disgust, Embarrassment, Fear, Happiness, Interest, Pride, Sadness, Shame, and Surprise). For each affect, 6 generations of 12 faces were generated from the Basel Face database, in which 199 coefficients modulate the structure of each face. After the 1st generation, 6 faces were created by combinations of the parameters of faces selected in the previous generation. The remainder were random to introduce genetic variability. In the 7th generation, participants selected one final face that best represented the target affect. The cosine distances for participant-generated coefficients for all affects were compared to the centroid of each affect using t-tests. There were significant differences ($p<0.05$) across all emotions, indicating that participants generated clusters of faces that were structurally similar for each affect and were significantly different from the other 12 affects. Additionally, the centroids of 10 affect pairs were significantly different for participants with high or low EQ scores suggesting that the perception of some affects may depend on empathy. These results quantify the space of similarity and variability in individual perceptions of facial affect expression, and reveal significant differences between high and low EQ scoring groups. This methodology may provide a novel tool for quantifying high-level visual function outcomes, and to monitor the efficacy of interventions for affective disorders.

Disclosures: B. Cui: None. **P. Bex:** None.

Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.12/M17

Topic: G.04. Emotion

Support: NIH Grant R21 MH133055

Title: Distinct time courses within the fronto-limbic response to psychosocial stress using stereoelectroencephalography in humans

Authors: *A. GOODMAN[1], **A. R. NOLAN**[1], K. E. DAVIS[1], D. JANKO[2], R. J. CHATFIELD[1], J. F. MAGNOTTI[3], J. P. SZAFLARSKI[1];
[1]Univ. of Alabama at Birmingham, Birmingham, AL; [2]Univ. of Potsdam, Potsdam, Germany; [3]Univ. of Pennsylvania, Philadelphia, PA

Abstract: *AMG and ARN contributed equally to this work.
A consistent network of brain regions has been implicated in the neural response to stress; however, the time course of the response in these regions has yet to be characterized in humans. Characterizing the temporal features of this network is critical to better understand how information is integrated and transferred in the brain during stress. Stereoelectroencephalography (sEEG) provides an opportunity to assess neural responses at a finer time course than fMRI and higher spatial specificity than EEG. The objective of this study is to determine the time course of responses in regions of interest (ROIs) within a stress processing network using sEEG. 12 participants undergoing sEEG monitoring for epilepsy surgical evaluation volunteered to complete a Montreal Imaging Stress Task (MIST) consisting of control and stress conditions during math performance. Data analysis was performed in R Analysis and Visualization of iEEG (RAVE). Signal from 0-2s post stimulus onset was extracted using a Morlet wavelet for the theta frequency band (4-8 Hz) based on prior evidence of emotion network coordination and processing. Electrodes were localized by normalizing CT and MRI images to a standard atlas (Desikan-Killiany). Signal was averaged for each of 5 ROIs, including right and left amygdala, right and left hippocampus, and anterior cingulate cortex (ACC). 4 linear mixed effects models compared conditions, corrected for multiple comparisons and inhomogeneity of variance, within each ROI separately for each time window (0-0.5s, 0.5-1s, 1-1.5s, 1.5-2s). Decreases in theta power during stressful math condition were observed during the 0-0.5s window for the right ($t(23)=3.3$, $p<0.01$) and left amygdala ($t(41)=3.3$, $p<0.01$), right hippocampus ($t(78.8)=3.1$, $p<0.01$), and anterior cingulate cortex (ACC; $t(22.9)=2.2$, $p<0.05$). Decreases in theta were also observed during the 0.5-1s ($t(23)=2.2$, $p<0.05$), and 1-1.5s ($t(41.4)=2.0$, $p<0.05$) windows for the right amygdala and the 0.5-1s ($t(13)=3.1$, $p<0.01$), and 1-1.5s ($t(13)=2.6$, $p<0.05$) windows for the ACC. The effect of conditions was greatest during the 0-0.5s window for the right amygdala ($\beta=0.58$) and during the 1-1.5s window for the ACC ($\beta=0.68$). Greater theta band responses to stress occurred in the first 0.5s during performance demands for amygdala and hippocampal regions, but later in the 1-1.5s for the ACC. These results provide evidence that medial temporal regions respond earlier to stress, followed by later medial frontal responses. These findings implicate bottom-up integration and transfer of stress relevant information during stressful performance demands in humans.

Disclosures: A. Goodman: None. **A.R. Nolan:** None. **K.E. Davis:** None. **D. Janko:** None. **R.J. Chatfield:** None. **J.F. Magnotti:** None. **J.P. Szaflarski:** None.

Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.13/M18

Topic: G.06. Anxiety Disorders

Support: CEN -- H2020-MSCA-ITN-2020 Number 956414

Title: Heritability of Moment-to-Moment Neural Variability During Emotion Recognition

Authors: *T. YILDIZ AHOLA[1], F. ÅHS[3], J. ROSÉN[4,3], G. KASTRATI[2], T. FURMARK[5], D. GARRETT[6,7], K. MÅNSSON[2,8];
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Abstract: Heritability, the extent to which differences in traits can be attributed to genetic factors, plays a fundamental role in shaping brain function. In functional magnetic resonance imaging (fMRI), the average blood-oxygen-level-dependent (BOLD) signal across time/trials (i.e., MEANBOLD remains the typical approach in task-based fMRI studies, while disregarding variability from trial to trial as unwanted noise, measured by SDBOLD. We investigated the heritability of moment-to-moment neural variability during emotion recognition, a critical aspect of brain function overlooked in genetic studies. We utilised a classical twin design with 144 pairs (69 monozygotic, 75 dizygotic) totaling 288 participants. Subjects performed an emotional recognition task during MRI scanning, including emotional face and geometrical shape matching blocks. We employed the fast and non-iterative APACE (Accelerated Permutation Inference for ACE model) method to estimate heritability, accounting for additive genetics (A), shared environment (C), and unique environment (E), while controlling for age and sex. Heritability (h2) is the measure of the proportion of phenotypic variance attributable to additive genetics and obtained through statistical methods embedded in APACE. We employed whole-brain voxel-wise analysis and revealed significant clusters of heritability with cluster-wise analysis with a family-wise-error correction (FWE) and alpha $P < .05$ threshold. Moment-to-moment whole brain neural variability (SDBOLD) during emotion recognition showed significant heritability ($h2 = 0.25$, 95% CI 0.12, 0.39; permuted $P = 0.029$), surpassing the heritability of average neural responses (MEANBOLD) ($h2 = 0.10$, 95% CI 0.05, 0.16; permuted $P = 0.240$). Significant

heritability clusters for SDBOLD included the occipital lingual gyrus (h2 = 0.69), anterior cingulate cortex (h2= 0.61), parahippocampal gyrus (h2= 0.56), and thalamus (h2 = 0.62). Our study highlights the significance of moment-to-moment neural variability in understanding genetic influences on brain function, particularly in emotion recognition. The identified heritable clusters provide valuable insights into the genetic basis of emotional face processing, with implications for understanding individual differences in socio-emotional abilities and potentially psychiatric disorders. SDBOLD could be a new and more powerful signature of how genetics influence brain function.

Disclosures: T. Yildiz Ahola: None. **F. Åhs:** None. **J. Rosén:** None. **G. Kastrati:** None. **T. Furmark:** None. **D. Garrett:** None. **K. Månsson:** None.

Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.14/M19

Topic: G.04. Emotion

Support: NIH Grant P20GM13044701A1
Program of Excellence Funds, University of Nebraska at Omaha

Title: Poor Emotional Awareness, Aging, and Gender: The Role of Testosterone

Authors: *J. BEADLE[1], D. E. WARREN[2];
[1]Univ. of Nebraska at Omaha, Omaha, NE; [2]Neurosciences, Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Although aging adults experience declines in many aspects of cognition, there are also changes to emotional function with age. Despite research pointing to clear age-related changes in sex hormones (e.g., testosterone), less is known about the impact of these changes on emotional functioning. The present study investigated relationships among emotional awareness, age, gender, and testosterone levels in a sample of healthy adults. Participants included 56 healthy adults aged 19-86 years (Mage=55.50, SD=22.37; Medu=16.04, SD=2.29). Participants were excluded if they had a history of neurological or psychiatric disease, were currently taking serotonin reuptake inhibitors, using corticosteroid cream, or had metal in their body that would prohibit neuroimaging. Emotional awareness was assessed through the Toronto Alexithymia Scale 20 (TAS-20). Higher scores were indicative of greater alexithymia (i.e., poorer emotional awareness). Salivary testosterone levels were collected using the passive drool method first thing in the morning over two consecutive days in the home. These values were averaged across the two days. We conducted a series of linear regression models examining relationships among TAS-20, average testosterone, age, and gender. The first model examined relationships among the total TAS-20 score, average testosterone, age, and gender. This model was significant

(R2=.14; p=.04). We found that testosterone was significantly, negatively associated with TAS-20 (p=.013), but age and gender were not significant. We conducted a separate linear regression investigating the relationship between TAS-20 Factor 2 (Difficulty Identifying Feeling), average testosterone, age, and gender and this model was significant (R2=.15, p=.03). In this model, TAS-20 Factor 2 was negatively associated with testosterone (p=.01) and age (p=.02). The other two TAS-20 Factor models did not reach significance. We found that lower average testosterone levels were related to higher alexithymia scores (which indicates poorer emotional awareness). In particular, we found that higher scores on TAS-20 Factor 2 (Difficulty Identifying Feeling) were associated with lower testosterone levels, whereas the models containing the other two factors were not significant. This suggests that healthy adults who have lower testosterone levels may also experience more difficulty identifying their feelings. Future research is needed to examine the degree to which this cross-sectional finding is corroborated by longitudinal studies that also examine additional social or health-related factors that could affect this relationship.

Disclosures: J. Beadle: None. **D.E. Warren:** None.

Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.15/Web Only

Topic: F.03. Stress and the Brain

Support: CIHR, PJT-166066
JPB Foundation, Grant to the JPB Research Network on Toxic Stress

Title: Protein network interaction polygenic risk score from nuclear insulin receptor predicts impulsivity

Authors: *A. GOMEZ-ILESCAS[1], P. PELUFO SILVEIRA[1], G. ELGBEILI[2], N. O'TOOLE[3], I. POKHVISNEVA[2];
[1]McGill Univ., Montreal, QC, Canada; [2]Douglas Res. Ctr., Verdun, QC, Canada; [3]Douglas Mental Hlth. Univ. Inst., Verdun, QC, Canada

Abstract: The established comorbidity between mental disorders and cardio-metabolic diseases following early life adversities suggests a shared developmental trajectory. Evidence indicates that poor fetal growth, an insulin-guided process, increases the risk for impulsivity. Notably, while systemic insulin regulates cellular glucose uptake via insulin receptor (IR) binding, brain IR activation predominantly confers neuroprotective effects. In this study, our aim was to detect the moderation of brain insulin-induced protein interactions in the association between poor fetal growth (a marker of prenatal adversity exposure) and the onset of comorbidities in adulthood. We utilized available brain mass spectrometry data, to identify a gene network from proteins binding to the IR after insulin administration. Analysis of the network by gene ontology terms

(GO) showed an enrichment for terms associated to DNA binding and lipid metabolism. Brain tissue-specific gene variants (SNPs) associated to the genes from the network were mapped to calculate a novel biologically informed polygenic score (mssIR-ePRS) in areas of the reward system for two different human cohorts using GTEx. mssIR-ePRS calculated in the substantia nigra/ventral tegmental area (SN/VTA) was found to moderate the association between poor fetal growth and impulsivity in the information sampling task (IST) $\hat{\beta} = 0.2637$, $p = .014$ at 48 months of age. Being born small for gestational age was associated with high impulsivity only in individuals with high mssIR-ePRS ($\hat{\beta} = 0.22$, $p = 0.05$, N=288). Moreover, poor fetal growth was associated with higher risk for mental and cardio-metabolic co-morbidities in UK Biobank adults especially in the high mssIR-ePRS group ($\hat{\beta} = -0.25$, $p < 0.001$, N=225839). These results suggest that the SN/VTA mssIR-ePRS network is highly associated with increased risk for the development of impulsivity and highlights its potential as a promising marker for clinical and interventional studies particularly in the context of early adversity.

Disclosures: A. Gomez-Ilescas: None. **P. Pelufo Silveira:** None. **G. Elgbeili:** None. **N. O'Toole:** None. **I. Pokhvisneva:** None.

Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.16/M20

Topic: H.03. Decision Making

Support: American Psychiatric Association RFM Award

Title: Developing a Neuroeconomically Informed and Biologically Constrained Regret Inventory

Authors: R. DURAND-DE CUTTOLI[1], A. FINK[5], A. BAGGETTA[5], G. CORICELLI[6], H. S. MAYBERG[7], *A. D. REDISH[8], J. MURROUGH[9], X. GU[2], I. SAEZ[7], L. MORRIS[3], J. DEPIERRO[3], B. M. SWEIS[4];
[1]Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Icahn Sch. of Med. at Mount Sinai, New York, NY, ; [3]Dept. of Psychiatry, [4]Psychiatry, Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [5]Icahn Sch. of Med. At Mount Sinai Grad. Training Program In Neurosci., New York, NY, ; [6]USC, Los Angeles, CA, ; [7]Mount Sinai, New York, NY, ; [8]Neurosci., Univ. of Minnesota, Minneapolis, MN; [9]Ichan Sch. of Med. At Mount Sinai, New York, NY.

Abstract: Regret is a poorly understood emotion that may contribute to nearly every mental illness. Regret describes a form of counterfactual thinking where one recognizes alternative decisions could have led to better outcomes. Despite being widely accepted that regret can be detrimental to emotional well-being, no description appears in the DSM nor is pathognomonic

for any disorder. Further, little is known about what aspects of regret if any carry utility worth preserving to restore healthy emotional processing and adaptive coping, even if evoking cognitive dissonance. Although psychologists, economists, and neuroscientists have been working toward understanding regret, this has historically occurred outside of a unified framework without a shared lexicon rooted in underlying neurobiology. Currently, there are limited clinical tools that move beyond plain language to describe regret. We propose the concept of a Neuroeconomic Regret Inventory (NRI) inspired by cross-species research efforts to resolve attributes of regret into discretely measurable computational units. The NRI characterizes multiple, orthogonal dimensions of regret. Question items examine cognitive domains derived from neuroeconomic principles, including aspects of reinforcement learning, foraging theory, and temporal discounting. Here, we collected data from 350 subjects online via the Prolific platform. We found subjects could complete the 115-item NRI survey in approximately 15 min. We found that overall, subjects ranked regret related to relationships as the most important life category compared to finance, health, career, and legal decisions. Interestingly, relationship regret-related decisions was the only category that interacted with sex and age. Across the 115 NRI items, questions elicited a wide distribution of responses that scored with varying direction and magnitude. This included within each of the 6 major themes of question items: general questions, regret recognition and registration, feeling and affect, mental operations, reactions and responses, and lastly, anticipation avoidance and learning. Our vision is that this tool could provide improved neuroeconomic language to be leveraged in multiple settings, e.g., structured interviews to guide psychotherapy strategies and inform computational models of task-based behavior and physiology. By enhancing the diagnostic nosology of psychiatric disorders through a description of one's decision narrative, we can develop more effective treatments based on a richer understanding of the psychological mechanisms mediating the perception and influence of one's prior actions.

Disclosures: R. Durand-De Cuttoli: None. **A. Fink:** None. **A. Baggetta:** None. **G. Coricelli:** None. **H.S. Mayberg:** None. **A.D. Redish:** None. **J. Murrough:** None. **X. Gu:** None. **I. Saez:** None. **L. Morris:** None. **J. DePierro:** None. **B.M. Sweis:** None.

Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.17/M22

Topic: H.03. Decision Making

Support: Leon Levy Fellowship in Neuroscience (JLA, BMS)
NARSAD Young Investigator 28240 (JLA)

Title: Diabetes alters neuroeconomically dissociable forms of mental accounting II: The effects of meal devaluation and insulin treatment on foraging behavior

Authors: C. A. NWAKAMA[1], R. DURAND-DE CUTTOLI[2], S. O. BROWN[2], A. MENDEZ[3], M. JODEIRI FARSHBAF[4], ***J. L. ABLES**[5], B. M. SWEIS[6];
[1]Med. Scientist Training Program, Icahn Sch. of Med. At Mount Sinai Grad. Training Program In Neurosci., New York, NY; [2]Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York City, NY; [5]Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York, NY; [6]Psychiatry, Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Those with diabetes mellitus are at higher risk of developing depression and other psychiatric disorders. Although diabetes is primarily characterized by chronic hyperglycemia, it remains unclear how impaired insulin function, which is known to have direct effects on neural activity within the reward circuitry, regulates motivated behavior.
We characterized value-based decision-making of an insulin-deficient diabetic mouse model on a naturalistic neuroeconomic foraging paradigm. 40 8-week old CB57BL/6J male mice were injected with either vehicle (VEH) or streptozotocin (STZ), an antibiotic that ablates insulin producing beta cells in the pancreas, to induce hyperglycemia. Mice were then tested across two months on the "Restaurant Row" task during which they foraged daily for their primary source of food while on a limited time budget. Mice learned to make serial decisions accepting or rejecting reward offers as a function of cost (delays cued by tone pitch) and subjective value (flavors cued by unique spatial contexts).Mice were trained on two different schedules during which the economic landscape (i) drastically or (ii) gradually progressed into an increasingly reward-scarce environment. In this poster, we analyzed the effects of a pre-feeding devaluation manipulation in tandem with saline vs. insulin co-treatment on economic choice behavior. Overall, we found that pre-feeding before task performance could devalue foraging behavior in a manner that depended on flavor specificity and meal size. Further, we found that STZ-treated mice generally displayed a decreased sensitivity to pre-feeding based on meal size that did not appear to be augmented by insulin paired with pre-feeding meals.
These findings suggest that complex relationships between glycemic regulation, hunger, meal bouts, and economic choice behavior interact to influence decision-making processes underlying unique aspects of reward value. These findings suggest that reward processing may be altered in diabetes, not all of which are augmented by simple insulin replacement therapy, and contribute to psychiatric illness.

Disclosures: C.A. Nwakama: None. **R. Durand-De Cuttoli:** None. **S.O. Brown:** None. **A. Mendez:** None. **M. Jodeiri Farshbaf:** None. **J.L. Ables:** None. **B.M. Sweis:** None.

Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.18/M23

Topic: H.03. Decision Making

Title: Diabetes alters neuroeconomically dissociable forms of mental accounting I: The effects of fed vs. fasted state on decision-making behavior

Authors: *C. NWAKAMA[1], R. DURAND-DE CUTTOLI[2], S. O. BROWN[2], A. MENDEZ[3], M. JODEIRI FARSHBAF[4], J. L. ABLES[5], B. M. SWEIS[6];
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Abstract: Those with diabetes mellitus are at higher risk of developing depression and other psychiatric disorders. Although diabetes is primarily characterized by chronic hyperglycemia, it remains unclear how impaired insulin function, which is known to have direct effects on neural activity within the reward circuitry, regulates motivated behavior. We characterized value-based decision-making of an insulin-deficient diabetic mouse model on a naturalistic neuroeconomic foraging paradigm. 40 8-week old CB57BL/6J male mice were injected with either vehicle (VEH) or streptozotocin (STZ), an antibiotic that ablates insulin producing beta cells in the pancreas, to induce hyperglycemia. Mice were then tested across two months on the "Restaurant Row" task during which they foraged daily for their primary source of food while on a limited time budget. Mice learned to make serial decisions accepting or rejecting reward offers as a function of cost (delays cued by tone pitch) and subjective value (flavors cued by unique spatial contexts).Mice were trained on two different schedules during which the economic landscape (i) drastically or (ii) gradually progressed into an increasingly reward-scarce environment. In this poster, we analyzed the effects of a pre-feeding devaluation manipulation in tandem with saline vs. insulin co-treatment on economic choice behavior. Overall, we found that pre-feeding before task performance could devalue foraging behavior in a manner that depended on flavor specificity and meal size. Further, we found that STZ-treated mice generally displayed a decreased sensitivity to pre-feeding based on meal size that did not appear to be augmented by insulin paired with pre-feeding meals. These findings suggest that complex relationships between glycemic regulation, hunger, meal bouts, and economic choice behavior interact to influence decision-making processes underlying unique aspects of reward value. These findings suggest that reward processing may be altered in diabetes, not all of which are augmented by simple insulin replacement therapy, and contribute to psychiatric illness.

Disclosures: C. Nwakama: None. **R. Durand-De Cuttoli:** None. **S.O. Brown:** None. **A. Mendez:** None. **M. Jodeiri Farshbaf:** None. **J.L. Ables:** None. **B.M. Sweis:** None.

Poster

PSTR416

Neural Mechanisms of Emotional and Social Experience Across Species

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.19/M24

Topic: H.03. Decision Making

Title: Ketamine reverses hypersensitivity to sunk costs in a mouse model of depression

Authors: R. DURAND-DE CUTTOLI[1], ***B. SWEIS**[2];
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Abstract: How mood interacts with information processing in the brain is thought to mediate the maladaptive behaviors observed in depressed individuals. However, the neural mechanisms underlying impairments in emotion-cognition interactions are poorly understood. While most of the discoveries in the neurobiology of depression stem from animal research, animal models are often limited in their ability to approximate the complexity of human cognition. Recent insights from the field of neuroeconomics offer novel approaches to study complex interactions between mood and decision-making in a manner that is biologically tractable and readily translatable across species. Using a neuroeconomic approach, it was recently discovered that rodents are sensitive to “sunk costs” - a feature of higher cognition previously thought to be unique to humans. The sunk costs bias describes the phenomenon in which an individual overvalues continuing an ongoing endeavor not solely based on future investment required to obtain a goal but also on irrecoverable (sunk) losses - information that, according to economic theory, should be ignored but instead is often honored as changing one's mind may be a source of cognitive dissonance. In the present study, we tested mice on a novel neuroeconomic task after being exposed to CSDS, a well-established model of depression. We found mice exposed to stress displayed an increased sensitivity to sunk costs that was renormalized following a single dose of ketamine. Mice were less sensitive to the effects of sunk costs 24 hr following ketamine treatment. These findings suggest that the antidepressant effects of ketamine may be mediated in part through changes in the processing of past-sensitive versus forward-looking information during complex decision-making.

Disclosures: R. Durand-De Cuttoli: None. **B. Sweis:** None.

Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.01/M25

Topic: G.06. Anxiety Disorders

Support: German Federal Ministry of Education and Research (BMBF: No.01EE1402)

Title: Neural Correlates of Fear Extinction in Patients with Anxiety Disorders: Reduced Activation Change in a Delayed Fear Extinction Paradigm

Authors: *Z. QIAN[1], Y. YANG[2], K. DOMSCHKE[3], K. KÖLKEBECK[4], J. DECKERT[5], M. HERRMANN[6], J. PLAG[7], B. STRAUBE[8];
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Abstract: Extinction learning is vital in the development, maintenance, and treatment of anxiety disorders (ADs). It involves continuous exposure to a previously conditioned stimulus (CS) without the expected negative outcome (US). Although neural activation changes from early to late CS+ presentations are believed to reflect improved inhibitory learning, this process is still not well understood in patients with ADs. In this study, we conducted a 24-hour delayed functional magnetic resonance imaging (fMRI) experiment to examine changes in neural responses from the early to late phases of the fear extinction paradigm. This experiment was preceded by a fear conditioning procedure on the previous day, allowing for overnight memory reconsolidation. Our quality-controlled dataset included 282 patients with ADs (146 females, 136 males, average age 31) and 103 healthy controls (49 females, 54 males, average age 33) from the PROTECT-AD sample. We used a two-way ANOVA to analyze group differences in reduction of neural activation from early CS+ to late CS+ and from early CS- to late CS-. The results revealed a significant main effect of group, indicating that healthy controls exhibited a more pronounced reduction in brain activation from early to late extinction compared to patients with ADs. This effect was observed in several brain regions, including the right superior temporal gyrus, the postcentral gyrus near the right posterior insula, the left postcentral gyrus near the anterior insula and putamen, and the left middle frontal gyrus and supplementary motor cortex. In terms of stimulus type, the left precentral gyrus and right postcentral gyrus showed more activation reduction for the CS+ compared to the CS-. Conversely, the right inferior occipital gyrus showed more activation reduction for CS- compared to CS+. We found no significant interaction between group and stimuli type in activation change from early to late extinction. In conclusion, our findings suggest that patients with ADs demonstrate reduced changes in neural activation from early to the late extinction phases, indicating a less adaptive processing of the CSs during extinction training, which could adversely affect treatment outcomes.

Disclosures: Z. Qian: None. **Y. Yang:** None. **K. Domschke:** None. **K. Kölkebeck:** None. **J. Deckert:** None. **M. Herrmann:** None. **J. Plag:** None. **B. Straube:** None.

Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.02/M26

Topic: G.07. Post-Traumatic Stress Disorder

Support: NIH Grant MH122387

Title: Neurobehavioral effects of counterconditioning in individuals with PTSD

Authors: *J. DUNSMOOR;
Univ. of Texas at Austin, Austin, TX

Abstract: While exposure therapy is a widely popular therapy for anxiety and stress disorders, the experimental analog to exposure (extinction) is well-known as a weak and transient form of learning. Strategies to enhance inhibitory learning to combat the retrieval of negative associations could help augment psychotherapy to prevent the relapse of fear. Counterconditioning is considered an enhanced form of extinction that involves replacing expected aversive events with positive events, rather than merely omitting the aversive event alone. This form of enhanced extinction could help compensate for deficiencies in the learning and retrieval of extinction memories, which are well described in PTSD populations. Here, we examined the neurobehavioral mechanisms of counterconditioning versus standard extinction in a population of adult individuals with post-traumatic stress disorder, and compared results to a psychiatrically normative population. METHODS: In a within-subjects fMRI study, we compared counterconditioning (CC; a form of rewarded-extinction) to standard extinction at recent (24 h) and remote (approximately one month) retrieval tests. We used univariate, functional connectivity, and multivariate pattern similarity to compare standard extinction versus counterconditioning in healthy adults and individuals with PTSD. RESULTS: Primary results confirmed that counterconditioning, as compared to standard extinction, diminishes fMRI BOLD activation in regions canonically involved in the appraisal and expression of conditioned fear, including the thalamus, brainstem, insula, and dorsal anterior cingulate cortex (whole-brain cluster corrected $p < .05$). Functional connectivity revealed dissociable trial-by-trial connectivity for CS+'s paired with a positive outcome versus a CS+ where the aversive outcome was merely omitted. Specifically, counterconditioning was associated with connectivity between the amygdala and ventral striatum, whereas extinction was associated with connectivity between the amygdala and ventromedial prefrontal cortex. However, these pattern of results were inconsistent between healthy comparison and PTSD groups. CONCLUSIONS: The ability to form and retrieve extinction memories can be affected by PTSD. Alternative strategies to enhance extinction through building new reward associations may bypass neurocircuitry compromised in PTSD to help enhance the learning and retrieval of inhibitory associations to combat retrieval of fear associations over time.

Disclosures: J. Dunsmoor: None.

Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.03/M27

Topic: G.06. Anxiety Disorders

Support: NIH Grant R21MH129851
NIH Grant R01MH121735

Title: Using a Novel Statistical "Hazard Rate" Approach to Model Anxiety

Authors: *E. A. VARGA[1], E. D. BOORMAN[2], A. S. FOX[3];
[1]UC Davis, Davis, CA; [2]Ctr. for Mind and Brain, Univ. of California, Davis, Davis, CA;
[3]Psychology, Univ. of California - Davis, Davis, CA

Abstract: Anxiety disorders are some of the most prevalent disorders in the world, with around one third of people being affected by them in their lifetime. Anxious people tend to experience the waiting period leading to an aversive event as more anxiogenic than the event itself. Indeed, various seminal studies indicate that uncertainty is a major contributor to anxiety, though the definition of uncertainty is often unclear and non-specific. Many of these studies have compared temporally certain and uncertain threats to support the claim that uncertainty causes anxiety, but we have identified a potential confound in this approach. Namely, as experimenters manipulate the uncertainty of threat, they implicitly manipulate the hazard rate (the probability of threat given that it has not happened yet). We address this using a novel uncertain threat anticipation paradigm that matches the probability of threat across two conditions, while varying the hazard rate and measuring resulting anxiety ratings and behavior. Specifically, we show that increased hazard rate, which can build during periods of uncertainty, promotes a tendency to avoid threatening contexts. Survival analysis demonstrates that participants were more likely to make threat avoidance decisions in our high hazard rate condition, forgoing cash rewards (log-rank test, N=42, χ;2=259.30, p<.005). Further, this high hazard rate condition increased self-reported fear/anxiety, with 19 of N=21 participants rating it as more anxiogenic than the lower hazard rate condition. These results reframe decades of past research by describing a potential explanation for why the anticipation of temporally uncertain threats is anxiogenic, providing a new framework through which to consider the causes of anxiety and anxiety-related psychopathology.

Disclosures: E.A. Varga: None. **E.D. Boorman:** None. **A.S. Fox:** None.

Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.04/M28

Topic: G.05. Mood Disorders

Support: ONR 00014-19-1-2149
Pritzker Neuropsychiatric Disorders Research Consortium Fund, LLC
Hope for Depression Research Foundation (HDRF)

Title: Predicting Resilience and Susceptibility to Stress in a College Freshman Sample: Validating the Affect Score

Authors: *H. KHALIL[1], V. MURPHY-WEINBERG[2], J. F. LOPEZ[3], S. J. WATSON[4], H. AKIL[2];
[1]The Univ. of Michigan, Ann Arbor, MI; [2]Univ. of Michigan, Ann Arbor, MI; [3]Univ. Michigan, Ann Arbor, MI; [4]MNI, Michigan Neurosci. Inst., Ann Arbor, MI

Abstract: We used a longitudinal approach to understand the various factors shaping resilience and vulnerability to stress, as indexed by the development of anxiety or depression symptoms in a sample of University of Michigan freshmen. At the start of their freshman year, subjects were genotyped and a polygenic risk score for depression (MDD-PRS) was calculated. At baseline we gathered information regarding their family history as well multiple psychological variables using survey instruments. Subjects were then sampled at multiple timepoints during their freshman year on clinical rating scales, including PHQ-9 for depression and GAD-7 for anxiety. Daily sleep levels and physical activity were collected as well. We found that while the MDD-PRS was useful in significantly predicting follow-up depression scores prior to the COVID-19 pandemic, its usefulness faded in the two cohorts (starting in Fall 2019 and Fall 2020) sampled during the pandemic. In particular, depression scores in female subjects with lower genetic risk increased the most dramatically during this pandemic period. We also used machine learning to determine which of the psychological instruments collected at baseline were best at predicting follow-up depression scores. These instruments, which included family history, state and trait variables, were then combined into a single index which we have termed the "Affect Score". This index proved highly predictive of follow-up depression scores. Here, we present data validating the Affect Score in two new cohorts (starting in Fall 2021 and Fall 2022). While the MDD-PRS is still not significantly predictive of follow-up depression scores in these new cohorts, the Affect Score continues to be highly predictive, independent of sex.

Disclosures: H. Khalil: Other; Pritzker Neuropsychiatric Disorders Research Consortium Fund, Hope for Depression Research Foundation (HDRF). **V. Murphy-Weinberg:** None. **J.F. Lopez:** None. **S.J. Watson:** Other; Hope for Depression Research Foundation (HDRF), Pritzker Neuropsychiatric Disorders Research Consortium Fund, LLC. **H. Akil:** Other; Pritzker Neuropsychiatric Disorders Research Consortium Fund, LLC, Hope for Depression Research Foundation (HDRF).

Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.05/M29

Topic: G.06. Anxiety Disorders

Support: NIH Grant DA051922

Title: Granger causality mapping of subgenual anterior cingulate connectivities and individual mental health traits: a Human Connectome Project Study

Authors: H.-T. LI[1], J. S. IDE[1], Y. CHEN[2], ***C.-S. LI**[1];
[1]Yale Univ., New Haven, CT; [2]Yale Univ. Sch. of Med., New Haven, CT

Abstract: Background The subgenual anterior cingulate cortex (sgACC) is implicated in individual variation in behavioral traits critical to mental health. Extant imaging studies have highlighted altered sgACC activity or connectivity during resting or behavioral challenges. However, as a hub of the limbic circuit that regulates emotion and motivated behavior, no studies have systematically investigated the functional inputs and outputs of the sgACC and how these connections relate to individual behavioral traits. **Methods** A total of 890 subjects' resting state fMRI data of the HCP were included in the analyses, after exclusion for severe head movements (Power et al., 2012) or non-stationary time series. Average BOLD time series were extracted for ROIs defined according to the Shen's atlas of 268 regions and for the sgACC. Granger causality (GC) between sgACC and each ROI was computed with the F-value representing the strength of "causality" for the GC influence of sgACC on other ROIs (*F-out*) and of the ROIs on the sgACC (*F-in*), respectively. Multiple comparisons across ROIs were corrected for FDR. We first evaluated the group results by counting the number of subjects with significant connections and performing a binomial test to identify the ROIs with significant sgACC outputs and inputs. Second, we performed Pearson regressions the GC measures of significant sgACC connections with the clinical metrics of the Achenbach Adult Self-Report (ASR) and evaluated the results with correction for multiple comparisons. **Results** 1) The findings showed right medial/posterior orbital gyri, precuneus, left entorhinal area, and bilateral cerebellum as significant output ROIs and right subcallosal area, medial/posterior orbital gyri, superior/middle frontal gyri, precentral gyrus, anterior insula, precuneus, left middle frontal gyrus, precentral gyrus, and bilateral cerebellum as significant input ROIs (all p's <0.00018). 2) Of these Granger causal connectivities, the GC outputs to the right posterior orbital gyrus was negatively correlated with anxiety scores ($p<0.005$, $r=-0.281$) as well as aggression, rule-breaking, and thought problems ($p<0.001$, $-0.369<r<-0.296$). The GC inputs from the right anterior insula was negatively correlated with anxiety score ($p<0.005$, $r=-0.282$). **Conclusions** The findings demonstrated for the first time functional outputs and inputs of the sgACC in resting state connectivities and highlight new neural markers of individual anxiety and comorbid behavioral traits across a large sample of young adults.

Disclosures: H. Li: None. **J.S. Ide:** None. **Y. Chen:** None. **C. Li:** None.

Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.06/M30

Topic: G.07. Post-Traumatic Stress Disorder

Title: Intracranial electrophysiological representations of traumatic autobiographical memories and their reappraisal in the posterior cingulate cortex

Authors: *C. LOPEZ RAMOS[1], A. ROCKHILL[2], M. SHAHIN[1], D. R. CLEARY[3], K. PARK[4], K. COLLINS[5], L. D. ERNST[1], A. M. RASLAN[6];
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Abstract: Introduction: The posterior cingulate cortex (PCC) is implicated in the processing of autobiographical memories (AMs) with alterations observed in patients with post-traumatic stress disorder (PTSD). However, their electrophysiological representations are less well understood. Using intracranial human recordings, we investigated the electrophysiological correlates of traumatic AMs and their modulation through cognitive reappraisal. Methods: A patient with complex PTSD (PCL-5 score 57) was implanted with intracranial electrodes for seizure localization and participated in an AM task paradigm consisting of recalling calm, sad, and PTSD-related traumatic memories. The patient was instructed to maintain or reappraise the AM for 20 seconds. Negative valence and arousal ratings were performed after each trial. Responses to script-driven imagery scores were highest for traumatic-related AMs, followed by sad (60 vs 27, respectively). We performed power spectral density analyses to evaluate changes in oscillatory activity associated with re-experiencing AMs. Results: Negative valence and arousal ratings were highest for traumatic AMs, although no significant decreases were observed in ratings with reappraisal. Theta oscillatory peaks were observed in the PCC during sad and traumatic AM recall. During reappraisal trials, theta power decreased for both negative memories. Conclusion: Cognitive reappraisal modulates theta activity in the PCC during both traumatic and sad memories in this patient with PTSD. Our preliminary findings shed insight into the electrophysiological representations of PTSD-related traumatic AMs. Further research is warranted to evaluate the intracranial neural correlates of reappraisal-based interventions for trauma-related disorders.

Disclosures: C. Lopez Ramos: None. **A. Rockhill:** None. **M. Shahin:** None. **D.R. Cleary:** None. **K. Park:** None. **K. Collins:** None. **L.D. Ernst:** None. **A.M. Raslan:** None.

Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.07/M31

Topic: G.06. Anxiety Disorders

Support: U01DA041022
R01MH128959
R01AG081144

Title: Functional connectivity of the salience network is related to symptoms of social anxiety in preadolescence

Authors: *R. HICKSON[1], E. M. MULLER-OEHRING[2], N. TOTAH[1], A. HERNANDEZ[1], A. CHEU[1], M. CALDERON[1], T. SCHULTE[3];
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Abstract: Preadolescence is a critical time window in social-emotional development. The salience network (SN) consists of limbic and prefrontal regions that are engaged in social connectedness and anxiety. Neurofunctional connections of the SN to other functional brain systems, such as the default mode network (DMN), play essential roles in bottom-up attention and top-down regulation of anxiety. This study investigated how internetwork functional connectivity (FC; i.e., synchronized activity) of the SN with other intrinsic networks is associated with the likelihood of an adolescent reporting symptoms of social anxiety. We used resting-state fMRI and self-report data from 7,652 participants (45.7% female assigned at birth, ages 10-13, 4,231 White, 1,021 Black, 1,452 Hispanic, 145 Asian, 803 Other) of the 2-year-follow-up of the Adolescent Brain Cognitive Development (ABCD) study. Internetwork FC of the brain was measured as the average correlation between two networks (e.g., average correlation between SN and DMN). Symptoms of social anxiety were measured by adolescents' self-report answers to questions on the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Aged Children (K-SADS). Logistic regression tested the relationship between symptoms of social anxiety and internetwork FC for the SN with 12 other networks. We found that greater SN-DMN FC increased the likelihood of reporting symptoms of social anxiety (OR = 30.68, $p < .05$, 95% CI = [1.52, 619.01]). Greater SN-retrosplenial temporal network (RTN) FC also increased the likelihood of reporting symptoms of social anxiety (OR = 6.92, $p < .05$, 95% CI = [1.52, 39.79]). Overall, our findings highlight the relevance of internetwork FC for social functioning in the maturing brain. Specifically, internetwork connectivity of the SN to the DMN and RTN are concomitant to experiencing symptoms of social anxiety. Future analyses are warranted on the longitudinal implications of brain functional network rewiring in early adolescence for later social-emotional development.

Disclosures: R. Hickson: None. **E.M. Muller-Oehring:** None. **N. Totah:** None. **A. Hernandez:** None. **A. Cheu:** None. **M. Calderon:** None. **T. Schulte:** None.

Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.08/M32

Topic: G.07. Post-Traumatic Stress Disorder

Title: Changes in functional connectivity of quantitative electrophysiology as a marker of treatment response in posttraumatic stress.

Authors: *J. PINTO;
Univ. of Sydney, Sydney, Australia

Abstract: Background: Posttraumatic stress disorder (PTSD) is characterized by abnormal functional connectivity (FC) during a state of rest, however, evidence for a prognostic role of candidate FC markers is scarce.**Aims:** This study investigated treatment-related changes in abnormal FC of quantitative electrophysiology (qEEG) among patients with chronic clinical and subclinical PTSD. **Methods:** War-exposed African refugees were assigned to a clinical and subclinical PTSD group (n=35) and a healthy control group (n=35). The final sample (N = 70) consisted of 31 males and 39 females aged 18 - 54 years (M = 33.64, SD = 10.54). All participants completed the Clinician-Administered PTSD Scale for DSM-5 (CAPS-5), and pre/post qEEG recordings seven days apart after a week-long self-guided digital trauma intervention. A full psychiatric evaluation to screen for co-morbid disorders was also conducted at baseline. Scalp qEEG was recorded with the Geodesic's 32-Channel HydroCel net according to the standard 10-20 international system. Recordings were plotted into a frequency power spectrum using Fast-Fourier Transform (FFT) for all brain networks and frequencies. EEG coherence was calculated to estimate abnormal FC. Independent t-tests and regression analyses were used to identify markers and symptom correlations. **Results:** Abnormal FC was documented in the PTSD group across several brain networks including centro-parietal, centro-occipital, fronto-central, fronto-parietal, fronto-temporal, parieto-occipital, and most predominantly, in the fronto-occipital network (44.2%, $p < .001$). Post-treatment, 50 of 61 identified FC metrics were no longer irregular when compared to healthy controls, with notable changes in alpha and theta oscillations within the fronto-occipital network, concurrent with significant PTSD symptom severity reductions. **Conclusion**: This study confirmed that patients with clinical and subclinical PTSD present abnormal brain activity in resting state FC, and supports preliminary evidence that brain coherence may be utilized as an objective marker to predict PTSD treatment response. Future studies should further investigate FC changes post-treatment within the fronto-occipital network among trauma-impacted populations to further our understanding of the pathophysiology of PTSD.

Disclosures: J. Pinto: None.

Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.09/M33

Topic: G.07. Post-Traumatic Stress Disorder

Support: VHA I01CX000994
IO1CX001937
NIGMS R25 GM069234 (BCM PREP)

Title: Resting State Functional Connectivity between the Habenula and the Globus Pallidus in PTSD

Authors: *J. TORANZO[1], T. W. SHAW, II[2], A. CASTELLANOS[3], R. SALAS[2], G. A. DE ERAUSQUIN[1];
[1]Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX; [2]Baylor Col. of Med., Houston, TX; [3]Dept. of Neurosci. Res., Michael E. DeBakey VA Texas Med. Ctr., Houston, TX

Abstract: According to The National Center for PTSD, approximately 6% of individuals will experience post-traumatic stress disorder (PTSD) at some point in their lives. Neuroimaging techniques, such as functional MRI (fMRI), have emerged as a powerful tool for identifying biomarkers associated with PTSD. The discovery of these biomarkers could potentially pave the way for future research and development of effective treatments for PTSD.This study investigates the link between Post-Traumatic Stress Disorder (PTSD) and Resting State Functional Connectivity (RFSC) between the habenula (Hb) and striatum components, specifically the caudate, putamen, and globus pallidus (GP).We used Functional magnetic resonance imaging (FMRI) to analyze RFSC in 35 PTSD patients and 269 psychiatric controls. Diagnoses and the number of different types of traumatic events were evaluated using the Structured Clinical Interview for Diagnostic - IV and the Stressful Life Events Screening Questionnaire (SLESQ), respectively.PTSD patients had lower connectivity (-0.048 ± 0.030) than psychiatric controls (0.074 ± 0.015) between the right Hb and left GP with a p-value of .001 meeting the Bonferroni correction (p = .004). The RFSC between the right Hb and left putamen and between the left Hb and left GP were also smaller in PTSD patients, with respective p-values of .014 and .049 not meeting the Bonferroni correction. RSFC between the right Hb and left GP showed a significant association with SLESQ Factor 1 (sexual/childhood abuse) in PTSD patients.Our findings reveal a lower RFSC between the right Hb and left GP in PTSD patients, suggesting its potential as a therapeutic target.**Keywords:** PTSD, habenula, resting state functional connectivity, globus pallidus, ketamine

Disclosures: J. Toranzo: None. **T.W. Shaw, II:** None. **A. Castellanos:** None. **R. Salas:** None. **G.A. de Erausquin:** None.

Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.10/M34

Topic: G.07. Post-Traumatic Stress Disorder

Support: Mary Gordon Roberts Fellowship
Cordeiro Summer Research Fellowship

Title: Brain region volumes and socioenvironmental factors in the symptom severity of adult post-traumatic stress disorder in the AURORA cohort

Authors: *C. W. MCFARLAND[1], H. GARRISON-DESANY[2], C. A. DENCKLA[3];
[1]Dept. of Neurosci., Harvard Univ., Cambridge, MA; [2]Dept. of Social and Behavioral Sci., Harvard T.H. Chan Sch. of Publ. Hlth., Boston, MA; [3]Harvard T.H. Chan Sch. of Publ. Hlth., CAMBRIDGE, MA

Abstract: *Background:* Brain morphology of key neural threat structures is implicated in adult post-traumatic stress disorder (PTSD). However, the interplay between differences in brain morphology and socioenvironmental factors in adult PTSD symptom severity has not yet been fully characterized. This presents a significant gap given known associations between socioenvironmental factors and brain morphology. To address this knowledge gap, this study aims to assess the predictive value of early post-trauma brain region volumes for later PTSD symptom severity and explore how socioenvironmental factors might modulate these relationships.
Methods: We conducted secondary analyses on 293 participants with complete case data in the AURORA Study, a longitudinal multi-site assessment of post-traumatic neuropsychiatric sequelae. Participants completed an MRI scan to evaluate brain morphology at 2 weeks post-trauma and the Post-Traumatic Stress Disorder Checklist for DSM-5 to assess PTSD symptom severity at 6 months post-trauma. Socioenvironmental variables were self-reported at study enrollment. We conducted zero-inflated negative binomial regression to estimate associations between early brain region volume and later PTSD symptom severity, adjusting for race/ethnicity, sex, age, educational attainment, and socioeconomic status (SES). Interaction terms were used to examine effect modification by race/ethnicity, sex, and SES strata.
Results: Larger volume of the left thalamus ($\beta = 5.266$, $p = 0.002$, 95% CI: 1.903 to 8.629) and right thalamus ($\beta = 4.565$, $p = 0.007$, 95% CI: 1.249 to 7.881), as well as increased cortical surface area of both hemispheres ($p < 0.05$) were observed to be predictive of an absence of later PTSD symptom severity. Decreased cortical thickness of both hemispheres and decreased volume of the right thalamus ($p < 0.05$) were also predictive of increased PTSD symptom severity. Interaction analyses revealed sex-specific impacts of brain region volume, including decreased right amygdala volume predicted increased PTSD symptom severity in men ($\beta = -2.055$, $p = 0.010$, 95% CI: -3.622 to -0.488), but with a reduced effect in women (interaction $\beta = 1.816$, $p = 0.047$, 95% CI: 0.025 to 3.608).
Conclusion: These results suggest that early neuroanatomical structures post-trauma are linked to later PTSD symptom severity, with associations influenced by socioenvironmental factors. This

highlights the importance of considering both biological and social dimensions in the understanding, diagnosis, and treatment of PTSD, and suggests that neuroanatomical features warrant further investigation as potential mechanisms in the pathogenesis of PTSD.

Disclosures: C.W. McFarland: None. **H. Garrison-Desany:** None. **C.A. Denckla:** None.

Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.11/M35

Topic: G.06. Anxiety Disorders

Support: NIH Grant R25NS080686
NIH Grant R56MH111700-01A1

Title: Characterizing EEG Connectivity Patterns in Anxious Adolescents to Identify Subgroups

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Abstract: There has been an increase of anxiety disorders amongst the general population but in adolescents especially (Malik, et al., 2022). Despite the upward trend, little is known about specific subtypes of anxious individuals and heterogeneity within the disorder that may provide insight to more personalized treatment and intervention. This current study attempts to characterize biologically-derived subgroups of anxiety to specific behaviors and symptoms using EEG connectivity patterns. Specifically, we examined theta connectivity, referring to the synchronization or coordination of neural activity within the theta frequency range across different brain regions, in a sample of anxious adolescents. Previous studies have shown certain theta connectivity to be an important marker for anxiety, specifically increased connectivity in the midline frontal channel in anxious individuals compared to control groups (Xing, et al., 2017). We hypothesize that these subgroups with specific functional connectivity maps differ in behavioral measurements of attentional bias and anxiety. The data was collected from teens (N=64) aged 12-14 (M =12.89) with mild to severe anxiety. Participants underwent a clinical interview (ADIS-5; Barlow and Brown,. 2014) and answered a set of self-report questionnaires such as Intolerance of Uncertainty Scale (IUS; Freestone et al,. 1994) and Screen for Child Anxiety Related Disorders (SCARED; Birmaher et al., 1997) accessing for different symptoms of anxiety before completing a baseline task and the dot probe while EEG was recorded. The baseline task measures the brain activity of participants at resting state, requiring participants to relax with each minute keeping their eyes open or closed. Trial Level Bias Scores (TLBS; Zvielli et al., 2015) were derived from the dot probe. Using Group Iterative Multiple Model Estimation (GIMME; Gates and Molenaar., 2012), two subgroups were identified: Subgroup A, was

characterized by more frontal connectivity and Subgroup B, was characterized by frontal and posterior connectivity. Compared to Subgroup A, Subgroup B scored consistently higher in anxiety scores and anxiety-related measures including: greater SCARED GAD, higher intolerance of uncertainty, and greater TLBS mean positive scores (indicated bias towards threat).The results suggest that there are distinct anxiety subgroups that vary in symptom severity and highlights the need for further research into heterogeneity within a disorder for intervention as well as the stability of subgroup classifications over time.

Disclosures: M. Mei: None. **P. Dave:** None. **S. Zalanyi:** None. **J. de Rutte:** None. **T.A. Dennis:** None.

Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: G.06. Anxiety Disorders

Support: NIH Grant P50MH115874
NIH Grant P50MH119467

Title: The role of stress peptides in the amygdala, understanding suicide and childhood trauma

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Abstract: Neural networks and signaling pathways associated involved in fear learning and stress responses have been postulated to mediate the effects of trauma, particularly early life adversity (ELA), on emotional dysregulation, in turn increasing suicide risk. To test this hypothesis, we focused on key stress-related signaling pathways, i.e. the corticotropin releasing hormone (CRH), nociceptin (NOP) and their respective receptors (CRH1R and OPRL1), within the central nucleus of the amygdala (CeA), hippocampus (HC), and prefrontal cortex (PFC). A cohort of 150 brain donors (donors with MDD (n=50), PTSD comorbid with MDD (n=50), and unaffected controls (n=50)) was available for these studies. Protein lysates were obtained from human post-mortem tissue samples containing the CeA, HC, and PFC. We performed western blots to measure expression levels of PACAP receptor (PAC1R), CRH, NOP, CRH1R, and OPRL1. Statistical analyses were carried out using multiple linear regression models and elastic net analysis accounting for the demographic and clinical variables. Comparisons between the 3

donor groups did not show significant differences in any of the proteins tested. Across the MDD and PTSD cohort, donors exposed to ELA who died by suicide had significantly higher CRH ($p=0.002$) and lower NOP levels ($p=0.018$) in the CeA compared to donors with ELA who died by other means. Regardless of diagnosis, donors who died by suicide had significantly higher CRH expression in CeA ($p=0.002$). Similarly, donors who experienced ELA also had significantly higher CRH expression in CeA ($p=0.0078$). No significant associations between primary diagnosis, suicide, CT, or sex and PAC1R or CRH1R were observed. Our results show that the expression of CRH and NOP in the CeA is disrupted in association to suicidal behavior and ELA. We put forth that a disruption of this relationship within the CeA may play a key role in emotional dysregulation following ELA and, later in life, contribute to psychiatric symptoms and suicidal behavior.

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Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: G.06. Anxiety Disorders

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National Center for Advancing Translational Sciences Grant UL1TR002369
NIH grant P51 OD011092

Title: Discrimination and Development: The Influence of Maternal Adversity on Child Neurobehavioral Development

Authors: *M. HAYES[1], E. WOOD[2], N. SRIDHAR[2], O. LASHLEY[2], H. C. GUSTAFSSON[3], E. L. SULLIVAN[4];
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Abstract: Adversities experienced by the mother during gestation can have a profound effect on child health, influencing both fetal brain development and infant behavior. The mechanisms by which types of adversity contributes to child development, however, are not fully established. We prospectively examined the effect of maternal perceived discrimination on maternal

psychological distress and offspring temperament at 1-, 6-, and 12-months postpartum. We hypothesized that maternal experiences of discrimination would increase both maternal distress and infant negative affect and that social support would serve as a protective factor, moderating the relationship between discrimination and infant negative affect.
Methods: Pregnant individuals' (N=302) mood was captured during the 2nd trimester using the Center for Epidemiological Studies-Depression Scale (CES-D), Beck Anxiety Inventory (BAI), Pregnancy Anxiety Questionnaire (PRAQ), State-Trait Anxiety Inventory (STAI), and Perceived Stress Scale (PSS). Confirmatory factor analyses resulted in a latent maternal distress variable composed of CESD, EPDS, BAI, STAI, and PSS scores. The Everyday Discrimination Scale, Macarthur Scale of Subjective Social Status, and Multidimensional Scale of Perceived Social Support were completed during the 2nd and 3rd trimesters, and 1 month postpartum. Infant neurodevelopmental was assessed by the Infant Behavioral Questionnaire-Revised at 1-, 6-, and 12-months.
Results: More discrimination experiences were associated with greater maternal distress ($\beta = 0.394$, $p = 0.001$). Social support moderated the relationship between discrimination and maternal distress: higher levels of social support was protective and dampened the relationship between discrimination and maternal distress ($\beta = -0.184$, $p = .004$). At 1-month postpartum, more maternal discrimination experiences were associated with increased infant negative affect ($\beta = 0.311$, $p = .006$), but no effects were present at 6- and 12-months postpartum.
Discussion: Adversity during gestation can influence both parent distress and offspring neurobehavioral development. As underserved populations disproportionately experience discrimination, maternal adversity may particularly challenge those children, underscoring the need for further research in this area.

Disclosures: M. Hayes: None. **E. Wood:** None. **N. Sridhar:** None. **O. Lashley:** None. **H.C. Gustafsson:** None. **E.L. Sullivan:** None.

Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.14/M38

Topic: G.05. Mood Disorders

Support: T32 G-RISE 1T32GM144873-01

Title: The correlations between hypothalamus and hippocampus predominantly in hippocampal body and stress-related reversibility in hippocampal head in trauma survivors

Authors: *R. M. HAMDAN[1], C.-H. SHIH[2], Q. SHAO[3], X. WANG[1], H. XIE[4];
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Abstract: The structural connections between the hypothalamus and hippocampus in humans remain largely unexplored. This study investigated these relationships following acute trauma and recovery, and during chronic stress from COVID-19 pandemic. Acute trauma survivors (n=189) were recruited after life-threatening experiences and underwent initial structural MRI scans within 2 weeks. Ninety-nine survivors returned for follow-up scans a year later. Forty-two survivors took additional scans during the pandemic. Partial correlation analyses were used to test the relationships between bilateral hypothalamic and hippocampal head/body volumes, as well as correlations between 5 hypothalamic nuclei and 7/8 subfields in hippocampal head/body (FreeSurfer v7.2), controlling for age, sex and intracranial volume. Chi-square analyses were used to test the changes of nuclei-subfield correlations under different physical conditions. Results revealed bilateral hypothalamic volumes were significantly positively correlated with hippocampal head/body volumes under acute and chronic stresses (ps: <0.001-0.029), and with hippocampal body in recovery (ps: <0.001-0.011). The number of significant nuclei-subfield correlations (threshold $p<0.01$) was greater in hippocampal body than in head (ps=0.009 and 0.005 for left/right hypothalamus). All nuclei-subfield correlations in the hippocampal body did not show differences in all conditions. However, nuclei-subfield correlations in the hippocampal head were observed only during chronic stress for the left hypothalamus. Conversely, they significantly increased during acute stress and diminished during recovery for the right hypothalamus (ps=0.004). This study indicates initial evidence of positive associations between hypothalamic nuclei and hippocampal subfields in humans, suggesting predominant structural connections within the hippocampal body and exhibiting reversibility in hippocampal head in response to stress.

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Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.15/M39

Topic: G.07. Post-Traumatic Stress Disorder

Support: IK2RX002490
I50RX003000

Title: Fronto-limbic activity and functional connectivity in post-traumatic stress disorder and mild traumatic brain injury

Authors: R. CLAAR[1], *D. LAMB[2,3];
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Abstract: Post-traumatic stress disorder (PTSD) and mild traumatic brain injury (mTBI) are complex, heterogeneous, and disruptive medical conditions that afflict millions yearly. mTBI often co-occurs or precedes PTSD, particularly in military contexts. PTSD and mTBI share many symptom domains, most pertinent to this work being emotional dysregulation and executive dysfunction. These shared symptoms create diagnostic and treatment ambiguity for clinicians. Investigations into the neurobiology of PTSD and mTBI could help distinguish the two conditions or prove a common pathway for their shared symptoms, thereby improving diagnostic clarity and creating personalized treatments. Current first-line treatments for PTSD do not reduce symptoms in a significant portion of patients, and much is still not understood about what underlies PTSD and mTBI symptoms, making the potential impact of this work significant. We enrolled 428 right-handed aged 18-45 years old combat-Veterans, 119 of which fully qualified after medical record review and completed our study. Participants' health and available service records were reviewed to confirm diagnoses and identify any potential confounding factors. Participants were administered a large battery of neuropsychological, cognitive, and emotional assessments along with structural magnetic resonance imaging (MRI) and task-based functional MRI. Five individuals had incomplete and/or poor data quality, leaving 114 individuals split across four groups: healthy controls (HCs), PTSD and no history of TBI, history of mTBI but no history of PTSD, both PTSD and history of mTBI. Analyses were conducted to assess group differences in functional connectivity between frontal and limbic regions. Relationships were also explored between functional connectivity and assessment scores. We found significant group differences in functional connectivity between key limbic and frontal brain regions including key regions implicated in the etiology of emotional dysregulation such as the insula and lateral prefrontal cortex. We also observed a significant group by symptom interaction in functional connectivity measures and symptoms of emotional dysregulation. These results help advance our models of how executive dysfunction and emotional dysregulation may be related share common underlying neurobiological characteristics in both PTSD and mTBI. In addition, this evidence implicates potential biomarkers of disease which could help improve diagnostic accuracy for individuals with PTSD or mTBI.

Disclosures: R. Claar: None. **D. Lamb:** A. Employment/Salary (full or part-time):; Department of Veterans Affairs, University of Florida. 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.; IK2RX002490 and I50RX003000.

Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.16/M40

Topic: G.06. Anxiety Disorders

Support: Mitacs

Title: Cortical and physiological modulations induced by non-invasive brain stimulation for anxiety.

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Abstract: A type of non-invasive brain stimulation using pulsed stimulation (NI-PBS) is a technology usedto treat anxiety and insomnia. A single session of NI-PBS has been shown to quiet activity in the Default Mode Network (DMN); however, its impact on physiological biomarkers, such as those related to anxiety, are unknown. It is critical to understand NI-PBS's physiological impact as a gauge of its efficacy to treat anxiety - a highly debilitating and prevalent condition. This study aimed to assess how one 30-minute NI-PBS session to the mastoid processes modulates electroencephalographic (EEG) activity, heart rate (HR), heart rate variability (HRV), blood oxygenation, skin conductance and perceived anxiety. Anxiety questionnaire (Generalized Anxiety Disorder 7-item, GAD-7; State-Trait Anxiety Inventory state and trait scales, STAI-S & STAI-T) and physiological data was collected from 32 healthy young adult participants ages 18-31 with moderate to severe anxiety (GAD-7 score 9+). Questionnaire, resting state and flanker task measurements were acquired pre- and post-NI-PBS. Physiological (i.e., skin conductance, HR, HRV) and EEG (i.e., power spectra) data were acquired at rest with eyes closed. Error-related negativity (ERN) and visual N2 event-related potentials (ERPs) were analyzed from an arrow-based flanker task due to their association with anxiety. It was hypothesized that alpha power and heart rate variability would increase, and beta power, ERP amplitudes, heart rate and skin conductance would decrease. Results showed: perceived anxiety (GAD-7 and STAI-S) to significantly decrease ($p<0.05$). Secondary analyses separated non-responders (whose GAD-7 or STAI-S scores increased; n=8) from responders (n=24). Post-NI-PBS, the overall group showed a large increase in ERN and N2 amplitudes ($p<0.05$). Responders alone showed a significant increase in frontocentral theta power ($p=0.03$), with an overall group trend toward increased global absolute theta power ($p=0.06$). Absolute frontocentral ($p=0.02$) and central ($p=0.05$) high beta power increased. Relative posterior high beta power significantly increased ($p=0.01$). Skin conductance increased ($p<0.001$). HR and HRV were unaffected ($p>0.05$). Increased ERP amplitudes and elevated high beta power may indicate potential cognitive enhancement after NI-PBS. Increased theta power may indicate an enhanced state of relaxation. Future work should assess potential physiological changes induced by longitudinal NI-PBS usage, as well as potential cognitive benefits of NI-PBS.

Disclosures: M. Faerman: None. **A. Smith:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Roga Life Inc. **R. Staines:** None.

Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.17/N1

Topic: G.07. Post-Traumatic Stress Disorder

Support: UTHSCSA CBN Pilot Award
5I01BX004693-02

Title: Preclinical and clinical outcomes of prefrontal cortex transcranial direct current stimulation in PTSD

Authors: *A. M. BOLEY[1], S. M. PEREZ[2], K. H. BUCCELLATO[3], C. L. STRAUD[4], D. A. MORILAK[5], D. J. LODGE[6], F. R. CARRENO[7];
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Abstract: Post traumatic stress disorder (PTSD) is a debilitating disorder that affects ~16 million people. Symptoms of PTSD often center around a traumatic event and can include fear, anxiety, and uncontrollable negative thoughts. Fear related information processing includes the prefrontal cortex (PFC), amygdala, and the hippocampus. The PFC is important for cognitive flexibility, extinction of conditioned fear, and volitional regulation of negative emotion - all being altered in PTSD. A consistent observation in functional imaging studies and in rodent models is hypoactivity of the PFC. Non-invasive brain stimulation is a novel approach to modulate PFC activity directly. Specifically, transcranial direct current stimulation (tDCS) can target superficial areas of the brain, such as the PFC. PFC activity can be modulated by anodal stimulation which causes depolarization and increases excitability. We posit that anodal tDCS will reverse PFC hypoactivity and may serve as a novel therapeutic approach for the treatment of PTSD. Here, we augment PFC activity using tDCS in rats and human subjects to investigate the potential therapeutic utility of this approach. Specifically, we observed an increase in c-fos expression in rats following PFC tDCS demonstrating target engagement. The consequence of this was examined in a rodent model used to study PTSD, namely the chronic unpredictable stress (CUS) model. In CUS rats, tDCS was administered as an adjunct treatment during sub effective extinction and reversed stress-induced deficits in cognitive flexibility. In parallel, a phase II, two arm, randomized clinical trial was conducted to examine tDCS vs. placebo delivered in combination with written exposure therapy (WET) in subjects with PTSD. Both arms completed five sessions of WET which is a first line exposure-based therapy known to be effective as a treatment for PTSD. The treatment arm received 30 minutes tDCS stimulation targeted to the dorsolateral PFC over five weekly sessions. Initial findings reveal a clinically relevant improvement in PTSD and depression symptoms across all groups. The treatment is well tolerated with no severe adverse effects and only one participant dropped out (with a large improvement in symptoms). Taken together these data suggest that tDCS augments PFC activity and may be effective as a safe, effective, and a promising novel adjunct treatment for PTSD.

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Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.18/N2

Topic: G.07. Post-Traumatic Stress Disorder

Title: Modulation of PTSD symptoms with focused ultrasonic waves

Authors: *C. LYBBERT[1], T. RIIS[1], E. WILDE[2], B. J. MICKEY[3], A. LOSSER[1], J. KUBANEK[1];
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Abstract: PTSD affects 1 in 15 people. One-third of these patients are resistant to current treatments. Here, we developed a new approach and system to directly modulate the deep brain regions involved in PTSD. This clinical study was pre-registered on ClinicalTrials.gov as NCT06135064. Within this protocol, two adult female patients (ages 27 and 35) with clinical diagnoses of PTSD were stimulated with a low-intensity ultrasound phased array. This system is unique in that it directly measures and compensates for the severe attenuation of ultrasound by the head and hair, thus delivering controlled, deterministic intensity of ultrasound into deep brain targets. We used previous protocol to modulate targets in the dorsal anterior cingulate and the subcallosal cingulate cortex. Each patient received a total of 40 minutes of stimulation. The PCL-5 served as the primary metric to evaluate the symptoms. In addition, subjective units of distress (SUD) scores measured acute changes in PTSD symptoms during the treatment. Both participants reported a clinically significant decrease in their PCL-5 scores, with a mean reduction of 30.5 points. This constitutes an average 55% decrease in PTSD symptom score. Moreover, during the treatments, there was a nearly immediate decrease in the SUD score, amounting to 32.5 points or a 41% reduction. These data provide an initial proof of concept that low-intensity transcranial focused ultrasound could be a safe and effective option for targeted, circuit-directed treatments of PTSD. The data collection is ongoing with a recruitment rate of about 1 patient / week.

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Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.19/N3

Topic: G.07. Post-Traumatic Stress Disorder

Title: Methylone, a rapid-acting neuroplastogen to treat PTSD and other CNS disorders

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Abstract: PTSD is a debilitating disorder with lifetime prevalence estimates of 9.7% in women and 3.6% in men. Current pharmacotherapies (two SSRIs) have limited efficacy and remission rates (20-30%). MDMA-AT shows promise as a potential new therapeutic for PTSD, but requires extensive concomitant psychotherapy. Methylone, a rapid-acting neuroplastogen and the beta-ketone analog of MDMA, is currently under clinical development for PTSD. Despite their structural similarity, methylone shows distinct pharmacological and subjective effects compared with MDMA. Both are triple reuptake inhibitors and releasers of 5HT, NE and DA. However, methylone has less effect on 5HT and DA release compared with MDMA, which may lead to lower side effects and improved efficacy. Both compounds show robust beneficial effects in PTSD patients, but methylone can be administered more frequently (i.e., weekly vs. monthly) and does not require adjunctive psychotherapy. Methylone is also highly selective and lacks activity at off-target receptors (e.g., serotonin, adrenergic) unlike MDMA. Methylone has rapid, robust and long-lasting antidepressant-like activity in both naïve and stressed rats, anxiolytic effects, and benefit in a model of PTSD. Methylone is non-hallucinogenic, evidenced by a lack of activity at 5HT2A receptors, no effect in the head-twitch model, and no reports of hallucinations across several clinical studies. Recent data from an open-label clinical trial of methylone in PTSD patients revealed rapid, robust and durable benefit. Patients with severe PTSD received 4 weekly doses. At the end of study (6w after the last dose), there was a mean change from baseline in CAPS-5 of -36.2 points and 61.5% of participants achieved remission. A placebo-controlled study is ongoing. Here we explore methylone's underlying mechanism of action. Neuroplasticity is a key mechanism underlying the activity of both classic (e.g., SSRI) and rapid-acting (e.g., ketamine) antidepressants. Methylone rapidly induces neuroplasticity-related gene expression in key brain areas linked to PTSD and depression, which may underlie methylone's rapid and durable behavioral and clinical effects. Neurotrophins, including BDNF, increased within 8-hours after a single dose. To determine the time course of these effects, we investigated the gene expression changes by RNA-seq for hours, days and weeks following a single dose of methylone in rats. We also explored methylone's neuroplastic effects *in vitro* by measuring neurite outgrowth in cultured neurons. Results offer important insights into the

mechanism of action of methylone, supporting its clinical development for PTSD and other CNS disorders.

Disclosures: J. Warner-Schmidt: A. Employment/Salary (full or part-time):; Transcend Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Transcend Therapeutics. **A. Jones:** A. Employment/Salary (full or part-time):; Transcend Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Transcend Therapeutics. **M. stogniew:** A. Employment/Salary (full or part-time):; Transcend Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Transcend Therapeutics. **B. Mandell:** A. Employment/Salary (full or part-time):; Transcend Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Transcend Therapeutics. **B. Kelmendi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Transcend Therapeutics.

Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.20/N4

Topic: G.07. Post-Traumatic Stress Disorder

Title: Scoping review on the neural markers of resilience to trauma and adversity

Authors: *N. HEDAYATI, Z. FOTOVATNIA, J. A. JONES;
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Abstract: Resilience is the ability to recover after experiencing a potentially traumatic event or adversity. Until recently, little was known about the neural markers of resilience to trauma and adversity. The purpose of this scoping review is to summarize the literature on the neural markers of resilience to trauma and adversity. Search terms were entered into the PsycINFO and MEDLINE databases. Articles were extracted from these databases and imported into Covidence, which was the software we used to remove duplicates, screen titles/abstracts, screen full-texts, extract data, and reach a consensus. Studies that met inclusion criteria and did not meet exclusion criteria throughout the screening process were eligible for data extraction and consensus. We used PRISMA reporting guidelines. We screened 977 studies. A preliminary data extraction and consensus was completed on 17 studies. An analysis of the results showed that higher resilience was associated with (a) increases in structural sizes of the subparietal sulcus, anterior cingulate cortex, cingulum fiber-density, hippocampus, and anterior corpus callosum; (b) greater functional activity involving the frontolimbic regions, (ventromedial) prefrontal

cortex, amygdala, bed nucleus of the stria terminalis, the left frontoparietal network, executive control network, and default-mode network; (c) reduced structural sizes in the left inferior parietal cortex, left subgenual anterior cingulate cortex, and right anterior mid-cingulate cortex; and (d) reduced functional activity in the locus coeruleus-norepinephrine system, amygdala, ventromedial prefrontal cortex, temporomesial regions, and in global synchronous neural interactions. These features of the structure of certain brain areas and their functions can serve as potential neural markers of resilience to trauma and adversity. Identifying these potential neural markers of resilience has implications for recognizing if people are resilient or if they may develop psychopathology like posttraumatic stress disorder.

Disclosures: N. Hedayati: None. **Z. Fotovatnia:** None. **J.A. Jones:** None.

Poster

PSTR417

Anxiety, Stress, and PTSD: Neural Mechanisms and Therapeutics in Humans

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.21/N5

Topic: G.06. Anxiety Disorders

Title: Sntx-axn, a novel fast acting anxiolytic discovered and developed from sensorium therapeutics ai-driven cns product engine

Authors: *J. BROWN, M. PLACZEK, G. DAIGLE, A. VAN HOOSER;
Sensorium Therapeut., Brighton, MA

Abstract: Anxiety represents a growing health problem with high unmet medical need. Serotonin selective reuptake inhibitors (SSRIs) and benzodiazepines (BDZ) represent the primary pharmacotherapy for this disorder. However, both options come with significant liabilities including delayed onset, black-box warnings, and sexual side effects (SSRIs) or tolerance, sedation, and abuse liabilities (BDZ). Development of safer and more effective pharmacotherapies has been unsuccessful using traditional CNS drug discovery approaches. New and innovative approaches to CNS drug discovery are needed to identify and develop novel therapeutics for this and other neuropsychiatric indications. Sensorium Therapeutics has developed an AI-Driven drug discovery product engine which leverages the intersection of human use of natural compounds, integrative biology, and machine learning to discover differentiated therapeutics for CNS disorders. SNTX-AXN is a fast-acting anxiolytic developed and validated from the Sensorium product engine. SNTX-AXN demonstrates nM potency at a well validated therapeutic target and precise pharmacological selectivity. Based on a natural compound with human use and clinical data to support fast on-set anxiolytic activity, SNTX-AXN is a new chemical entity with improved CNS-drug like properties. Human efficacy data was validated in behavioral (marble-burying) and phenotypic (Smart Cube) models of anxiety. Moreover, SNTX-AXN did not cause motor deficits or sedation and differentiates from SSRIs

and BZDs. Consistent with this finding, SNTX-AXN does not bind GABAergic targets including the GABAa receptor. Preclinical target engagement PET studies show >80% target occupancy at low brain exposures confirming the in vitro potency and selectivity. Robust non-GLP data supports a clean safety profile in both rat and dog species with a large therapeutic index. SNTX-AXN is rapidly advancing toward clinic and represents a differentiated approach to treating anxiety and depression.

Disclosures: J. Brown: A. Employment/Salary (full or part-time):; Sensorium Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sensorium Therapeutics. **M. Placzek:** A. Employment/Salary (full or part-time):; Sensorium Therapeutics. **G. Daigle:** A. Employment/Salary (full or part-time):; Sensorium Therapeutics. **A. Van Hooser:** A. Employment/Salary (full or part-time):; Sensorium Therapeutics.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.01/N6

Topic: G.08. Other Psychiatric Disorders

Support: NIH Grant R01AG05798
NIMH Grant MH107487
NIMH Grant MH121102
NIA Grant AG057598

Title: Kinomic analysis of glutamate excitoxicity in primary neuronal cultures

Authors: *J. NGUYEN[1], A. S. IMAMI[2], A. LUNDH[3], W. S. MESSER, Jr.[4], I. T. SCHIEFER[5], R. E. MCCULLUMSMITH[6];
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Abstract: Glutamate excitotoxicity plays a critical role in neurodegeneration by triggering NMDA receptor hyperactivation, leading to elevated synaptic calcium levels and subsequent neuronal death. To better understand how glutamate affects neurons in neurological diseases, we conducted a comprehensive analysis of molecular changes at the transcriptome and kinome levels. Our research used primary cortical cultures from rat embryos to study glutamate excitotoxicity. Intermediate doses of glutamate (250 μM) had significant neurotoxic effects, while high and low doses resulted in less cell mortality, aligning with previous findings related to calcium influx. Transcriptional analysis identified B-cell translocation gene 2(BTG2), neuronal

PAS domain protein 4(NPAS4), and cellular communication network factor 1(CCN1) as top significantly differentially expressed genes following glutamate treatment in neurons. Dkk2, a Wnt antagonist, had the highest log fold change among our most significantly differentially expressed genes. Gene set enrichment analysis identified 1,127 pathways exhibiting significant alterations. Perturbagen analysis identified 2,811 unique concordant signatures and 1,071 unique discordant signatures. Kinome array profiling indicated activation of PKA and PKG kinases; these kinases regulate signaling pathways essential for synaptic plasticity-related gene expression. Multi-omic integration of transcriptome and kinome data identified enrichment of response to oxidative stress, actin filament organization, and regulation of apoptotic processes pathways. The Wnt signaling pathway serves as a pivotal factor in the early stages of axon differentiation and growth, as well as in shaping axonal behavior and dendrite development. Moreover, the interplay between MAPK and Wnt signaling pathways likely impacts cellular differentiation processes. Our investigation revealed an elevation in the p38/MAPK and stress-activated MAPK pathways, indicating the activation of the MAPK/ERK signaling pathway in response to excitotoxic neuronal damage in vitro. In conclusion, glutamate excitotoxicity causes molecular changes at the transcriptome and kinome level that include elements of the MAPK and WNT biological pathways.

Disclosures: J. Nguyen: None. **A.S. Imami:** None. **A. Lundh:** None. **W.S. Messer:** None. **I.T. Schiefer:** None. **R.E. McCullumsmith:** None.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.02/N7

Topic: G.08. Other Psychiatric Disorders

Support: NIMH Grant MH107487

Title: Metabolic insights into neuropsychiatric illnesses and ketogenic therapies at the transcriptomic level

Authors: *S. SAHAY, P. PULVENDER, M. RAMI REDDY, R. E. MCCULLUMSMITH, S. M. O'DONOVAN;
Dept. of Neurosciences, The Univ. of Toledo Col. of Med. and Life Sci., Toledo, OH

Abstract: Disruptions in brain energy metabolism, influencing synaptic signaling, neural circuitry, and neuroplasticity, are associated with severe mental illnesses like schizophrenia, bipolar disorder, and major depressive disorder. While the therapeutic potential of ketogenic treatments suggests a connection between metabolic disturbances and disease pathology, the precise mechanisms and therapeutic effects remain unclear. This study employs an in silico analysis of transcriptomic data to explore the disruption in 12 specific metabolic pathways in the

brain across these disorders. We also investigate the same pathways in ketosis models, comparing them with disease states. Our analysis reveals significant perturbations in metabolic pathways, notably glycolysis, the tricarboxylic acid (TCA) cycle, and the electron transport chain (ETC) across all disorders. Furthermore, we observe discordant gene expression patterns between disease states and ketogenic intervention studies, hinting at a potential role for ketone bodies in regulating abnormal metabolic changes. These findings emphasize the importance of considering metabolic dysregulation in severe mental illnesses and the therapeutic potential of ketogenic interventions in re-establishing metabolic equilibrium. This study provides insights on the relationship between metabolism and neuropsychiatric disorders and provides a foundation for further experimental investigations to appreciate the implications of our transcriptomic findings in hopes of developing targeted therapeutic approaches for these complex severe mental illnesses.

Disclosures: S. Sahay: None. **P. Pulvender:** None. **M. Rami Reddy:** None. **R.E. McCullumsmith:** None. **S.M. O'Donovan:** None.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.03/N8

Topic: G.08. Other Psychiatric Disorders

Support: NIH NIMH grant number R01MH107487
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NIH NIA grant number R01AG057598

Title: A Functional Protein Kinase Atlas of Disorders of Cognition

Authors: *A. S. IMAMI[1], N. HENKEL[2], P. PULVENDER[1], E. A. DEVINE[3], B. SICILIANO[4], Z. WEN[5], R. E. MCCULLUMSMITH[6];
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Abstract: A Functional Protein Kinase Atlas of Disorders of Cognition
Ali Sajid Imami, Nicholas D. Henkel, Priyanka Pulvender, Emily Devine, Ben Siciliano, Zhexing Wen, Robert E. McCullumsmith
Protein kinase activity plays a pivotal role in cellular signaling pathways, influencing various physiological processes including cognition. However, protein kinase-mediated signaling events are not linear cascades. They include myriad protein kinases, phosphatases, and signal

integration molecules that form networks that coordinate cellular functions and pathological fates of cells. It follows that the “active kinome” represents the composite of protein kinase activity for the 100s of kinases in the human genome. Active kinome networks include protein kinases and targets of these enzymes that mediate and coordinate cellular functions.
We conducted a series of experiments across a variety of substrates using a high throughput kinase activity assay to explore the nature of these global signaling. We have used postmortem brain samples from disease-free controls, people with mild cognitive impairment (MCI) and Alzheimer's Dementia (AD), and Schizophrenia. We also assayed patient-derived induced pluripotent stem cells (iPSCs) to develop a global understanding of the state of these signaling networks in the normal brain compared to the disease state.We have analyzed these datasets using established methodologies to generate a putative “map” of disease-related kinase network activity that can help guide future
research and prioritize drug target selection. We will present the top kinase nodes common to across AD and Schizophrenia as well as active kinome signatures unique to each disease. Assessment of active kinome networks provides a promising new approach to extend our understanding of the disorders of cognition. Interrogation of the active kinome using multi-omics approaches provides datasets unbiased by prior work, yielding novel active kinome profiles for validation, replication, and causal studies, highlighting novel pathophysiological insights for this devasting disease.

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Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.04/N9

Topic: G.08. Other Psychiatric Disorders

Title: Multiomics Analysis of Intracellular Signaling Dysregulation in GRIN1 Channelopathy

Authors: *P. PULVENDER[1], N. HENKEL[2], A. S. IMAMI[3], R. E. MCCULLUMSMITH[1], A. J. RAMSEY[4], S. M. O'DONOVAN[1];
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Abstract: The NMDA subtype glutamate receptor subunit 1 gene (GRIN1) encodes an obligatory subunit of the glutamatergic N-methyl-D-aspartate (NMDA) receptor. Individuals with pathogenic variants in this gene have GRIN1-related neurodevelopmental disorder, a rare

and debilitating channelopathy featuring intellectual disability, epilepsy, autism, movement disorders, and cortical visual impairment. To develop treatments for this neurodevelopmental disorder, a deeper understanding of the molecular changes that occur in the brain in GRIN1 disorder is needed. We applied a functional proteomics approach to explore perturbations of signaling in the frontal cortex of patient variant Grin1 Y647S heterozygous miceand their age- and sex- matched wild-type littermates (WT) (n = 3 per genotype). We deployed a protein kinase activity array platform (PamStation 12, PamGene Inc) to assay serine/threonine protein kinase activity. Data were analyzed using a comprehensive bioinformatic workflow developed to identify kinases with significantly altered activity profiles. For proteomic analysis, samples were enriched for phosphopeptides and then analyzed by label-free mass spectrometry (LC-MS/MS). Pathway enrichment analyses were performed with EnrichR. Assessment of protein kinase activity profiles on the kinome array found 14 kinases meeting our threshold for significance in *Grin1* mutant mice compared to WTs. Protein kinase C (PKCH), was the only significant kinase identified in male and female mice, suggesting sex-dependent changes in kinase signaling networks in *Grin1* mutants. Phosphoproteomic analyses of the prefrontal cortex also found significant sex-differences in *Grin1* mutants. Pathway analysis of significantly differentially expressed phosphopeptides ($p<0.05$; abundance ratio >2.0) revealed significant changes in proteins involved in synaptic signaling in female *Grin1* mice ($p<0.001$), while cellular recycling processes were among the top dysregulated pathways in male mice ($p<0.001$). In summary, we found sex-specific perturbations signaling substrates in the frontal cortex in *Grin1* Y647S heterozygous mice. Future work will integrate these multiomics datasets to elucidate the pathological mechanisms underlying *Grin1* channelopathies.

Disclosures: P. Pulvender: None. **N. Henkel:** None. **A.S. Imami:** None. **R.E. McCullumsmith:** None. **A.J. Ramsey:** None. **S.M. O'Donovan:** None.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.05/N10

Topic: G.08. Other Psychiatric Disorders

Title: Atypical Activity of SGK1 in Schizophrenia

Authors: *S. HANNA[1], A.-R. HAMOUD[2], L. STERTZ[3], A. S. IMAMI[2], R. E. MCCULLUMSMITH[2], C. WALSS-BASS[3];
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Abstract: Abstract: Schizophrenia (SCZ) is a neuropsychiatric disorder affecting approximately 0.45% of adults worldwide in which affected people experience symptoms such as

hallucinations, delusions, and altered cognition. Perturbations of established insulin pathways could contribute to an increased risk of SCZ. The role of constituents within canonical insulin signaling pathways is poorly understood in SCZ. Serum-glucocorticoid kinase 1 (SGK1), an inhibitor of glycogen synthase kinase 3β, regulates insulin signaling and drives formation of neurites during development. Thus, we studied SGK1 expression in a SCZ cell culture model. hiPSC-derived cell lines were generated from SCZ subjects from the Central Valley of Costa Rica. hiPSCs, neural precursor cells from four healthy controls and seven SCZ subjects assayed for SGK1 expression by western blot analysis. We specifically measured phospho-SGK1 (pSGK1) and SGK1 proteins levels for each subject. Expression levels were quantified using the Odyssey Imaging System and Image Studio. pSGK1 and SGK1 expression values of each subject were normalized to VCP expression. Differences between SCZ and control groups were analyzed through GraphPad Welch's t-test. We found increased ($p < 0.05$) pSGK1, but not total SGK1, in SCZ compared to control subjects. Our findings suggest an abnormality in canonical insulin signaling pathways, consistent with prior findings of altered AKT1 expression and activity in SCZ. Next steps include measuring SGK1 protein kinase activity, as well as developing novel modulators of SGK1 activity.

Disclosures: S. Hanna: None. **A. Hamoud:** None. **L. Stertz:** None. **A.S. Imami:** None. **R.E. McCullumsmith:** None. **C. Walss-Bass:** None.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.06/N11

Topic: G.08. Other Psychiatric Disorders

Support: National Institute of Mental Health (MH107487 REM)
American Foundation for Suicide Prevention (YIG-1-139-20)

Title: Differential expression of ATP-binding proteins in major depressive disorder postmortem human brain tissue

Authors: *M. RAMI REDDY, W. RYAN, J. VERGIS, X. WU, R. E. MCCULLUMSMITH, S. M. O'DONOVAN;
Neurosciences, Univ. of Toledo, Toledo, OH

Abstract: Major Depressive Disorder (MDD) is a prevalent psychiatric disorder characterized by persistent low mood, loss of interest or pleasure, and significant impairment in social, occupational, or other important areas of functioning. The neurobiological mechanisms underlying MDD remain poorly understood. One hypothesis suggests that dysregulation of energy (ATP) utilization may contribute to the pathophysiology of MDD. Here, we investigated differences in expression of the complement of ATP-binding proteins (the "ATPome") in the

anterior cingulate cortex (ACC) of MDD patients and age- and sex-matched non-psychiatrically ill controls (n=3) using mass spectrometry (LC-MS/MS). All samples were enriched for ATP-binding proteins (Thermoscientific ActivX #88310) and non-specific protein binding was assessed for each sample using a “no probe” negative control. In total, 109 “ATPome” proteins were identified in the ACC. Identified proteins (abundance ratio of ≤ 0.85 or ≥ 1.15 in MDD compared to control) were heavily enriched for protein kinases including cAMP-dependent protein kinase type II-alpha regulatory subunit (PRKAR2A) and Proto-oncogene tyrosine-protein kinase Src (SRC). Additionally, we observed dysregulation of other ATP-binding proteins such as High mobility group box 1 (HMGB1), a nuclear protein involved in DNA repair and inflammation, and Hypoxia up-regulated protein 1 (HYOU1), a molecular chaperone implicated in protein folding and cellular stress response. These findings suggest dysregulation of ATP-binding proteins in the ACC in MDD, offering insight into energy dysregulation contributing to the pathophysiology of this serious mental illness. Further research is needed to elucidate how these protein perturbations contribute to disturbances in specific biological and molecular pathways underlying MDD.

Disclosures: M. Rami Reddy: None. **W. Ryan:** None. **J. Vergis:** None. **X. Wu:** None. **R.E. McCullumsmith:** None. **S.M. O'Donovan:** None.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.07/N12

Topic: G.08. Other Psychiatric Disorders

Support: NIH Grant P20GM125508
NIH Grant R01GM148960
Hawaii Community Foundation 18CON-90818

Title: Genetic and Metabolic Regulation of Repetitive Behavior in the Asocial Teleost Fish.

Authors: *M. YOSHIZAWA[1], K. LACTAOEN[1], S. SINGH[1], X. BARRAGAN[1], M. WONG[2], R. LEE[3], M. IWASHITA[1];
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Abstract: Stereotypic repetitive behaviors are observed in mammalians and teleost fish, and are thought to be an obstacle to executing complex tasks, including social behaviors and learning. These repetitive behaviors are frequently seen in animals exposed to a stress-associated environment. Chronic stress is known to change the neurocircuit property as well as increase the blood glucose level—high blood glucose suppresses the metabolic state of ketosis. Accordingly, the ketogenic diet reduced repetitive behaviors in disorder model animals. However, there is a

significant knowledge gap regarding how repetitive behaviors are mitigated under ketosis. To fill these knowledge gaps, we chose the Mexican teleost fish, *Astyanax mexicanus*, as an experimental model. *A. mexicanus* exists in two forms: cave-dwelling (cavefish) and surface-dwelling (surface fish). Cavefish are reported as asocial and prone to repetitive circling, while surface fish are social and do not exhibit repetitive circling. Cavefish exhibit 1,839 of the shared directional gene expression changes (up- or down-regulations) seen in patients with autism spectrum disorder, whose diagnosis includes repetitive behavior. Ketosis induced by either fasting or ketogenic diet-feeding suppressed repetitive circling in cavefish. Three independently evolved cave populations of *A. mexicanus* revealed different levels of repetitive circling among them, which did not represent distinct nutrient availabilities between these three caves (*i.e.*, not nutritional history of populations). Instead, they were aligned with the degree of their phylogenetic divergences from surface fish. Response to the ketogenic diet seemed to correspond to the genetically diversified metabolic state (insulin resistance) instead of phylogenetic divergences, suggesting the link between ketosis, metabolic condition, and repetitive behavior. Indeed, the brain transcriptome indicated that the ketogenic diet feeding shifted the brain gene expression patterns in cavefish toward the surface fish type. Our quantitative trait (QTL) mapping using 416 F_2 hybrid from a pair of surface fish and cavefish revealed that only one genomic locus is responsible for repetitive circling (~20 candidate genes within this genomic locus). In conclusion, our results indicate a simple genetic architecture of repetitive behavior, and that the ketosis treatment for repetitive behavior can be efficient in a group with metabolic alteration. We are generating CRISPants of each candidate gene in surface fish, and investigating their repetitive circling, serum metabolism, and brain transcriptome.

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Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.08/N13

Topic: G.08. Other Psychiatric Disorders

Support: R35 ES031686
U01 ES026697
P30 ES017885
K01 ES035064

Title: Pirna machinery (piwil) expression in the mouse brain and its association with gestational lead exposure: potential for epigenetic editing applications

Authors: *J. S. SMITH, D. C. DOLINOY;
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Abstract: Gestational lead exposure is a persistent public health concern due to its lasting neurotoxic effects on psychiatric and cognitive health, possibly through DNA hypomethylation in the brain (Dou et al., 2019; Hill et al., 2015). However, precise mechanisms, and subsequently, therapies to mitigate DNA hypomethylation resulting from metal exposures are underdeveloped. piRNAs, a class of short ncRNA, are best known for their role in maintaining genomic stability through the germline by inducing DNA methylation at transposable elements (Perera et al., 2019). However, the presence and functions of piRNAs and their associated regulatory proteins outside of the germline, and specifically in the brain, remain largely unknown.

The study aims to uncover epigenetic mechanisms influencing early life exposures and persistent adverse outcomes, with potential implications for intervention and therapeutic strategies, including epigenetic editing approaches. To do this, we sought to characterize the piRNA machinery (*Piwil)* gene expression profile in the developing mouse brain *in vivo* as well as piRNA expression in the adult brain of mice gestationally exposed to lead.

We explored the expression of *Piwil* mRNA in the mouse embryonic head during 13-15 days post conception. Adult C57BL/6 mice were time mated and 10 embryos (5 M, 5 F) were harvested from 8 different litters. No more than 1 male and 1 female were used per litter. RT-qPCR was conducted using Taqman probes to analyze *Piwil1*, *2*, and *4* expression revealing present but low expression in the fetal brain. In a parallel study of gestational lead exposure (32 ppm in the water), we identified 52 differentially expressed piRNAs in the adult mouse cortex, 3 of which remain significant after multiple testing correction, all downregulated in the male brain $q<0.05$. Interestingly, *TAO Kinase 3* (*Taok3*), a downstream mRNA target for one of the lead-sensitive piRNA, exhibited increased expression ($p<0.04$) and decreased DNA methylation ($p<0.01$).

The findings suggest the presence of *Piwil* machinery in the mouse brain during embryonic development and demonstrate that gestational lead exposure stably influences the piRNA pathway. The existence of *Piwil* machinery in the brain has inspired research exploring targeted piRNA induced DNA methylation with application to epigenetic editing therapies in response to toxicant exposures. Future research will select commonly differentially over-expressed genes in response to lead exposure with a focus on those resulting from DNA hypomethylation *in vitro* and *in vivo*. These findings will be used to optimize piRNA delivery systems for gene silencing through targeted DNA methylation.

Disclosures: J.S. Smith: None. **D.C. Dolinoy:** None.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.09/

Topic: G.08. Other Psychiatric Disorders

Support: Florida Department of health 6JK-08

Title: Granulocyte-colony stimulating factor gene therapy reverses behavioral and psychological symptoms of dementia in AD mouse model

Authors: *S. BHANDARI[1], H. SONTAG[2], K. MADLIN[2], J.-Y. WU[2];
[1]Col. of Med., Florida Atlantic Univ., Boca Raton, FL; [2]Florida Atlantic Univ., Boca Raton, FL

Abstract: Alzheimer's disease (AD) is characterized by the gradual accumulation of misfolded proteins, such as amyloid plaques and neurofibrillary tangles, leading to cognitive impairment and brain atrophy. However, in the majority of patients, a significant and under-addressed aspect of AD is the emergence of heterogeneous neuropsychiatric symptoms, commonly referred to as behavioral and psychological symptoms of dementia (BPSD). BPSD encompasses a wide range of symptoms, such as anxiety, aggression, and stereotypy, which have an adverse impact on patients' quality of life and impose a significant burden on caregivers and healthcare systems. While recent advances in disease-modifying therapies for AD offer hope, there is a critical lack of effective treatment options for BPSD. We recently identified the force plate actometer (FPA), a computerized open field apparatus, as an instrument sensitive enough to comprehensively assess BPSD-related functional alterations in preclinical AD rodent models. We demonstrated that the frequency domain of the largest amplitude-whole body-power spectra wave exhibited a distinct shift in 3xTgAD in a pathology-dependent manner. This research uses a 3- and 12-month cross-sectional design to examine the impact of non-invasively administered gene therapy on modulating these non-cognitive behaviors. Granulocyte-colony stimulating factor (G-CSF) gene therapy restored functional impairments, such as increased locomotor activity and curiosity-like behavior, as well as reduced amplitude and frequency of power spectra during low mobility bouts (LMBs), the incidence and latency of LMBs, as well as spatial confinement, stereotypy, and anxiety-like behavior. In conclusion, these findings suggest G-CSF gene therapy's potential to modulate BPSD and AD pathology, warranting further investigation.

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Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.10/N14

Topic: G.03. Motivation

Support: Intramural Research Grant (3-Y2) for Neurological and Psychiatric Disorders of NCNP
JSPS KAKENHI Grant Number 24K20365

Title: Effects of epidural labor analgesia on maternal brain reward system activation in the postpartum period

Authors: *K. HISHIKAWA[1], M. ABE[2];
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Abstract: Administration of epidural labor analgesia during labor is considered the standard for pain relief during labor. In Japan, there is a traditional belief that pain accompanied with labor's pain plays a crucial role in developing maternal bonding with their own infants. Recent evidence suggests that events of pregnancy and childbirth trigger reorganization of the whole brain networks in the maternal brain. In fact, the dopaminergic reward system highly responds to their own infant. However, it is not investigated whether administration of epidural labor analgesia for the purpose of reducing labor's pain would result in decreased activation of the dopaminergic reward system in the maternal brain. In the present study, we observed neuronal activity in the reward systems when mothers saw visual stimuli presenting faces of their own's baby, using functional MRI (fMRI). We analyzed brain activity of mothers who underwent normal delivery or those who had experienced analgesia during labor. We recruited 29 women who had experienced their first childbirth within the past three months (normal delivery: 17, analgesia delivery: 12). We used functional MRI to measure brain activity in response to photographs of their own baby's face and the face of an unfamiliar baby. Our analysis focused on the ventral tegmental area (VTA) and the nucleus accumbens, key components of the brain's reward system. We compared brain activities of these two conditions (own baby's face vs. unfamiliar baby's face) in these regions, as well as the functional connectivity between theses regions. An outlier in brain data was defined as any data point that was 1.5 times greater than the interquartile range above the upper quartile or below the lower quartile, using Tukey's method. Functional connectivity between the VTA and the nucleus accumbens increased significantly when participants viewed their own baby's face compared to an unfamiliar baby's face. However, this increase was significant only in the normal delivery, with no significant change in the analgesia delivery. While the brain activity in the nucleus accumbens when viewing one's own baby's face was not significantly different from that when viewing an unfamiliar baby's face across both groups, however, a significant increase in activity was noted only in the normal delivery, with no significant increase in the analgesia delivery. This study reveals enhanced brain activity and connectivity within the reward system in the normal delivery group, whereas no significant increase was observed in the analgesia delivery. Our findings suggest that epidural labor analgesia may influence the activation of the maternal reward system.

Disclosures: K. Hishikawa: None. **M. Abe:** None.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.11/N15

Topic: G.03. Motivation

Title: Autonomous exploration trajectories reflect motivational determinants linked to subsequent memory performance

Authors: *T. YANG[1,2], B. CHEN[3], Q. HUANG[3], S. QIN[3];
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Abstract: Motivation plays a crucial role in shaping effective learning and memory. Psychological views emphasize the merits of motivational factors on cognitive and academic performance, including intrinsic autonomy and extrinsic reward. Few studies, however, explore the dynamic nature of motivational states during autonomous learning and its impact on memory performance. Recent advances in computational ethology offer a powerful approach to uncover latent factors underlying motivated learning and memory. Here we set up an autonomous learning experiment with 2 (autonomy vs. follow) *2 (reward vs. no reward) factorial design to address above question. During autonomous learning, participants used a joystick to explore a set of 25 items placed into a 5-by-5 grid (six grids in total, 60 seconds per grid) and memorize these items for subsequent memory tests (old/new recognition and spatial locations). Participant's autonomous exploration trajectories was recorded in a resolution of 17 milliseconds. Fifty-five college students were participated in this study. Using computational ethology approach, we extracted several latent features from participants' dynamic exploration trajectories. First, mixed-effects linear model revealed that entropy of autonomous trajectories predicted better memory performance, whereas stop times, trajectory disturbance value (TDV) and number of explored items predicted poorer memory performance. Compared to passive exploration, autonomous learning led to stronger beneficial effect for entropy, but less pronounced effects of TDV and impulsive errors on memory performance. Second, mediation analysis revealed that reward enhanced memory performance by reducing TDV specifically during autonomous exploration. Moreover, K-Means analysis clustered trajectories into two distinct patterns that were linked to better and poorer memory performance respectively. Finally, we examined dynamic trend in latent factors over time by fitting trajectories into polynomial functions with functional principal component analysis. Six components were extracted, explaining 95% of the trajectory's variance, with two of them in relation to the two distinct patterns identified by K-Means clustering.Our findings provide preliminary evidence into the dynamic nature of human autonomous learning and memory systems, offering an innovative approach to uncover motivational determinants of subsequent memory performance. This may inform feasible strategies to improve effective

learning and memory in the realm of education, including how to optimize and maintain equal distribution of learning time, and control the amount of learning tasks.

Disclosures: T. Yang: None. **B. chen:** None. **Q. Huang:** None. **S. Qin:** None.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.12/N16

Topic: H.13. Schizophrenia

Support: Children's Health Research Institute - Whaley & Harding Fellowship
Canadian Institute of Health Research (CIHR)
Natural Sciences and Engineering Research Council of Canada (NSERC)

Title: Sex-dependent effects of early life stress and prenatal thc exposure increases neuropsychiatric risk: evidence for the double-hit hypothesis of schizophrenia

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Abstract: The prevalence of cannabis use during pregnancy among young women (18-24 years) has risen by 19% between 2009-2016. Notably, maternal cannabis use has been linked to increased maternal rejection and withdrawal from offspring. Prenatal exposure to cannabis heightens susceptibility to co-occurring environmental stressors during or after prenatal life, potentially escalating the risk of schizophrenia later in life. This combination represents a "double-hit" hypothesis for schizophrenia risk in children, whereby a prenatal neurodevelopmental insult might make offspring more vulnerable to environmental stressors post-natally, dramatically increasing neuropsychiatric risk. We hypothesized that prenatal THC exposure combined with early life stress (ELS) would exacerbate schizophrenic-like behaviours through dysregulation of neuronal activity states and schizophrenia-related molecular signalling pathways in the Prefrontal Cortex (PFC)- Hippocampal circuitry. In this study, pregnant Wistar rats were exposed to edible THC (5 mg/kg) or vehicle from gestational day 7 to 21. Subsequently, litters were separated into two groups; a maternal separation group (daily 3-hour maternal separation from postnatal day 2-15) or a control group without separation. Behavioural phenotypes of male/female offspring were assessed during adulthood using various preclinical tests (social interaction, spontaneous alternation, novel object recognition, open field, and pre-pulse inhibition). Following behavioural testing, electrophysiological recordings and proteomic

analyses were performed in the PFC-ventral hippocampal circuit. Our finding revealed that prenatal THC exposure resulted in reduced body weight in male and female offspring, similar to fetal growth restriction effects observed in human populations. Additionally, prenatal THC induced sensorimotor deficits in both sexes, with male rats showing a selective increase in intracellular AKT/GSK3 signaling in the PFC. Interestingly, the combination of prenatal THC and ELS impaired spatial short-term memory and working memory in males, accompanied by decreased firing and bursting rates in the ventral hippocampus. These effects were also associated with increased expression of fatty acid amide hydrolase and decreased phosphorylation ERK 1-2 and mTOR expression in ventral hippocampus of male cohorts. Thus, prenatal THC induces sensory motor gating impairments in both male and female offspring, while the combination of prenatal THC and ELS selectively impaired working and spatial memory in males, demonstrating sex-selective effects of prenatal THC and early life stressors.

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Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.13/N17

Topic: H.03. Decision Making

Support: NIH Grant K99AA029454
NIH Grant P50AA012870
State of Connecticut Department of Mental Health and Addiction Services

Title: Individual differences in model-based and model-free decision making strategies differentially predict future habitual seeking behavior in mice

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Abstract: Value-based decision making is fundamental to adaptive behavior and is frequently altered in psychiatric disorders. One aspect of decision making that is associated with addiction is the balance of model-based and model-free learning strategies. However, the literature regarding the relationship between these strategies and alcohol use lacks consensus. To date, no studies have assessed these relationships in animal models, which allow for controlled alcohol exposure. Here, we used our recently developed multi-stage decision-making (MSDM) task in mice to assess how individual differences in model-based and model-free decision making

predict future alcohol use behaviors. Forty-seven adult male and female C57BL/6J mice were trained and tested on the MSDM task with 20% sweetened condensed milk as the reinforcer. Following MSDM testing, mice self-administered either 0.1% saccharin (CTRL) or 10% ethanol, 0.1% saccharin (EtOH) for four weeks on an escalating fixed-ratio schedule. Outcome devaluation was performed to assess habitual seeking during the final self-administration session. Reinforcement learning models were fit to these MSDM choice data to quantify model-based and model-free contributions to decision making and metrics of escalation and habitual alcohol seeking were calculated for individual mice. We found that female mice learned the training version of the task more rapidly, but performance approached convergence between the sexes by the end of training. During MSDM testing, no sex differences were observed in the contribution of model-based or model-free strategies to decision-making strategies. Across self-administration, EtOH mice escalated responding as the ratio requirement increased to a greater degree than CTRL mice. Female mice of both groups self-administered more reinforcer per bodyweight than male mice. Outcome devaluation revealed that EtOH mice were more habitual in their seeking behavior than CTRL mice. Higher model-based decision making predicted future goal-directed seeking, regardless of sex or reinforcer group. By contrast, model-free decision making exhibited sex- and reinforcer-dependent relationships. Higher model-free decision making predicted greater future habitual seeking within CTRL male mice but predicted greater goal-directed seeking within ETOH male mice. Overall, we identified sex differences in the learning trajectory for a MSDM task, patterns of self-administration, and how model-based and model-free decision-making processes informed future seeking patterns.

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Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.14/N18

Topic: G.03. Motivation

Support: Duke Institute of Brain Sciences Germinator Award

Title: Whole-brain networks support activation of the ventral tegmental area during motivational thinking with real-time fMRI neurofeedback

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Abstract: People often attempt to motivate themselves using specific thoughts and imagery, with mixed effectiveness. Real-time fMRI neurofeedback training can be a powerful tool to train

individuals to link specific mental processes with brain activity. We have previously demonstrated that individuals can learn to upregulate and sustain BOLD activation in the ventral tegmental area during neurofeedback using self-generated motivational thoughts and imagery. The present study aimed to replicate these findings in a larger sample and extend these findings by examining whole-brain activity during neurofeedback (compared to partial-volume data in the original study). In our current study (n=29), participants were instructed to motivate themselves while increasing a thermometer display of real-time VTA fMRI signal (activate trials) or to count backwards (count trials). To examine transferability, participants completed test trials without neurofeedback before and after training. Whole-brain and ROI analyses replicated the main finding that individuals can learn to increase VTA activity during real-time neurofeedback using motivating thoughts and imagery. Whole-brain analyses of neurofeedback trials compared to control trials revealed significant activation in widespread brain regions including: posterior cingulate cortex, superior frontal gyrus, supramarginal gyrus, temporal occipital cortex, thalamus, angular gyrus, middle frontal gyrus, insular cortex, dorsolateral prefrontal cortex, and primary visual cortex. Such widespread brain activation during neurofeedback trials reflects the complex mental processes involved in upregulating motivation and evaluating feedback information. To further examine how brain networks support VTA upregulation specifically, functional connectivity analyses revealed a functional network comprised of left hippocampus, left amygdala, temporal occipital cortex, and lingual gyrus associated with VTA activation. We propose that this network serves the role of generating motivational imagery (temporal occipital cortex/lingual gryus, hippocampus) and increasing arousal (amygdala) associated with heightened motivational states. Individual differences in trait measures of motivation and reward experiences may relate to the ability to engage the VTA during real-time neurofeedback.

Disclosures: R.N. Wright: None. **R. Adcock:** None. **J. Poh:** None.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.15/N19

Topic: G.03. Motivation

Support: NIH Grant K08DA053441

Title: Time-frequency measures index associations between reactivity to racial stigma cue images and perceptions of lived experiences of discrimination

Authors: *D. BUTLER[1], C. RISCO[1], S. NAHABEDIAN[1], M. VALENÇA[1], E. BERNAT[2]; [1]Univ. of Maryland, Col. Park, College Park, MD; [2]Psychology, Univ. of Maryland, College Park, MD

Abstract: Background. Experiences of racial discrimination may shape neurophysiological processes associated with heightened stress reactivity in African American communities (AA). However, well-controlled, lab paradigms have been underutilized, limiting our understanding of core cognitive-affective processes underlying stress reactivity in the immediate context of a racial stigma event—as well as factors that amplify (e.g., cumulative experiences of perceived racial discrimination) or mitigate (e.g., racial identity) effects.**Current Study.** We used a Racial Stigma Cues Task (RSCT; assessing real-time cognitive-affective reactivity to racial stigma) to 1) examine associations between RSCT reactivity and individual difference variables related to perceptions of lived experiences of discrimination 2) explore the extent that time-frequency alpha and theta measures, relative to conventional time-domain, can index this association.**Methods.** 114 AA young adults. Continuous EEG recording. Viewed 60 racial stigma images (e.g., negative AA stereotypes, police brutality, racial segregation, and more). Measures of racial identity (3 subscales of the Multidimensional Inventory of Black Identity; MIBI) and frequency of experiences of discrimination (2 subscales of the Experiences of Discrimination scale; EOD) were assessed. Associations were explored across stigma images in conventional ERP averages. To assess individual image contributions, we assessed trial level ERP data from each image. Each image was correlated with the MIBI and EOD, with a *t*-test then conducted across the correlations to determine if there were significantly more correlations than chance.**Results.** Conventional ERP averaged time domain P3 & LPP RSCT reactivity was not correlated with MIBI or EOD. *t*-tests across correlations showed significant associations with P3 and MIBI [*t* value range: 2.1 to 3.3, *p'*s < .05], but nonsignificant in the LPP [*p'*s for MIBI and EOD > .05]. Time-frequency results were stronger: alpha showed significant associations [MIBI- *t'*s 3.43 to 15.08, *p*'s < .001; EOD- *t's* –6.4 to –11.32, *p'*s < .001], as did theta [MIBI- *t's* 2.41 to 10.63, *p'*s .01 to < .001; EOD- *t's* –5.8 to –7.89, *p*'s < .001].**Discussion**. Standard time domain ERP measures did not demonstrate a relationship to the MIBI or EOD. Trial level analyses provided new information: time domain identified some P3 significance; the strongest effects emerged from time-frequency theta and alpha, showing consistent relationships to the MIBI and EOD, if relatively small in magnitude. Thus, among the 60 images, the majority showed effects in the same direction, validating their sensitivity to the individual differences.

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Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

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Topic: G.08. Other Psychiatric Disorders

Support: NIH Grant R01 MH129742
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Health Research Board, Ireland (HRA-POR-324)
Irish Research Council Postgraduate Scholarship (LN)

Title: Comparison of machine learning models trained on brain volume and radiomics features to predict bipolar disorder diagnosis and treatment

Authors: *Y. IM[1], M. KANG[1], L. NABULSI[1], S. I. THOMOPOULOS[1], B. MWANGI[2], M.-J. WU[2], J. C. SOARES[2], S. DAHAN[3], D. CANNON[3], O. A. ANDREASSEN[4], P. M. THOMPSON[1], C. CHING[1];
[1]USC, Los Angeles, CA; [2]UTHealth Houston, Houston, TX; [3]Univ. of Galway, Galway, Ireland; [4]Oslo Univ. Hosp., Oslo, Norway

Abstract: Bipolar disorder (BD) is associated with smaller subcortical volumes, such as the hippocampus, which are influenced by commonly prescribed medications. Machine learning (ML), applied to structural brain markers, may be useful to predict BD diagnosis and potential treatment effects, but prior studies have been challenged by underpowered samples and limited model generalizability. In this preliminary analysis from the ENIGMA Bipolar Disorder Working Group (ENIGMA-BD), we compared ML models trained on subcortical volumes and radiomics features to predict BD diagnosis and treatment in three cohorts. 3D T1-weighted brain MRI from 3 sites, with 100 participants from the University of Galway (CHRM2: 44BD/56HC, 54% Female) and 179 and 153 participants across 2 scan sites at the University of Texas Health Science Center at Houston (HOU1: 74BD/105HC, 61% Female; HOU2: 90BD/63HC, 54% Female) were segmented using the ENIGMA-standard FreeSurfer (v5.3) protocol to derive 8 bilateral subcortical volumes (thalamus, hippocampus, amygdala, caudate, putamen, pallidum, nucleus accumbens, and lateral ventricles). PyRadiomics (v3.0) was used to extract 102 shape and gray level features from bilateral hippocampal regions. Brain features were adjusted for potential site differences using ComBat. Nine ML models (decision tree, random forest, ridge, k-NN, SVM, LASSO, logistic regression, elastic net, MLP) were tested to predict BD diagnosis and treatments including lithium, second generation antipsychotics (SGA), and antidepressants (AD). Model hyperparameters were optimized via grid search and significance was evaluated using balanced accuracy (BAC) and area under the receiver operator curve (AUC). FDR (q=0.05) was used to adjust for multiple comparisons. MLP trained on ComBat-adjusted hippocampal radiomics features showed significant BD classification (AUC=65.9%; 95% CI=58.4-73.5; BAC=65.9%). MLP trained on raw hippocampal radiomics features predicted AD (AUC=70.2%; 95% CI=64.8-75.7; BAC=70.2%) and SGA treatment (AUC=72.4%; 95% CI=63.2-81.6; BAC=72.4%). Decision tree classifier trained on raw hippocampal volume showed significant SGA classification (AUC=80.8%; 95% CI=66.1-95.6; BAC=80.8%). MLP trained on site-adjusted hippocampal radiomics features outperformed other models for diagnostic prediction on a held-out test site, with BAC performance in line with prior ENIGMA-BD ML analyses. MLP and decision tree classifiers trained on raw hippocampal volume and radiomics features outperformed other models in predicting AD and SGA use, and future analyses are underway to include the larger ENIGMA-BD sample to replicate these findings.

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Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.17/N21

Topic: G.08. Other Psychiatric Disorders

Title: A discrete subset of VTA dopamine neurons receives tonic inhibition from the ventral pallidum: relevance to psychosis

Authors: *O. YANG[1], K. LILLY[2], H. ELAM[2], A. MCCOY[2], S. M. PEREZ[2], D. J. LODGE[2]; [1]Univ. of Texas Hlth. Sci. Ctr. San Antonio, San Antonio, TX; [2]Pharmacol., Univ. of Texas Hlth. Sci. Ctr. San Antonio, San Antonio, TX

Abstract: Symptoms of psychosis are present in numerous neuropsychiatric disorders, including PTSD and schizophrenia, and are thought to be driven by aberrant dopamine transmission. Using rodent models, we have previously demonstrated that this aberrant dopamine system function is attributable to an increase in dopamine neuron population activity, defined as the number of spontaneously active dopamine neurons. Dopamine neuron population activity provides a gain of function to the dopamine system whereby the salience of a signal can be augmented or attenuated based on environmental context, novelty, etc. Furthermore, in models of psychosis, dopamine neuron population activity is pathologically enhanced resulting in aberrant salience signaling. We have previously demonstrated that this is due to a loss of GABAergic tone from the ventral pallidum (VP) leading to disinhibition of VTA dopamine neurons. However, it is unclear whether neurons in the VP modulate all dopaminergic VTA neurons or a specific subset that receive VP innervation. This knowledge is critical for understanding the neurocircuitry underlying the increased mesolimbic dopaminergic transmission observed in patients with psychosis. Using *in vivo* electrophysiology, we recorded spontaneously active dopamine neurons in the VTA while electrically stimulating the VP and found that approximately 23% of these dopamine neurons were inhibited by VP stimulation. Interestingly, following NMDA-activation of the vHipp (a known modulator of VP activity), we observed an increase in the number of neurons inhibited by VP stimulation (~42%). These data suggest that the VP regulates a discrete subpopulation of VTA dopamine neurons. To examine this anatomically, we used a transsynaptic herpes simplex virus 1 strain H129 (HSV129) to visualize the dopaminergic neurons in the VTA receiving inputs from the VP. Taken together, these data demonstrate that only a subset of VTA dopamine neurons receive tonic inhibition from the VP, suggesting that the gain of function ascribed dopamine neuron population activity engages a specific sub-population of dopamine neurons. The phenotype and innervation (both afferent and efferent) of these neurons will be the focus of future studies.

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Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

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Program #/Poster #: PSTR418.18/N22

Topic: G.08. Other Psychiatric Disorders

Support: BBRF Grant 2023A004169

Title: Mechanism based augmentation of accelerated-TMS: Preliminary analysis of a 1-day iTBS protocol

Authors: *P. GANESH[1], J. KWEON[1], J. TOM[1], H. KIM[1], A. M. FUKUDA[1,2], M. RAZAFSHA[1,2], J. BROWN[1,2];
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Abstract: Repetitive transcranial magnetic stimulation (rTMS) is an FDA-cleared treatment for depression. Accelerated-TMS (aTMS) and n-methyl-d-aspartate receptor (NMDAR)-mediated pharmacologic augmentation of TMS using d-cycloserine (DCS) have both been shown to markedly increase depression remission rates, and DCS enhances TMS-induced motor plasticity. We hypothesized that combining DCS with aTMS will yield greater modulation of motor-evoked potentials (MEPs) - a measure of brain plasticity. We conducted a double-blind, placebo-controlled study with depressed adult subjects aged 19 to 69 (n = 9, 5 male). Participants underwent one day of accelerated intermittent theta burst stimulation (iTBS) (10 treatments, 1800 pulses at 50 Hz/5Hz and 50-minute inter-session interval) over the left dorsolateral prefrontal cortex. aTMS was combined with a single dose of placebo or 250 mg DCS administered the night before. MEPs were recorded before and after each iTBS treatment and normalized to baseline. Quick Inventory of Depression Symptomology (QIDS-SR16) and Patient Health Questionnaire (PHQ-9) were self-administered at baseline and 1-week post-TMS. Repeated measures ANOVA and pair t-tests analyzed differences between groups. We observed significant differences in MEP amplitude between the two groups ($p = 6.14e\text{-}6$, t-statistic = -5.23) when normalized to baseline. Interestingly, despite only 1 day with 10 treatments, we also found significant clinical improvements in both QIDS-SR16 (15.0 ± 5.2, 10.4 ± 5.7, $p = .011$, 33% response) and PHQ-9 (16.4 ± 4.6, 12.9 ± 5.1, $p = .018$, 22% response) for both groups at 1-week post-TMS compared to baseline. This preliminary analysis indicates statistical differences between the two groups in terms of motor plasticity and excitability. Although we cannot determine the specific impact of the single dose of 250mg DCS with aTMS until unblinding occurs, these findings suggest that either the presence or absence of the DCS influences motor outcomes. Though preliminary, ten sessions in one day of aTMS appears to produce clinical benefit one week following treatment.

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Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

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Topic: G.08. Other Psychiatric Disorders

Support: Brain and Behavior Research Foundation NARSAD 30738
NIH R01 MH110447
NSF CAREER 2042251
NIH MSTP T32 GM140935

Title: A hominoid-specific brain structure is related to a transdiagnostic predictor of psychopathology

Authors: *S. A. MABOUDIAN[1], E. H. WILLBRAND[3], G. KELLERMAN[2], M. V. ELLIOTT[2], S. L. JOHNSON[2], K. S. WEINER[2,1];
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Abstract: Identifying neuroanatomical correlates of psychopathology is a major neuroscientific goal. Of all neuroanatomical features, tertiary sulci are particularly intriguing: they emerge late in gestation, continue to develop after birth, and are located in association cortices, which have expanded most throughout evolution and therefore, may be related to human-specific aspects of cognition. Recent work shows that tertiary sulci — including likely the most widely studied tertiary sulcus, the paracingulate (PCGS) — are related to cognition. Thus, in the present study, we tested whether the presence or absence of the PCGS is related to individual differences in a prevalent predictor of psychopathology: emotion-related impulsivity (ERI), the tendency to react impulsively during elevated emotional states. Three factors contribute to ERI: two are emotionally driven (Feelings Trigger Actions and Pervasive Influence of Feelings), while the third (Lack of Follow Through, LFT) does not explicitly refer to emotion.
We examined the relationship between ERI and PCGS presence in a transdiagnostic sample with a range of internalizing and externalizing syndromes (N=120, age 18-55, 66% female). At least one PCGS was present in 66% of participants (76% of left, 56% of right hemispheres). Further, as is common in the field, we defined three PCGS patterns [symmetric, leftward (LW) or rightward (RW) asymmetric] and quantified hemispheric PCGS asymmetry, finding a LW bias in PCGS presence (P=.001).
To quantitatively examine if the PCGS presence was related to individual differences in any of the 3 ERI facets, we ran an ANOVA with left and right hemisphere PCGS presence as factors. This approach revealed a main effect of left PCGS presence for LFT ($F(1, 117)=23.87$, $P<.00001$, $\eta_p^2=.17$), in which PCGS presence was associated with LFT severity. A second ANOVA relating PCGS pattern to LFT scores revealed a main effect of pattern (F(2,

117)=12.69, P<.0001, η_p^2=0.18); post hoc pairwise comparisons showed participants with RW asymmetry had lower LFT scores than symmetric (P<.00001) and LW asymmetric (P<.001) patterns. To account for differences in sample size between PCGS pattern groups, we iteratively sampled a size-matched subset of the symmetric and LW groups to the RW sample 1000 times, confirming the behavioral difference.
This work expands the understanding of neuroanatomic correlates of impulsivity by suggesting the morphological organization of the anterior cingulate cortex relates to this transdiagnostic marker.

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Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

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Topic: G.08. Other Psychiatric Disorders

Support: NIH Grant R16GM149498
NIH Grant P20GM103436

Title: Early-life risperidone increases locomotor activity later in life: reconsideration of dose and timing

Authors: I. G. CARR, M. A. GOEPPER, A. C. HARRELL, A. L. REY CALDERA, T. DOWNNEN, *M. E. BARDGETT;
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Abstract: Antipsychotic drugs are sometimes used in children to mitigate externalizing symptoms of ADHD, disruptive disorders, and autism. However, basic research evaluating the possible long-term effects of childhood antipsychotic drug treatment is limited. We have shown in several published studies using rats that early-life administration of the antipsychotic drug, risperidone, leads to elevated locomotor activity in adulthood. Given the influence of dopamine on locomotor activity, this outcome raises concerns that childhood antipsychotic drug treatment permanently alters dopaminergic tone. However, our previous work was based on a relatively high dose of risperidone (3.0 mg/kg) that was administered daily over 28 days, and relied on a once-a-day regimen that did not fully account for risperidone's relatively quick metabolism in rats. Therefore, this study examined locomotor activity in young adult rats administered a lower risperidone dose (0.75 mg/kg) twice a day for 14 days prior to puberty. Female and male Long-Evans rats received subcutaneous injections of either risperidone or vehicle twice a day from postnatal day 14 through postnatal day 28. Beginning on postnatal day 49, an age approximating young adulthood in humans, locomotor activity was tested for one hour a day for four

consecutive days. Young adult rats administered risperidone prior to puberty were significantly more active than controls regardless of sex. These results show that administering risperidone in a more clinically relevant manner early in life still leads to significant behavioral changes later in young adulthood. The increased activity seen in this model could imply that early-life antipsychotic drugs significantly and permanently impact dopaminergic tone.

Disclosures: I.G. Carr: None. **M.A. Goepper:** None. **A.C. Harrell:** None. **A.L. Rey Caldera:** None. **T. Downnen:** None. **M.E. Bardgett:** None.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.21/N25

Topic: G.08. Other Psychiatric Disorders

Title: Sensory Processing Deficits of MK-801 Responds to Risperidone Across ASSR-EEG, PPI and 5-CSRTT

Authors: A. M. BERNARDO[1], J. R. HUXTER[2], L. DLUGOSZ[3], L. GIGGINS[4], ***G. A. HIGGINS**[5];
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Abstract: A common untreated symptom in Schizophrenia (SZ) is difficulty processing sensory information. Auditory Steady State Response (ASSR) is an EEG based response reflecting ones ability to integrate auditory sensory information by cortical networks. Pre-Pulse Inhibition (PPI) considers cortical processing using auditory sensory information and the resulting reflex motor output. 5-Choice Serial Reaction Time Task (5-CSRTT) uses sensory stimuli to elicit a learned response and considers processing related to attention and impulse control. The NMDA antagonist MK-801, modulates response and performance across ASSR, PPI and 5-CSRTT. Atypical antipsychotics such as risperidone (RISP) aim to overcome processing impairments by targeting the serotonin system. Male, Sprague Dawley rats were used in: ASSR: N=7 (crossover), PPI: N=12 (parallel), 5-CSRTT: N=32 (crossover). A dose response of MK-801 (0.01-0.3 mg/kg) was established followed by reversal effects of RISP. ASSR: Rats were implanted with DSI telemetry and presented with click-train stimuli. PPI: Startle response was quantified as %PPI. 5-CSRTT: Rats perform a nose poke into an illuminated aperture to receive a food reward with 5s intertrial interval over 100 trials. ASSR: MK-801 reduced ASSR dose dependently, significantly enhanced 50-100 Hz high-gamma power and supressed frequencies below 30Hz ($F(1.11,7.8=22.59$; $p=0.001$). RISP partially reversed the MK-801 ASSR deficit ($F(1.12,7.86)=22.89$; $p=0.001$), and reversed increased high-gamma dose dependently

(F(2.96,20.74)=60.18; p<0.001). PPI: MK-801 reduced %PPI in a dose dependant manner (F(3,44)=33.49; p<0.001), RISP reversed this dose dependently (F(6,49)=5.4; p=0.0002); Veh: 64.1 ± 4.6, MK-801: 29.4 ± 7.1, MK-801+Risp1: 64.1 ± 5.2. 5-CSRTT: MK-801 increased premature (F(3,42)=3.5; p=0.02) and perseverative responding (F(3,123)=10.75; p<0.0001) relating to increased impulsivity and compulsivity respectively. RISP reversed premature responses, (F(3,124)=6.8; p=0.0003); Veh: 10.8 ± 2.2, MK-801: 73.6 ± 21.0, RISP: 9.6 ± 1.8. MK-801+RISP: 25.0 ± 6.3. RISP also reversed perseverative responding: Veh: 23.6 ± 2.4, MK-801: 95.6 ± 18.6, RISP: 31.9 ± 2.9, MK-801+Risp: 47.8 ± 6.0. The sensitivity of different sensory processing assays varies such that ASSR-EEG and 5-CSRTT are highly sensitive requiring low doses of MK-801 to produce a change in response and behaviour. PPI requires higher doses of MK-801 to elicit a change in behavioural output. In conclusion, MK-801 impairs processing of environmental stimuli across multiple assays. RISP improves some aspects of sensory processing across ASSR-EEG, PPI, and 5-CSRTT.

Disclosures: A.M. Bernardo: Other; Transpharmation Ltd. **J.R. Huxter:** Other; Transpharmation Ltd. **L. Dlugosz:** Other; Transpharmation Ltd., Transpharmation Ltd. **L. Giggins:** Other; Transpharmation Ltd. **G.A. Higgins:** Other; Transpharmation Ltd..

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.22/N26

Topic: G.08. Other Psychiatric Disorders

Support: NIH Grant UH3NS100549

Title: Investigating Approach-Avoidance Behavior in Obsessive Compulsive Disorder Patients Undergoing Deep Brain Stimulation

Authors: *R. BECHTOLD[1], A. GANDHI[2], J. KANG[3], T. B. LIU[4], Z. NAQVI[5], E. STORCH[5], W. K. GOODMAN[6], S. A. SHETH[7], N. R. PROVENZA[7], J. A. HERRON[8];
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Abstract: Obsessive Compulsive Disorder (OCD) is a debilitating psychiatric condition affecting approximately 2% of the United States population. Among emerging treatment options, deep brain stimulation (DBS) targeting the ventral capsule/ventral striatum (VC/VS) has shown promise in ameliorating symptoms in treatment-resistant cases. However, the mechanisms underlying the therapeutic effects of VC/VS DBS remain incompletely understood. Approach-avoidance behavior, a fundamental aspect of decision-making, plays a crucial role in various

psychiatric disorders. Patients with OCD often exhibit aberrant approach-avoidance tendencies, characterized by excessive caution and avoidance of perceived threats. Understanding the nuances of approach-avoidance behavior in the context DBS treatment holds immense clinical relevance. By employing a probabilistic approach-avoidance task (PAAT), we aimed to understand the impact of VC/VS DBS on approach-avoidance behavior in OCD patients. The PAAT, designed to simulate real-world decision-making scenarios, provides a nuanced assessment of risk-taking behavior, offering insights into patients' willingness to engage with potentially rewarding stimuli despite associated risks. Preliminary analyses suggest intriguing differences in approach-avoidance behavior between responders and non-responders to VC/VS DBS treatment. Responders demonstrate a greater inclination towards approach behavior, characterized by reduced reaction times (reaction time = 2.36 ± .12 seconds) and increased willingness to accept risk for reward (65.2% chance to select options that provide increased probability of reward for an increase in risk). Comparatively, non-responders exhibit heightened avoidance tendencies, as evidenced by prolonged decision-making (reaction time = 2.92 ± .10 seconds) and reluctance to engage with uncertain outcomes (52.4% chance to select options that provide increased probability of reward for an increase in risk). By characterizing the behavioral correlates of VC/VS DBS treatment, our study aims to provide crucial insights into treatment response mechanisms. These insights hold promise for informing targeted interventions and refining treatment strategies for individuals with treatment-resistant OCD. Furthermore, recognizing approach-avoidance behavior as a potential metric for treatment response sets the stage for future developments in closed-loop adaptive neuromodulation methods. This envisioned closed-loop approach holds tremendous promise for improving overall treatment outcomes by precisely titrating therapy to individual patient needs.

Disclosures: R. Bechtold: None. **A. Gandhi:** None. **J. Kang:** None. **T.B. Liu:** None. **Z. Naqvi:** None. **E. Storch:** None. **W.K. Goodman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nview, LLC, OCDscales, LLC. **S.A. Sheth:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder of Motif. F. Consulting Fees (e.g., advisory boards); Boston Scientific, Neuropace, Zimmer Biomet, Max Sensoria Therapeutics, Varian Medical. **N.R. Provenza:** None. **J.A. Herron:** None.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.23/N27

Topic: G.08. Other Psychiatric Disorders

Support: Vulnerable Brain Project Grant
Baszucki Group Grant

Title: Ketogenic diet reduces activity-based anorexia (ABA) vulnerability of adult mice by minimizing wheel activity and weight fluctuation

Authors: *Y. DONG[1], L. KHATRI[2], M. V. CHAO[3], C. J. AOKI[4];
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Abstract: Anorexia nervosa (AN) is a complex mental disorder with no known effective pharmacological treatment. The main symptoms of AN include significantly low body weight due to voluntary food restriction, persistently compulsive exercise that interferes with weight gain, and body dysmorphia (American Psychiatric Association, 2013). Activity-based anorexia (ABA) is an animal model that captures two main characteristics of AN: compulsive physical activity and voluntary restriction of food intake, leading to significant body weight loss. In human clinical trials, ketogenic diet has been used to treat many neurological dysfunctions successfully. A previous study on human AN patients showed that adopting a ketogenic diet followed by ketamine infusion led to a complete recovery, and the remission persisted for more than 6 months (Scolnick et al., 2020). Studies on ABA animals also showed that 1 or 3 injections of ketamine during adolescence prolonged resilience in female ABA mice by increasing food intake, decreasing wheel running activity, and reducing anxiety-like behavior (Chen et al., 2018., Goodwin-Groen et al., 2023). Since ketamine is a controlled drug (Schedule III), we asked whether adopting a ketogenetic diet alone during the 5 days preceding ABA1 and recovery phase from ABA1 could be effective in eliminating maladaptive behaviors during relapse (ABA2) (N = 33, 16 males, 17 females, half fed ketogenic diet (KG), remaining fed standard rat pellet and balanced wet food (CON)). Hyperactivity was measured hourly as wheel running; body weight was measured on a daily basis; anxiety-like behavior was measured by the elevated plus-maze (EPM) after recovery from relapse. During relapse (ABA2), KG decreased food anticipatory activity (FAA) on food restriction day 2 (FR2) ($p = 0.02$) and FR3 ($p = 0.005$); dark phase running on FR1 ($p < 0.0001$) and FR3 ($p = 0.003$); and total running on FR1 ($p = 0.04$), FR2 ($p = 0.019$) and FR4 ($p = 0.04$), when compared to CON. KG retained more body weight on FR2, 3 and 4 ($p < 0.0001$), when compared to CON. Grimace was present in 7 out of 17 CON at the end of ABA1 and in 2 out of 17 at the end of ABA2. None of the KG exhibited grimace. Moreover, KG showed more open arm duration on EPM than CON ($p = 0.03$), indicating increased exploratory behavior and decreased anxiety-like behavior. These behaviors reflect reduced ABA vulnerability and indicate the prophylactic effect of ketogenic diet on AN relapse. Based on data from another KG and CON cohort, BDNF levels in hippocampus correlated significantly with wheel running ($p = 0.009$) but was not different between the 2 diet groups, suggesting that KG's protective mechanism is independent of up-regulation of BDNF in the hippocampus.

Disclosures: Y. Dong: None. **L. Khatri:** None. **M.V. Chao:** None. **C.J. Aoki:** None.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.24/N28

Topic: G.08. Other Psychiatric Disorders

Support: CIHR MFE - 187903

Title: Neighborhood-level disadvantage, genetic pathways, and mental health

Authors: *D. E. VOSBERG[1], I. ALAMEDDINE[2], Z. PAUSOVA[1], T. PAUS[1];
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Abstract: Introduction: Neighborhood disadvantage may confer vulnerability to mental health problems, particularly during adolescence. Here, we investigate the associations between neighborhood-level exposures and individual-level mental health as outcomes, drawing on an administrative database in Ontario (Institute for Evaluative Clinical Sciences [ICES]) and a cohort study (Saguenay Youth Study [SYS]) in Quebec. While the ICES administrative dataset has a very large sample size, the SYS cohort study participants are all assessed and genotyped, providing insights on potential mechanisms. Methods: The ICES sample comprised 988,951 youth (ages 12-22; 48.6% female) living in Ontario, with area-level measures obtained from Statistics Canada and the Canadian Urban Environmental Health Research Consortium. The SYS sample included 822 adolescents (ages 12-18; 51% female), with mental health measures from the Diagnostic Interview Schedule for Children Predictive Scales. First, we conducted principal component analyses (PCA) of 42 social environmental variables (e.g., income, employment, education) in ICES, deriving the first PC for each neighborhood (dissemination area). Following the ICES analyses, we focused on neighborhood (census tract) income in SYS, which loaded strongly on the social PC1. Moreover, we computed a polygenic score for educational attainment (PGS_{EA}) using PRSice-2, and tested its association with area-level income (gene x environment correlation) and whether area-level income moderated an association between individual-level PGS_{EA} and psychopathology. Results: In ICES, the PC1 social (i.e., higher social disadvantage, lower neighborhood income) was associated with a greater probability of a psychiatric diagnosis ($OR = 1.30$, $p < 1 \times 10^{-300}$). Similarly, in SYS, lower area-level income was associated with higher general psychopathology ($\beta = -0.09$, $p = 0.039$). Moreover, higher PGS_{EA} scores were associated with higher area-level income, indicating a gene x environment correlation ($\beta = 0.11$, $p = 0.0098$). Finally, there was a trend ($\beta = 0.07$, $p = 0.068$) for an interaction between PGS_{EA} and area-level income vis-à-vis psychopathology. Conclusions: Neighborhood disadvantage was associated with a greater risk of mental health disorders in Ontario, and with higher psychopathology scores in a Quebec cohort. This effect may stem from both environmental and genetic pathways, given the identified gene x environment correlation. Finally, a trend indicated that the protective effect of PGS_{EA} on mental health may be stronger in neighborhoods with higher (area-level) income; this is being investigated with additional genetic instruments.

Disclosures: D.E. Vosberg: None. **I. Alameddine:** None. **Z. Pausova:** None. **T. Paus:** None.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.25/N29

Topic: G.08. Other Psychiatric Disorders

Support: R56 MH130006

Title: The sinking platform test: a novel paradigm to measure persistence in animal models

Authors: *M. BORTOLATO[1], G. FLORIS[2], I. S. PIRAS[3], A. L. RAVENS[1], M. HUENTELMAN[3], G. BRACCAGNI[1];
[1]Univ. of Utah, Salt Lake City, UT; [2]Sch. of Med., Temple Univ., Philadelphia, PA;
[3]Neurogenomics, TGen, Phoenix, AZ

Abstract: Persistence is the tendency to continue pursuing goal-directed actions despite facing obstacles. While this characteristic plays a critical role in reducing the risk of depression, its underlying neurobiological basis is not fully understood. It is essential to develop behavioral tasks that can effectively measure persistence in animal models to gain insight into its molecular foundations. In this study, we present the Sinking Platform Test (SPT), a new high-throughput method designed to assess persistence.During the SPT, mice were trained to exit a water-filled tank by climbing onto a platform above the water's surface. Throughout training, mice encountered occasional "failure trials," where the platform was submerged just after the mouse reached it, requiring the mouse to locate and ascend a newly introduced platform. After training, mice underwent a 5-minute test consisting solely of failure trials. Both male and female mice demonstrated similar levels of persistence during this test, as measured by the number of platforms they successfully climbed.Additionally, we observed that chronic administration of fluoxetine or imipramine increased persistence, while acute and chronic haloperidol decreased it. Social isolation for six weeks resulted in reduced performance on the SPT, but this effect was mitigated by imipramine treatment during the last two weeks of isolation. Furthermore, a four-week regimen of voluntary wheel running improved persistence in socially isolated mice.Finally, comparing the transcriptomic profiles of the prefrontal cortex between mice with high and low SPT performance revealed significant enrichment of immediate-early genes associated with susceptibility to chronic stress. These findings underscore the potential of the SPT as a valuable tool for investigating the biological mechanisms underlying persistence and for assessing new interventions aimed at enhancing this trait.

Disclosures: M. Bortolato: F. Consulting Fees (e.g., advisory boards); Asarina. **G. Floris:** None. **I.S. Piras:** None. **A.L. Ravens:** None. **M. Huentelman:** None. **G. Braccagni:** None.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.26/N30

Topic: G.08. Other Psychiatric Disorders

Support: CONAHCYT grants No.1038794
CONAHCYT grants No. 252808

Title: Study of oxidative stress biomarkers in the hippocampus of individuals who committed suicide.

Authors: *A. J. VÁZQUEZ HERNÁNDEZ[1], G. FLORES[2], F. GARCIA[3];
[1]Neuropsychiatry Lab., Inst. de Fisiología, BUAP, Puebla, Mexico; [2]Univ. Autonoma de Puebla / Inst. de Fisiologia, Puebla, Mexico; [3]Inst. of Forensic Sci. DF, Ciudad DE Mexico, Mexico

Abstract: The hippocampus is a subcortical brain region with a large number of glucocorticoid receptors. This structure is part of the limbic-cortical-hypothalamic circuit and is also highly sensitive to stress. Therefore, this region has been studied in relation to the pathophysiology of suicide. Neuroimaging studies have demonstrated a reduced hippocampus volume in individuals who committed suicide. In addition, histological studies have found significant changes in the hippocampus associated with suicide, such as alterations in stress regulation, which lead to overproduction of reactive species of oxygen and nitrogen. All this can compromise cell homeostasis and cause oxidative stress and inflammation, and even cell death.In addition, glia are non-neuronal cells with various support and regulatory functions vital for the proper functioning of the nervous system. Microglia, a part of glia, is considered the immune cells of the brain, which are involved in processes such as apoptosis, synaptic pruning, and neuroinflammation, among others. Astrocytes, which are also part of glia, regulate the chemical environment surrounding neurons. Our study focuses on determining changes in biomarker activity associated with oxidative stress and glial activity, aiming to evaluate cellular response to stress. Since, changes in the activity of astrocytes and microglia have been shown in several psychiatric pathologies. In the present study we carry out immunohistochemistry studies using markers such as 3-NT, glucocorticoid receptors, IBA1 and GFAP.For conducting the experiments, hippocampal samples were obtained according to the criteria of a forensic physician, a bioethics committee, Mexican official norms, and the Helsinki Declaration of 1975. Included data are age, sex, and cause of death. Before sample processing, the samples were divided into control and suicide groups. Samples were fixed in paraformaldehyde for subsequent paraffin embedding, 10 μm sections were made using a microtome, and the methodology for immunohistochemistry on fixed tissue was followed. Our results suggest that hippocampus of the individuals who committed suicide show changes in glial activity with alterations in GFAP and Iba1 expression. Additionally, the activity of the biomarker 3-NT and glucocorticoid receptors varied between control samples and samples from subjects who committed suicide. Therefore, these post -mortem findings show that there are changes in oxidative stress and inflammatory process in hippocampus regions in individuals who committed suicide.

Disclosures: A.J. Vázquez Hernández: None. **G. Flores:** None. **F. Garcia:** None.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.27/N31

Topic: G.08. Other Psychiatric Disorders

Title: The dual-hit model of schizophrenia-like behavior in the female Wistar rat.

Authors: *K. WALKER, E. ESNEAULT, C. FROGER-COLLEAUX, E. SABLÉ, A. HERNIER;
Porsolt S.A.S., Le Genest Saint Isle, France

Abstract: Schizophrenia is a severe mental disorder characterized by the presence of symptoms usually grouped into three categories: positive, negative and cognitive signs. While both human genders are affected by schizophrenia, sex differences are clearly reported, particularly on age of onset, with a peak age of 15-24 years in men and 20-29 years in women.
We previously set-up the neonatal Phencyclidine (PCP) exposure and post-weaning social isolation dual-hit model in the male Wistar rat. This model takes into consideration the neurodevelopmental origin of schizophrenia and combines an adverse early-life challenge followed by stress during adolescence to induce behavioral deficits related to some signs of schizophrenia at adulthood. The aim of this study was to evaluate the behavioral changes occurring in this model in the female rat at adulthood and to compare the results with some of those observed in the male rat.
Female Wistar rat pups were treated with PCP (10 mg/kg s.c.) or physiological saline on postnatal days 7, 9 and 11. At weaning, neonatal PCP-treated rat pups were placed in individual cages for social isolation for the rest of the study while control rats were group housed. Behavioral assessment was performed at 2 months of age using locomotor activity, pre-pulse inhibition (PPI), sociability, and Y-maze tests related to positive, negative. and cognitive signs, respectively.
Neonatal PCP treatment and post-weaning social isolation did not affect locomotor activity, percent of PPI at 3 intensities level, or the percent of time spent in the novel arm of the Y-maze, when compared with the vehicle housed grouped controls. A significant increase in the time spent in the social side as well as interaction time with the cage containing the stranger in the 3-chamber sociability test suggests a normal social approach.
Our results reveal that the dual interventions at two crucial early-life neurodevelopmental stages do not lead to behavioral deficits relevant to schizophrenia in female the rat at early adulthood. These data are in contrast with persistent hyperactivity and deficits related to information processing in PPI observed in the male rat. These findings confirm the presence of sex related differences at early adulthood, in accordance with human pathology.

Disclosures: K. Walker: Other; Porsolt S.A.S. **E. Esneault:** Other; Porsolt S.A.S. **C. Froger-colleaux:** Other; Porsolt S.A.S. **E. Sablé:** Other; Porsolt S.A.S. **A. Hernier:** Other; Porsolt S.A.S..

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.28/N32

Topic: G.08. Other Psychiatric Disorders

Support: NIH Grant 5R00MH121355
Bruce/Jones Graduate Fellowship in Addiction Biology

Title: Assessing the role of ventral hippocampal parvalbumin interneurons in alcohol intake and schizophrenia-like behaviors

Authors: *J. L. PALMER[1], J. J. DONEGAN[2];
[1]Inst. for Neurosci., UT Austin, Austin, TX; [2]Psychiatry, Dell Med. Sch. at UT Austin, Austin, TX

Abstract: Alcohol use disorder (AUD) affects one in ten Americans and is one of the leading risk factors for disability worldwide. Although AUD can be difficult to manage on its own, one area that remains understudied is the co-occurrence of AUD with psychotic disorders, namely schizophrenia. Schizophrenia, a disorder characterized by multiple symptom domains including positive (e.g. hallucinations), negative (e.g. social withdrawal), and cognitive (e.g. disorganized thinking) symptoms, is a debilitating psychiatric disorder that carries a massive economic burden. However, people with schizophrenia are also at a higher risk for heavy alcohol use, which diminishes medication adherence, can exacerbate psychiatric symptoms, and results in poorer social and health outcomes. The anterior hippocampus is heavily implicated in schizophrenia, with hippocampal hyperactivity consistently observed in patients. This hippocampal hyperactivity is thought to be caused by a loss of inhibitory interneuron function, particularly in the parvalbumin (PV)-expressing subtype. Therefore, in the current experiments, we will test the hypothesis that hippocampal PV interneuron dysfunction is necessary and sufficient to produce schizophrenia-like behavioral deficits and increased binge-like drinking. In the first experiment, maternal immune activation (MIA) is used to produce schizophrenia-like behavioral deficits and alter interneuron function. In adult MIA offspring, chemogenetics was then used to activate vHipp PV cells. We hypothesized that activating vHipp PV interneurons would reduce binge-like alcohol consumption. Our preliminary results suggest that MIA increases binge-like drinking in males but not females, and this increased consumption is not altered by vHipp PV interneuron activation. In the second experiment, chemogenetics was used to inhibit vHipp PV cells in healthy mice to determine if reduced activity is sufficient to produce

behavioral shifts. Our preliminary data suggest that vHipp PV interneuron inhibition decreases alcohol intake in males. Together, these results suggest that PV interneuron activity may alter binge-like drinking behavior in some contexts. Understanding the contribution of vHipp PV interneurons to binge drinking behavior may lead to improved prevention and treatment strategies for AUD.

Disclosures: J.L. Palmer: None. **J.J. Donegan:** None.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.29/N33

Topic: G.08. Other Psychiatric Disorders

Support: Stanley Center for Psychiatric Research

Title: Molecular and Behavioral Changes in Zmym2 Mutant Mice, a Genetic Animal Model of Schizophrenia and Neurodevelopmental Disorder

Authors: *W.-C. HUANG, S. ARYAL, B. J. SONG, M. H. SHENG;
Broad Inst., Cambridge, MA

Abstract: Loss-of-function mutations in the ZMYM2 gene have been associated with an increased susceptibility to schizophrenia and neurodevelopmental disorders. ZMYM2 is known to interact with the subunits of the complexes regulating histone modifications and gene expression. However, ZMYM2's functions within the brain remain unclear. Here, we studied the impact of ZMYM2 loss in a mouse model through transcriptomic, proteomic, and behavioral analyses. Transcriptomic analysis revealed that the Zmym2 knockout leads to the de-repression of genes across widespread brain regions and alteration of diverse molecular pathways, such as those related to cytoskeleton and RNA processing. Consistent with the finding, synaptic proteomics analysis unveiled dysregulation within cytoskeleton-related pathways. Additionally, the expression of immediate early genes was downregulated across most examined brain regions. Finally, young Zmym2 +/- males exhibit hyperactivity in behavioral tests. Our results underscore the role of Zmym2 mutation in eliciting behavioral deficits and dysregulations of molecular pathways in the brain.

Disclosures: W. Huang: None. **S. Aryal:** None. **B.J. Song:** None. **M.H. Sheng:** None.

Poster

PSTR418

Preclinical Models, Human Studies, and Therapeutic Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.30/N34

Topic: G.08. Other Psychiatric Disorders

Support: FORDECYT-PRONACES/83/2021/Conahcyt

Title: Potential biomarkers and psychosocial factors associated with suicidal behavior in psychiatric patients

Authors: *G. PÉREZ SÁNCHEZ[1], E. BECERRIL[2], L. PAVON-ROMERO[3], M. PONCE[4], G. MARTINEZ[5], S. JACINTO[2], J. MALDONADO-GARCIA[5], J. CORTEZ SANCHEZ[6], S. BRITO JHEMAN[7], E. BAUTISTA RODRIGUEZ[8], I. CAMACHO[9], M. CHIN[9], M. CHIN[9]; [1]Natl. Inst. OF PSYCHIATRY RAMON DE LA FUENTE MUÑÍZ, Tlalpan, Mexico; [2]INPRFM, Ciudad de Mexico, Mexico; [3]Natl. Inst. of Psychiatry. Mexico, Mexico, Mexico; [4]UDG, Guadalajara, Mexico; [5]UNAM, INPRFM, CIUDAD DE MÉXICO, Mexico; [6]Univ. Autónoma de Tlaxcala, Tlaxcala, Mx., Mexico; [7]Univ. Autónoma de Campeche, Campeche, Mx., Mexico; [8]Fac. of Hlth. Sci., UPAEP, Tlaxcala, Mexico; [9]Univ. Autonoma de Campeche, CAMPECHE, Mexico

Abstract: Suicide is defined as the act of a person intentionally causing their own death. The complex phenomenon of suicide is frequently linked to mental health issues. Studies reveal a robust association between mental health conditions and suicide. The fact that people with psychiatric problems constitute a sizable at-risk category for suicide attempts and completed suicides highlights the connection between psychiatric diseases and suicide even more. According to the WHO, about 800,000 individuals commit suicide annually, making it the second most common cause of death globally for those between the ages of 15 and 29 and the fifth most common among those between the ages of 30 and 49. Biomarkers are compounds that exhibit differential expression in a given state, such a disease. The aim of this study was to determine serotonin levels in psychiatric patients with suicidal behavior. Two study groups were included: a) psychiatric patients who exhibited suicidal behavior, ideation, or attempts, and b) controls who did not exhibit any of these behaviors. A serum sample was obtained from each participant and they were also administered the Beck Depression and Suicidal Ideation Scale. HPLC was used to analyze serum serotonin. Patients and controls were recruited at the Psychiatric Hospital of Campeche, Mexico. This protocol was approved by the Research and Ethics Committee in accordance with international guidelines. Our results showed that psychiatric patients with suicidal behavior had lower serum serotonin concentrations than controls. In addition, we analyzed psychosocial factors that may be associated with this behavior.

Disclosures: G. Pérez Sánchez: None. **E. Becerril:** None. **L. Pavon-Romero:** None. **M. Ponce:** None. **G. Martinez:** None. **S. Jacinto:** None. **J. Maldonado-Garcia:** None. **J. Cortez Sanchez:** None. **S. Brito Jheman:** None. **E. Bautista Rodriguez:** None. **I. Camacho:** None. **M. Chin:** None. **M. Chin:** None.

Poster

PSTR419

Neurobehavioral Mechanisms of Addiction

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA037744
NIH Grant F99 NS130870

Title: Comparison of c-Fos in cortical, striatal, and thalamic brain areas after contingent vs. noncontingent footshock during cocaine seeking in rats

Authors: *A. CRUZ[1], R. J. SMITH[2], P. KAHANEK[3], A. MILLER[2], S. HANDEL[1];
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Abstract: In an animal model of compulsive drug seeking, a subset of rats continues to seek cocaine despite footshock consequences (i.e., punishment resistance). In contrast, another subset of rats suppress cocaine seeking in response to footshock (i.e., punishment sensitivity). We recently showed that noncontingent footshock, unlike contingent footshock, does not suppress cocaine seeking. Here, we investigated c-Fos activity following exposure to contingent vs. noncontingent footshock. We hypothesized that contingent footshock, but not noncontingent, would drive c-Fos activity in brain regions associated with behavioral flexibility, particularly in rats sensitive to punishment. We trained male Sprague Dawley rats to self-administer cocaine on a seeking-taking chained schedule of reinforcement. Rats were sacrificed 30 min after a self-administration session in which they experienced contingent footshock punishment (0.7 mA, 0.3 sec, 1/3 of trials), noncontingent footshock (3-6 shocks at variable intervals), or no punishment. We assessed c-Fos expression in brain regions involved in behavioral flexibility, including the medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), intralaminar thalamus (ILN), and striatum (STR). We found no group differences in average c-Fos expression across subregions of mPFC and OFC. However, we found that prelimbic cortex (PL) c-Fos was negatively correlated with the number of footshocks, infusions, and normalized trials in the contingent footshock group, indicating PL was related to behavioral flexibility during punishment. Lateral OFC (lOFC) c-Fos was positively correlated with normalized trials in the noncontingent footshock group, which may indicate lOFC was involved in processing lack of contingency. In ILN, we observed lower average c-Fos in the noncontingent footshock group, but no correlations with behavior, which may indicate ILN suppression is involved in processing lack of contingency. When we examined correlations for c-Fos across brain regions, we found positive correlations after contingent footshock or no punishment within OFC subregions, within STR subregions, within ILN subregions, and between nucleus accumbens and ILN. Interestingly, we found negative correlations after noncontingent footshock between mPFC and several areas, including OFC, STR, and ILN. This may indicate that disengagement of mPFC control is involved in

learning the lack of control over footshock. Overall, these results show that footshock engages distinct brain regions when it is contingent vs. noncontingent.

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Poster

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Support: NIH Grant R01 DA046457

Title: Comparing the influence of fixed (FR, FI) versus random (RR, RI) reinforcement schedules on habitual responding and punishment resistance in cocaine-seeking rats

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Abstract: Random ratio (RR) and random interval (RI) schedules of reinforcement have long been shown to bias goal-directed and habitual responding, respectively. Recently, our lab found that the RI60 schedule also drives greater resistance to punishment of cocaine self-administration compared to the RR20 schedule. However, the mechanisms underlying the bias for habitual responding or punishment resistance with RI schedules is unknown. RI schedules have been hypothesized to bias habits due to: 1) the nonlinear response-outcome (R-O) association, or 2) poor R-O contiguity (i.e., increased time between response and outcome). Here, we tested the influence of fixed interval (FI60) and fixed ratio (FR20) schedules on habits and punishment sensitivity, given that the FI60 schedule has nonlinear R-O associations (like RI60) but strong R-O contiguity (unlike RI60). Both FI60 and FR20 have strong predictability, unlike RI60 and RR20. We trained male Sprague-Dawley rats to self-administer cocaine on a seeking-taking schedule of reinforcement with seeking reinforced via FR20, FI60, RR20, or RI60 in a between-subjects design. We used outcome devaluation to assess whether responding was goal-directed or habitual prior to and after 4 days of footshock punishment testing (0.4 mA, 0.3 sec, 1/3 of trials after completion of seeking). We observed greater punishment resistance with FI60 and RI60 compared to FR20 and RR20, which may indicate that a nonlinear R-O association biases punishment resistance. Interestingly, prior to punishment, rats trained on FR20 or FI60 were sensitive to outcome devaluation (i.e., goal-directed), while rats trained on RR20 and RI60 were insensitive (i.e., habitual), which may indicate that unpredictability biases habitual responding. After punishment, all groups exhibited goal-directed behavior. Therefore, interval schedules led

to higher punishment resistance and random schedules led to greater habitual responding, indicating that the features of schedules that bias habitual responding are dissociated from the features that bias punishment resistance. Moreover, punishment-resistant drug-seeking may invoke both goal-directed and habitual strategies. Addiction involves procurement of drugs, which can take the form of performing actions to obtain them or waiting to perform an action once drugs become available. Thus, schedules of reinforcement may be useful to model these different forms of drug seeking.

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Poster

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Neurobehavioral Mechanisms of Addiction

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Title: The influence of fear conditioning prior to cocaine self administration on cocaine seeking in adult rats

Authors: *Y. A. PEREZ-PEREZ[1], R. J. MORALES SILVA[2], G. RODRIGUEZ-TORRES[3], L. J. GODOY-MUÑOZ[4], S. FAZAL[3], N. RIVERA AVILES[3], M. T. SEPULVEDA-ORENGO[5]; [1]Basic Sci., Ponce Hlth. and Sci. Univ., Ponce, Puerto Rico; [2]Basic Sci., Ponce Hlth. Sci. Univ., Ponce, Puerto Rico; [3]Ponce Hlth. and Sci. Univ., Ponce, Puerto Rico; [4]Basic Sci., Ponce Hlth. Sci. Unirversity, Ponce, Puerto Rico; [5]Dept. Basic Sci., PHSU/Ponce Res. Inst., Ponce, Puerto Rico

Abstract: Comorbidity of cocaine use disorder (CUD) and trauma-related disorders is frequently observed, showing a marked relationship between trauma exposure and cocaine use. However, the mechanism by which prior exposure to traumatic events increases the risk of developing CUD remains unclear. Our study seeks to evaluate the impact of fear conditioning (FC) as a traumatic event exposure on cocaine-seeking behavior. We hypothesize that adult male and female Sprague Dawley rats subjected to a traumatic event (stressed group) will exhibit increased drug-seeking behavior compared to non-stressed rats. Rats underwent a single session of FC, followed by 12 days of short access cocaine self-administration (2 hours/day) and then 15

days of extinction training (2 hours/day). Twenty-four hours after the final extinction session, rats underwent cue-primed and cocaine-primed reinstatements. Results showed that stressed male rats displayed increased active lever presses during cue and cocaine-primed reinstatements compared to non-stressed male rats. Conversely, there was no difference in active lever presses between stressed and non-stressed female rats during either cue or cocaine-primed reinstatement. Our findings suggest that a traumatic event before cocaine exposure may influence the transition from recreational cocaine use to the development of CUD, in a sex-specific manner. We are testing whether the prelimbic cortex (PL) is responsible for the increased active lever presses during cue-primed reinstatement by inactivating it immediately after FC. We will study the intrinsic excitability changes in the PL of both stressed and non-stressed rats after cue-induced seeking behavior.

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Poster

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Neurobehavioral Mechanisms of Addiction

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Program #/Poster #: PSTR419.04/N38

Topic: G.09. Drugs of Abuse and Addiction

Title: Differential expression of immediate early genes (IEGs) in the dorsal and ventral striata of rats with punishment-associated compulsive and non-compulsive methamphetamine taking phenotypes

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Abstract: Differential expression of immediate early genes (IEGs) in the dorsal and ventral striata of rats with punishment-associated compulsive and non-compulsive methamphetamine taking phenotypes
Nasser Adjei*, Atul P. Daiwile, Bruce Ladenheim, Michael T. McCoy, and Jean Lud CadetMolecular Neuropsychiatry Research Branch, NIDA-IRP, Baltimore, MD 21224
Methamphetamine (METH) is an extremely addictive drug that continues to wreak havoc in the lives of individuals who suffer from METH use disorder (MUD). Differences in patterns of abuse, amounts of METH taken, and in relapse rates exist among METH users who meet DSM criteria for MUD. We are actively investigating the behavioral and molecular differences

between compulsive and non-compulsive METH self-administration (SA) by rats in the presence of an electric shock barrier. In the present study, we used male SD rats that were trained to self-administer METH on an FR-1 schedule for 22 days using a pattern of three 3-h sessions/day. After 22 days of METH SA, rats continued to self-administer METH in the presence of contingent footshocks (0.18mA to 0.36mA) for 9 more days. Animals were euthanized at 2 hours after the last SA session and different brain regions were used for quantitative PCR analysis. Rats escalated their METH intake during the first 22 days of SA training. Increased intensity of foot-shock punishment resulted in some rats (n = 7) that continue compulsive METH intake despite the footshocks; these rats were termed shock-resistant (SR). There were other rats (n = 6) that decreased their lever pressing during the punishment phase and were named shock-sensitive (SS) or non-compulsive. Quantitative PCR revealed decreases in the expression of *c-fos, fra2, Nr4a1, Nr4a2, and Nr4a3* mRNAs in the dorsal striatum of both compulsive and non-compulsive rats, indicating that chronic exposure to METH caused similar changes in these IEGs in that brain region. In the nucleus accumbens, there were significant decreases in the levels of c-fos, fra1, fra2, Egr1, Egr2, Nr4a1, and Nr4a3 mRNAs in both METH SA phenotypes. Together, these observations suggest that chronic exposure to METH via self-administration might suppress IEG expression in a region-specific fashion.
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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Title: Punished versus effortful self-administration of cocaine using various concentrations of histamine & reinforcement schedules.

Authors: *P. A. FOGEL, T. M. MOSCHAK;
Biol. Sci., Univ. of Texas at El Paso, El Paso, TX

Abstract: Substance use disorder (SUD) represents a pressing public health crisis, affecting millions of lives across the United States, and drug overdose deaths are at an all-time high. As the toll of drug abuse continues to escalate, understanding the neurobiological underpinnings of addiction becomes paramount. One complex relationship in SUD is the interplay between a drug of abuse and the role of cost. The role of cost applied to self-administration can be categorized as punished or effortful. Punished self-administration involves an adverse consequence of drug use (i.e. withdrawal, jail time) while effortful self-administration involves requirements to obtain a

drug (i.e. paying money for a drug). Here, we examine the interplay between cocaine, sex, and punished v. effortful self-administration in adult Long Evans rats. Rats were equipped with intrajugular catheters, and, after recovery, underwent two weeks (14 days) of cocaine or water/saline self-administration; one 6hr session per day. Following the two weeks of self-administration, rats began either punished or effortful self-administration. For punished self-administration, histamine was co-administered with the reinforcer. For effortful administration, increasing responses were required per unit of reinforcer. Preliminary data demonstrate that punishment and effort both suppress responding for reinforcement, and suggest that further exploration is needed to realize the neurobiological mechanisms underlying these interactions. Future directions include analyzing neural activity of Prelimbic (PrL) to Dorsomedial Striatum (DMS) projections during punished and effortful self-administration.

Disclosures: P.A. Fogel: None. **T.M. Moschak:** None.

Poster

PSTR419

Neurobehavioral Mechanisms of Addiction

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Topic: G.09. Drugs of Abuse and Addiction

Title: Differential gene networks are activated in in the rat nucleus accumbens of punishment-resistant compulsive methamphetamine takers that show incubation of drug craving

Authors: *A. DAIWILE[1], M. T. MCCOY[2], B. N. LADENHEIM[2], J. L. CADET[2];
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Abstract: Differential gene networks are activated in in the rat nucleus accumbens of punishment-resistant compulsive methamphetamine takers that show incubation of drug craving.Atul P. Daiwile, Ceiveon Munoz, Michael T. McCoy, Bruce Ladenheim & Jean Lud CadetMolecular Neuropsychiatry Research Branch, NIH/NIDA IRP, Baltimore, MD.AbstractMethamphetamine (METH) use disorder (MUD) is a neuropsychiatric disorder characterized by loss of control over drug taking despite negative consequences. We are actively investigating the molecular substrates of the behavioral differences between compulsive and non-compulsive METH self-administration (SA) by rats in the presence of an electric shock barrier. In the present study, we initially trained rats to self-administer METH for 22 days followed by 8 days with foot-shocks and then 15 days of forced abstinence. After it the rats underwent a second period of 12 days of METH SA followed by 3 days of foot-shocks, and then 15 days of forced abstinence. The first 8 days of footshocks divided the METH SA groups into namely Always shock-resistant (ASR), and shock-sensitive (SS) rats. ASR rats showed greater incubation of METH craving than the SS rats. During the second METH SA sessions with

footshocks, 36% percent of the SS rats developed into a shock-resistant and were named as “Delayed shock-resistant (DSR)”. Whereas the rest of the SS rats reduced their METH intake and are named as “Always sensitive (AS)”. During the second abstinence period, ASR rats showed greater incubation of METH craving than the DSR and AS animals. Rats were euthanized 24 hours after the last relapse tests and their nucleus accumbens were used in RNA-sequencing. RNA-seq revealed significant differentially expressed genes (DEGs) in the nucleus accumbens of ASR, DSR and AS compared to control animals. The METH self-administering phenotypes, ASR, DSR and AS, also showed significant alterations in the mRNA expression of multiple genes in comparison to each other. We then used a stringent cut-off of 1.5-fold and generated a Hierarchical cluster using 564 DEGs that documented clusters of genes that were impacted differentially in the METH SA groups. We used DAVID annotation and Sankey diagrams to further delineate interactions between DEGs in the 3 groups. IPA Analysis revealed the involvement of several DEGs in learning and cognition processes, affective and delusional disorders, and addictive behaviors. The present behavioral paradigm should help to elucidate the molecular substrates of early and late addiction-like behaviors in substance use disorders (SUDs).Acknowledgements: This work is supported by the DHHS/NIH/NIDA IRP, Baltimore, MD, USA.

Disclosures: A. Daiwile: None. **M.T. McCoy:** None. **B.N. Ladenheim:** None. **J.L. Cadet:** None.

Poster

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Topic: G.09. Drugs of Abuse and Addiction

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Title: Incubation of methamphetamine craving is associated with cell type and pathway specific synaptic adaptations in the rat nucleus accumbens core

Authors: *E.-K. HWANG[1], M. M. BEUTLER[2], M. E. WOLF[2];
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Abstract: A major problem in treating methamphetamine (meth) use disorder is a high rate of relapse even after prolonged abstinence. In a rat model of drug craving and relapse, cue-induced drug seeking progressively intensifies after withdrawal from drug self-administration (SA) (incubation of craving). Our lab previously demonstrated that incubation of meth craving is associated with synaptic calcium-permeable AMPA receptor (CP-AMPAR) upregulation in nucleus accumbens core (NAcc) medium spiny neurons (MSN) and that CP-AMPAR activation

is required for expression of incubated meth craving. However, the type of MSN (D1 or D2 receptor expressing) and the specific glutamate inputs that participate in CP-AMPAR plasticity are unknown. Here, we examined CP-AMPAR plasticity during meth incubation in a cell type- and pathway-specific manner in the NAcc. Rats self-administered saline or meth under extended-access conditions and received cue-induced seeking tests on withdrawal day (WD) 1 and WD21 to establish incubation. Whole-cell patch clamp recordings were performed after WD21. To enable identification of MSN subtypes, we use D1-Cre or A2a-Cre rats (the A2aR colocalizes with the D2R) crossed with reporter rats. We found upregulation of CP-AMPAR in D1+ but not D1- or A2a+ MSN after withdrawal from meth SA compared to saline controls. Furthermore, combining electrophysiology and optogenetics, we assessed AMPAR transmission in NAcc synapses originating from medial prefrontal cortex (mPFC), basolateral amygdala (BLA) and posterior paraventricular thalamus (PVT). We found potentiated glutamatergic synaptic transmission in mPFC and PVT to NAcc pathways, but not the BLA to NAcc pathway after meth incubation. Interestingly, PVT-D1+ NAcc synapses showed altered glutamatergic synaptic transmission through upregulation of CP-AMPAR and NMDAR. In contrast, mPFC-D1+ synapses showed increased AMPAR-mediated synaptic responses accompanied by an increased ratio of AMPAR-to-NMDAR-mediated EPSCs without significant changes of CP-AMPAR levels. Our data suggest that abstinence from meth induces pathway-specific synaptic plasticity in the NAcc and increased excitatory tone through CP-AMPAR upregulation at PVT-D1+ NAcc synapses may contribute to expression of incubated meth craving. We are currently extending these studies to explore the effect of meth incubation on excitatory synapses on D1+ and A2a+ neurons in the central nucleus of the amygdala.

Disclosures: E. Hwang: None. **M.M. Beutler:** None. **M.E. Wolf:** Other; Founder of Eleutheria Pharmaceuticals LLC.

Poster

PSTR419

Neurobehavioral Mechanisms of Addiction

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA025634

Title: Vta dopaminergic cell body activity in response to cues and mfb stimulation by amphetamine

Authors: *A. PAGE[1], R. M. DONKA[2], M. R. KROLL[1], M. F. ROITMAN[1], J. D. ROITMAN[1]; [1]Psychology, Univ. of Illinois Chicago, Chicago, IL; [2]Psychology, Univ. of Illinios Chicago, Chicago, IL

Abstract: Amphetamine (AMPH) is a commonly used drug of abuse largely due to reinforcing effects within the mesolimbic dopamine system. AMPH has been shown to reverse the action of the dopamine transporter (DAT) at the axon terminal, resulting in an efflux of dopamine into the synapse. Previous work has also suggested that within the mesolimbic dopamine system, AMPH inhibits cell body firing in the ventral tegmental area (VTA) *in-vitro*, but these results have not been validated *in-vivo*. Here, we use a Pavlovian paradigm to examine VTA dopaminergic cell activity in response to medial forebrain bundle (MFB) stimulation and predictive cues with acute AMPH exposure using *in-vivo* fiber photometry.
To measure activity of dopamine neurons in the VTA, a Cre-dependent GcAMP6f was injected and a fiber optic cannula was implanted in the VTA (AP −5.4, ML−0.7, DV −8.15 mm relative to bregma) of Long Evans rats (TH:Cre^{+}). An bipolar stimulating electrode was also implanted in the MFB (AP -2.8, ML -1.7, DV -7.8 relative to bregma). Experimentation began four weeks post-surgery to allow for recovery and viral expression. Each trial consisted of presentation of an auditory tone followed by stimulation in the MFB. Five different stimulation frequencies were used (0 Hz, 50 Hz, 63 Hz, 79 Hz, 141 Hz) each paired with a unique tone. Each session consisted of 10 blocks with 5 trials, each with the cue-stimulation pairing presented in a randomized order. Animals were trained for 10 days before experimentation began. We conducted three recording sessions per week across three consecutive weeks; a retraining session, followed by a saline control session, and an AMPH exposure session on day three. One dose of AMPH was administered each week in randomized order (1.0, 2.0, 5.0 mg/kg, i.p.) immediately before the session began. Data were normalized to the pretrial baseline. Responses to each cue and stimulation were analyzed by trial type and compared between saline and AMPH sessions. Overall, VTA dopaminergic cell activity increased across the range of MFB frequencies. Cue-responses proportional to predicted MFB frequency developed over the course of training. Further, AMPH treatment did not inhibit VTA dopaminergic cell body activity in response to MFB stimulation. Future experiments will measure dopamine release in nucleus accumbens (NAc) sensors with different binding properties in the same paradigm.

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Poster

PSTR419

Neurobehavioral Mechanisms of Addiction

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA053328

Title: Incubated cocaine- and sucrose-craving are not associated with changes in total dopamine receptor 1 or 3 expression levels within the medial prefrontal cortex of male and female rats

Authors: *D. GONZALEZ[1], F. CANO[2], L. L. HUERTA SANCHEZ[3], K. K. SZUMLINSKI[4];
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Abstract: Throughout abstinence from a drug or natural reward, cue-elicited craving for the reinforcer strengthens across time - a phenomenon known as the "incubation of craving". Previous research has observed a decrease in cue-induced dopamine release within the medial prefrontal cortex (mPFC) across a period of withdrawal from cocaine, making dopaminergic transmission within mPFC a possible key regulator in the development of incubated craving. The current study sought to investigate changes in expression of dopamine receptors 1 (D1R) and 3 (D3R) within the mPFC of rats with a history of cocaine or sucrose self-administration as a function of withdrawal length. Male and female Sprague-Dawley rats were trained to self-administer cocaine or sucrose (6h/day x 10 days) before undergoing a period of early (WD1 for sucrose, WD3 for cocaine) or late withdrawal (WD30). During a single 2h cue test following the withdrawal period, rats' responding was recorded but yielded no reinforcer, and subjects' brains were immediately extracted for immunoblotting of D1R and D3R expression within prelimbic (PL) and infralimbic (IL) cortex. An incubation of craving for cocaine and sucrose was observed, but there were no associated changes in D1R or D3R expression in either PL or IL. PL D3R expression positively correlated with sucrose seeking, and there was a time-dependent decrease in D3R expression in control but not cocaine-experienced females. Possible explanations for these results and avenues for future research are discussed.

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Poster

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Title: Methamphetamine-induced behavioral deficits are not associated with prefrontal microglia: potential role of COX-2 signaling in cortical neurons

Authors: *A. ACUNA, E. PEACOCK, S. E. RODARTE, R. WHITTINGTON, E. NAGY, J. LEGG, H. SIDDIQUI, M. WHITAKER, M. OLIVE;
Arizona State Univ., Tempe, AZ

Abstract: Methamphetamine (METH) use disorder (MUD) is characterized by recurring relapse, cognitive deficits, risk of long-term health effects, and overdose. Because there are no FDA approved medications to treat MUD, novel pharmacologic targets are currently being investigated. Immunomodulation has recently become an important potential strategy for the treatment of substance use disorders including MUD. Neuroinflammatory responses to METH have been observed, including increases in immune signaling, glial activation, and oxidative stress. Recently, our lab observed microglial immune activation and elevated pro-inflammatory cytokines such as IL-6, IL-18, CX3CL1 (Fractalkine), IFN-γ, CCL2, and leptin in the prefrontal cortex (PFC) following 3 weeks of abstinence from binge-like access to METH (96 consecutive hrs/week for 3 weeks) via intravenous self-administration (IVSA) at 0.05 mg/kg/infusion in both sexes. Control animals self-administered saline under the same conditions (n = 4-6/group). Behavioral flexibility deficits in attentional set shifting were observed at the same time point, which were attenuated by the COX-2 inhibitor, parecoxib (PXB) in both sexes (n = 9-13/group). Previous work has indicated that COX-2 and downstream prostaglandins are expressed by microglia and can serve as pro-inflammatory signals. Given our behavioral findings and co-occurring microglial immune activation in the PFC, we hypothesized that COX-2 expression would be elevated in microglia in this region following METH IVSA, and that PXB would attenuate microglial activation. Following 3 weeks of abstinence from METH IVSA, perfused brain sections were immunohistochemically stained for COX-2, neurons (NeuN), and microglia (Iba1). Preliminary immunohistochemical analysis indicates that COX-2 colocalizes with neurons and not microglia. Further, microglial morphology showed no effect of PXB treatment in the third week of abstinence compared to those treated with saline as a control. Colocalization of COX-2 with specific neuronal cell subtypes is on-going. Outside of its inflammatory function, COX-2 is expressed constitutively in neurons following glutamatergic activation, providing a potential alternative explanation for our behavioral findings and a novel avenue for investigation.

Disclosures: A. Acuna: None. **E. Peacock:** None. **S.E. Rodarte:** None. **R. Whittington:** None. **E. Nagy:** None. **J. Legg:** None. **H. Siddiqui:** None. **M. Whitaker:** None. **M. Olive:** None.

Poster

PSTR419

Neurobehavioral Mechanisms of Addiction

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.11/O5

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH NIDA Grant DA051551
NIH NIDA Grant DA056592
NIH NINDS Grant T32NS115704

Title: Granulocyte-colony stimulating factor (G-CSF) is a neuroimmune signaling molecule driving behavioral and molecular responses to opioids

Authors: *D. CAZAREZ, J. P. SENS, W. J. BROOKS, E. J. WISER, R. S. HOFFORD, D. D. KIRALY;
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Abstract: Opioid use disorder (OUD) is a major public health crisis leading to tens of thousands of deaths annually. Currently approved medications for the treatment of OUD remain ineffective or intolerable for too many, and the need for novel therapeutics is great. While all current FDA-approved pharmacotherapies have direct effects on opioid receptors, alternative strategies hold great promise. There is considerable literature showing changes in immune function in patients with OUD. Previously, our group has identified granulocyte-colony stimulating factor (G-CSF), a pleiotropic cytokine, as a potential mediator of drug induced behavioral and molecular adaptations. Manipulations of G-CSF alter both drug taking and drug seeking, thus suggesting translational potential. However, effects of G-CSF on models of opioid use remain to be studied. We assessed the behavioral effects of G-CSF treatment on conditioned place preference and locomotor sensitization in male and female C57BL6/J mice. G-CSF (50 µg/kg) or saline was injected one hour prior to behavioral tests. We also assessed transcriptional changes due to morphine and G-CSF treatment in brain punches of the nucleus accumbens. G-CSF treatment potentiated conditioned place preference at a low dose morphine (3.75 mg/kg) for male & female mice, and at morphine doses (7.5 and 15 mg/kg) for female mice. Development of locomotor sensitization to morphine was attenuated by G-CSF treatment at both low and high doses. Molecularly, we analyzed levels of expression for the FosB transcription factor, which was specifically decreased in female mice treated with high dose morphine. Levels of the Oprm1 gene which encodes the mu opioid receptor were differentially altered by G-CSF and morphine treatment in male and female mice. These results demonstrate G-CSF has dose and sex-dependent effects on behavioral responses to morphine and morphine-induced gene expression changes.

Disclosures: D. Cazarez: None. **J.P. Sens:** None. **W.J. Brooks:** None. **E.J. Wiser:** None. **R.S. Hofford:** None. **D.D. Kiraly:** None.

Poster

PSTR419

Neurobehavioral Mechanisms of Addiction

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.12/O6

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Maternal nutritional programming by hypercaloric diets programs IFN type I signaling and microglia complexity in the NAc shell and activates a systemic inflammation leading to development and transgenerational heritance of addiction-like behavior in Wistar rats.

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Abstract: Obesity and maternal energy-dense foods consumption during pregnancy programs aberrant behaviors resembling addiction in offspring and activates a systemic and central inflammation. This study aimed to characterize the central and systemic molecular immune networks, as well as, microglia reactivity in the nucleus accumbens (NAc) shell affected by fetal nutritional programming leading to addiction-like behavior in three offspring generations of Wistar rats. Females Wistar rats were exposed to cafeteria (CAF) diet or control diet for 9 weeks (prepregnancy, pregnancy and lactation), then male offspring (F1) at 2 months of age were diagnosed with food addiction-like behavior using operant conditioning. F1 male offspring diagnosed with addiction-like behavior were mated with virgin females to generate the F2 and the F3 offspring, respectively. Global microarray analysis, RTqPCR, proinflammatory plasma profile and microglia immunostaining were performed in the NAc shell of male rats. SIM-A9 microglia cells were stimulated with IFN-α and palmitic acid. Then, microglia activation and phagocytosis were determined by RTqPCR and incubation of green-fluorescent latex beads, respectively. Microarray analysis showed increasing in the type I interferon-inducible gene, Ift1, gene network. Genomic and cellular characterization also confirmed microglia hyperreactivity and upregulation of the Ifit1 in the NAc shell of males with addiction-like behavior. In-vitro models demonstrated that microglia do respond to IFN-α promoting a time-dependent genomic expression of Ift1, IL-1β and IL-6 followed by increased phagocytosis. Finally, we found that addiction-like behavior shown by F1 male offspring is transgenerational inherited to the F2 and F3 generations and it is associate with a decreased IL-10 levels in plasma.

Disclosures: L.J. Montalvo Martinez: None. **R.A. Maldonado:** None.

Poster

PSTR419

Neurobehavioral Mechanisms of Addiction

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.13/O7

Topic: G.09. Drugs of Abuse and Addiction

Title: Drugs of abuse increase serum- and glucocorticoid-inducible kinase 1 expression in the ventral tegmental area

Authors: *S. CAICO[1], V. BALI[2], S. C. SIMMONS[3], M. S. MAZEI-ROBISON[4];
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Abstract: Activity within the mesolimbic dopaminergic (DA) circuitry in the brain is essential for motivated behavior and drugs of abuse can hijack this system by increasing DA signaling. Drug-induced cellular and molecular changes within the ventral tegmental area (VTA) can promote motivation to use drugs and drug-induced behavior. Our lab seeks to identify cellular and molecular changes in the VTA that promote drug responses. We found that repeated injections of morphine or cocaine increase the expression of serum- and glucocorticoid-inducible kinase 1 (SGK1) in the VTA using RNA sequencing. However, it was unclear whether similar regulation occurs with acute drug exposure and how persistent SGK1 expression changes are. To investigate this, we treated mice acutely with morphine or cocaine and isolated RNA from VTA for RT-PCR. We observed that VTA SGK1 gene expression is increased both 1 and 24 hours following both repeated and acute drug injections. We are currently exploring whether increased SGK1 expression persists during withdrawal and if it is differentially regulated by drug re-exposure. Given that the VTA is heterogenous structure containing multiple cell types besides DA neurons, we are determining which cell populations contribute to this drug-induced increase in SGK1 expression. We performed translating ribosome affinity purification studies to examine cell type-specific changes in SGK1 expression. Interestingly, we did not find an increase in SGK1 expression in either DA or GABA neuron pulldowns following morphine administration, suggesting that induction is occurring in alternative VTA cell populations or that SGK1 is not being actively translated. To assess whether SGK1 expression is occurring in specific neuronal populations or non-neuronal cells such as glia we are utilizing RNAScope analysis. Together, these studies will define how drugs of abuse alter expression of SGK1 in the VTA, a necessary first step to explore the role of this gene in drug behavior.

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Poster

PSTR419

Neurobehavioral Mechanisms of Addiction

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.14/O8

Topic: G.09. Drugs of Abuse and Addiction

Support: This work was supported by NIH grant 1R01AA030061 to MFO

Title: Sustained methamphetamine-induced impairment of cognitive flexibility in rats: the behavioral effects of prostaglandin E2 (PGE2) synthase inhibition

Authors: *S. RODARTE, A. M. ACUNA, S. BICKLEY, E. PEACOCK, E. NAGY, J. KARUGU, J. LEGG, A. PATIBANDA, H. SIDDIQUI, M. WHITAKER, M. OLIVE; Arizona State Univ., Tempe, AZ

Abstract: Methamphetamine (METH) Use Disorder (MUD) and METH-related overdose deaths have more than doubled since 2018. There are currently no FDA approved medications to aid in METH abstinence. Neuroinflammation occurs in response to METH and has been linked to cognitive impairment. Recently, our lab investigated the effect of methamphetamine on behavioral flexibility using the Attentional Set Shifting Task (ASST) in which rats retrieved a food reward by digging in bowls using odor or digging media to determine the bowl containing the reward. The number of trials it takes to meet criterion (6 consecutive correct choices) was quantified, with more trials equating to a deficit in behavioral flexibility. We found that administration of parecoxib (PXB), a cyclooxygenase-2 (COX-2) inhibitor, attenuated deficits in the extradimensional shift (ED) phase of the ASST following abstinence in rats given binge-like access to self-administered METH. Several prostaglandins are synthesized downstream of COX-2, including prostaglandin E2 (PGE2). We hypothesized that the attenuation of behavioral deficits through COX-2 inhibition is due to decreased synthesis and signaling of PGE2, which others have shown to be elevated following METH use. Therefore, we investigated the behavioral effects of UT-11, a PGE2 synthase inhibitor. Female Long-Evans rats (n=10) underwent the ASST both prior to and following 96 hour/week/3 week METH access, or saline as a control. Following abstinence and prior to post-METH behavioral assessment, animals were administered daily 5 mg/kg injections of UT-11 for 5-7 days. Male treatment is currently underway. We compared four groups: METH-administering rats treated with saline (METH-saline), PXB (METH-PXB), or UT-11 (METH-UT-11) and saline-administering rats treated with saline (saline-saline). METH-UT-11 animals did not differ in the ASST ED from any other group. The lack of differences between METH-UT-11 and saline-saline groups suggests that UT-11 attenuates behavioral deficits. However, the lack of differences between METH-saline and METH-UT-11 indicates a less pronounced impairment of behavioral flexibility as compared to METH-PXB. These results show that behavioral flexibility in METH self-administering rats is slightly improved with UT-11 administration, but not to the extent of PXB treatment. Further work could investigate if a higher dose of UT-11 would result in greater attenuation of deficits or analyze the effect of inhibiting the synthesis of other prostaglandins. These results reinforce the notion that pharmacological therapeutics may promote cognitive recovery and facilitate behavioral treatment for MUD patients.

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Poster

PSTR419

Neurobehavioral Mechanisms of Addiction

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.15/O9

Topic: G.09. Drugs of Abuse and Addiction

Title: The role of the anti-reward pathway in alprazolam withdrawal induced behavioral despair and addiction related gene signaling

Authors: *N. I. VARDELEON[1], A. M. CARDONA-ACOSTA[2], M. LOBO[3], C. A. BOLANOS-GUZMAN[4];
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Abstract: Benzodiazepines (BZD) such as alprazolam (ALP) are the most prescribed and abused anxiolytic medication worldwide. There has been a stark 6x increase in BZD related deaths in the last decade, and a near 200% increase in BZD prescriptions dispensed from 2021-2022. Despite these unfortunate trends, research investigating the neural circuitry and molecular mechanisms of ALP withdrawal is critically limited. Recently, the lateral habenula (LHb) to ventral tegmental area (VTA) pathway, coined as the 'anti-reward pathway,' has become a potential target for treatment of drug withdrawal and depression. Activation of this pathway induces behavioral despair and aversive states in non-human primates and rodents. Therefore, to address gaps in the BZD withdrawal literature, we established a robust behavioral measure of ALP withdrawal. Adult male and female mice (n=6/group) were pretreated with either ALP (6.0 mg/kg s.c.) or vehicle (VEH; 0.0 mg/kg s.c.) for 8 days and received flumazenil (FLZ; 5.0 mg/kg, i.p.), a BZD antagonist, to precipitate withdrawal 2h after the last ALP injection. FLZ-induced behaviors reflecting despair were recorded and scored under blind treatment conditions. Twenty-four hours after withdrawal, brain punches from the LHb and the VTA were taken for qPCR analysis. Mice pretreated with ALP showed robust signs of behavioral despair as measured by jumping, head twitches, and forepaw tremors when compared to VEH controls ($p<0.001$, $p<0.01$, $p<0.001$, respectively). This effect was rescued when mice received a single injection of NMDA antagonist ketamine (KET) (10.0 mg/kg i.p.) with ALP+KET mice returning to baseline levels of behavioral despair when compared to ALP alone ($p<0.01$). The qPCR analyses revealed decreased expression activity related metabotropic glutamate receptor 5 (mGluR5) protein in the VTA when compared to VEH controls ($p<0.05$), effects that were not seen in the female mice. We observed trending increases in the extracellular signal-regulated kinase1/2 (ERK2), addiction related gene, in the VTA and LHb of male and female mice in the ALP pre-treatment condition. Precipitating withdrawal with FLZ induced robust behavioral symptoms in adult ALP-pretreated male and female mice. We hypothesized that the LHb-to-VTA 'anti-reward pathway' may be driving these behaviors as the LHb sends feedforward inhibition onto the VTA. Together, our qPCR and behavioral data suggest increased activity of this pathway and addiction related signaling in the LHb, and a sex-dependent decrease in the VTA after withdrawal. Studies utilizing chemogenetic DREADDS are underway to test our hypothesis that LHb inhibition recues ALP-induced withdrawal.

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Poster

PSTR419

Neurobehavioral Mechanisms of Addiction

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.16/O10

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R01 DA048208

Title: Investigating effects of concurrent chronic unpredictable stress (CUS) and cocaine self-administration on neuronal and microglial remodeling in the rat prefrontal cortex

Authors: *D. B. NOWAK[1], M. K. ESTES[2], E. THOMPSON[1], M. MEYERINK[1], D. R. OLIVEIRA, Sr.[1], C. GARCIA-KELLER[3], J. R. MANTSCH[3];
[2]Pharmacol. & Toxicology, [3]Pharmacol. and Toxicology, [1]Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Substance misuse is a leading cause of disability and mortality among adults in the United States. People experiencing substance use disorders (SUDs) are at greater risk for the diagnosis of a co-occurring mental health condition, including major depressive disorder (MDD). Exposure to chronic stress, which often accompanies the onset of depressive disorders, may also precipitate or sustain the misuse of addictive drugs. Exposure to either chronic stressors or repeated psychostimulant use leads to changes in neuronal function and cortical connectivity. Rodent models of chronic stress-induced depression have implicated microglia as pivotal actors in promoting regressive cortical plasticity and behavioral deficits following repeated stress. Although chronic cocaine exposure results in similar regressive cortical plasticity, involvement of microglia in this process remains unknown. Understanding the potentially shared or related neurobiological mechanisms through which chronic stress and cocaine use promotes cortical remodeling is essential for developing treatments for stress-related drug misuse. To probe the neurobiological and behavioral effects of chronic unpredictable stress (CUS) on drug taking and seeking, we have employed a concurrent exposure design, whereby adult rats were trained to self-administer cocaine and then allowed daily access (2hr/14days), while subject to twice daily unpredictable stressors (AM stress/12pm SA/PM stress). Previous unpublished work from our lab has demonstrated that, although rats exposed to CUS do not show differences in drug taking, exposure to CUS during the drug taking phase results in augmented drug-primed reinstatement and sensitivity to shock-induced reinstatement after a period of extinction training. Our current studies aim to investigate the role of microglia and neuronal remodeling in stress-related changes in drug seeking. Preliminary results indicate that CUS and cocaine self-administration independently produce significant reductions in dendritic spine density among neurons in the prelimbic area of medial prefrontal cortex. These findings corroborate previous literature reports. Ongoing work is investigating the effects of concurrent cocaine self-administration and CUS on microglial morphology and neuronal remodeling in prefrontal cortex in both male and female rats. Uncovering the role of microglia in stress-related drug misuse may unlock novel therapeutic approaches for treating patients whose drug misuse is enhanced by stress exposure.

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Poster

PSTR419

Neurobehavioral Mechanisms of Addiction

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA Grant R37-DA051686
NIH/NIDA Grant R25-DA057786
NIH/NIDA Grant T32-DA007278

Title: Modulation of Cocaine Escalation by CRFR1 Antagonists in Male and Female Rats

Authors: *J. JONES[1], L. J. GORDON-FENNELL[2], P. E. PHILLIPS[3];
[1]Univ. of Washington, seattle, WA; [3]Psychiatry & Behavioral Sci., [2]Univ. of Washington, Seattle, WA

Abstract: Chronic use of highly reinforcing drugs, such as cocaine, can result in an escalation of drug consumption. Escalation is related to some of the most severe consequences associated with substance use disorder (SUD), including overdose. Chronic substance use leads to neurobiological changes including the signaling of the stress-related peptide corticotropin-releasing factor (CRF). Previous work has implicated CRF dysregulation in alcohol, psychostimulant, nicotine, and opioid dependence. CRF release in extrahypothalamic regions contributes to anxiety-like symptoms of withdrawal that can motivate individuals to consume drugs. There is limited evidence addressing whether CRF receptor (CRFR) activation alters cocaine consumption in individuals who have escalated their cocaine consumption, especially in female subjects. The present study examines the underlying neurobiology of escalated cocaine consumption in male and female rats. Wistar rats were trained on long access (6hr) cocaine self-administration paradigm in which a subset demonstrated an escalation in their cocaine consumption. At the end of this paradigm, rats were subject to systemic administration of one of two CRFR1 antagonists, antalarmin (25 mg/kg, i.p.) or N,N-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethyl-pyrazolo[1,5a]pyrimidin-7-amine (MPZP; 10 and 27.5 mg/kg, s.c.), counterbalanced with vehicle. These treatments were based on a previous study where there was a small reduction in cocaine consumption. However, our findings to date have not found significant effects of antalarmin treatment ($p=0.34$, paired t-test, $n = 9$).

Disclosures: J. Jones: A. Employment/Salary (full or part-time):; University of Washington.
L.J. Gordon-Fennell: A. Employment/Salary (full or part-time):; University of Washington.
P.E. Phillips: A. Employment/Salary (full or part-time):; University of Washington.

Poster

PSTR420

Nicotine-Physiological, Behavioral, and Cognitive Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.01/Web Only

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant DA047678
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Title: Adolescent-onset volitional nicotine exposure promotes sex-dependent expression of nicotine seeking and dependence in adulthood

Authors: *T. G. FREELS, L. NORTON, A. R. TAPPER;
Brudnick Neuropsychiatric Res. Institute, Univ. of Massachusetts Chan Med. Sch., Worcester, MA

Abstract: Rates of nicotine vaping among U.S. adolescents have been likened to an epidemic; however, our understanding of the protracted effects of adolescent-onset intrapulmonary nicotine exposure on nicotine-seeking and dependence is lacking. This deficit is due to the limited translational value of previous research engendered by use of intravenous, subcutaneous, intraperitoneal, and oral nicotine administration in primarily male preclinical models. We address this knowledge gap using an adolescent-onset volitional nicotine vapor exposure approach in male and female C57BL/6J mice and testing nicotine seeking and dependence in adulthood. Following 20 days of vapor exposure starting in middle - late adolescence (4 - 6 weeks of age), female mice that self-administered 6 mg/ml [nicotine] vapor (NIC6) exhibited enhanced nicotine seeking compared to males that administered NIC6 indicated by higher break points during progressive-ratio tests. Volitional NIC6 exposure enhanced nicotine seeking compared to vehicle irrespective of sex. Additionally, volitional NIC6 exposure promoted nicotine dependence particularly in male mice after 24 hours and 2 weeks of nicotine vapor abstinence illustrated by increased somatic nicotine withdrawal symptoms. Ongoing experiments are being conducted to elucidate aberrations in locomotion, anxiety, novelty-seeking, and fear learning following 3 weeks of vapor abstinence. Together, our findings suggest that there are sexually dimorphic patterns of volitional nicotine vapor exposure and nicotine dependence which could inform adolescent vaping interventions. Future studies will integrate advanced *in-vivo* neuroscience approaches with adolescent-onset volitional nicotine vapor exposure to uncover the neural mechanisms of nicotine’s sex-dependent effects.

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Poster

PSTR420

Nicotine-Physiological, Behavioral, and Cognitive Effects

Location: MCP Hall A

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Program #/Poster #: PSTR420.02/O12

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH NIDA Grant K01DA056854
The State of Connecticut, Department of Mental Health and Addiction Services

Title: Chronic pain promotes nicotine intake and enhances dopamine signaling in adult male rats

Authors: *D. BAGDAS, B. KUMA, E. J. NUNES, T. NELSON, N. A. ADDY;
Psychiatry, Yale Univ., New Haven, CT

Abstract: Background & Aims: Chronic pain patients demonstrate high comorbidity of negative affect (such as depression and anxiety). Pain-related negative affect is also a risk factor for tobacco use. In this study, we modeled the comorbidity of chronic pain and nicotine use in rats. We aimed to determine whether a chronic pain state increases the reinforcing efficacy of nicotine. Given the role of dopamine in nicotine reinforcement, we also sought to determine whether chronic pain alters nicotine-mediated dopamine signaling in the ventral tegmental area (VTA) to nucleus accumbens (NAc) pathway.
Methods: We induced neuropathic pain in adult male Sprague Dawley rats by using chronic constrictive nerve injury (CCI) model. We assessed the pain status using the Von Frey test. Two weeks post-CCI surgery, we used a combined passive and vapor self-administration procedure with e-liquids containing 0, 3, and 6 mg/ml freebase nicotine in a propylene glycol and vegetable glycerin vehicle (50:50). E-liquids were administered through an e-cigarette vapor delivery system set for 3-second vapor puff at 50W power and 400°F. Rats passively received 15 puffs daily for 5 days of conditioning. After initial conditioning, rats underwent 18 days of vapor self-administration (VSA), receiving a puff with a 30-second timeout for one hour daily. VSA sessions occurred on fixed-ratio (FR) schedules: FR1 for 6 days, FR3 for 5 days, and FR5 for 7 days. In a separate group of rats, we also evaluated behavioral response to stress, using the forced swim test, two weeks after the surgery. The following day, we examined nicotine (0.7 mg/kg, subcutaneous) effects on VTA stimulated dopamine signaling in NAc, using fast-scan cyclic voltammetry.
Results: The CCI rats acquired stable nicotine self-administration at the 3 mg/ml nicotine concentration, but not at 6 mg/ml. Sham rats did not show a strong intake for any nicotine concentration. Furthermore, CCI rats exhibited increased immobility time in the forced swim test and higher nicotine-stimulated dopamine release in the NAc, compared to sham rats.
Conclusion: Chronic pain promoted nicotine self-administration at low concentration of nicotine. Sham rats developed only a moderately weaker association with nicotine taking. Chronic pain also induced a negative affective state associated with depression-like behavior, alongside an increase in dopamine signaling in the NAc following nicotine administration. The greater brain

nicotine reward in CCI compared to sham animals could be responsible for the increase in nicotine taking behavior in chronic pain animals.

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Poster

PSTR420

Nicotine-Physiological, Behavioral, and Cognitive Effects

Location: MCP Hall A

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Topic: G.09. Drugs of Abuse and Addiction

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DePauw University - Dr. Frederick Hendricks Student Travel Award
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Title: Behavioral Tests for the Characterization of Zebrafish Acetylcholine Receptor Mutants

Authors: *H. SCHNEIDER[1,2], A. R. DELGADO[1], T. VIERLING[1], M. ZAHID[1], M. BARKER[1];
[1]DePauw Univ., Greencastle, IN; [2]Department of Biology, Depauw University, Greencastle, IN

Abstract: Nicotine-use behavior such as smoking and vaping represents one of the main causes of preventable diseases worldwide. Nicotinic acetylcholine receptors mediate nicotine-induced changes in the central nervous system associated with nicotine dependence. The nicotinic acetylcholine receptor subunits alpha 3 (chrna3), alpha 5 (chrna5) and beta 4 (chrnb4) have been linked to heavy smoking and early smoking onset in humans. Larval zebrafish represent a model for studying the role of chrna3, chrna5 and chrnb4 in nicotine-seeking and avoidance behavioral tests using pharmacological methods and gene-knockouts or gene-modifications. Since the chrna3 and chrnb4 genes are likely to be expressed in both the central and the peripheral vertebrate nervous system knockout mutants for these acetylcholine receptor genes could impact the development, health and behavioral responsiveness of zebrafish that would affect nicotine-seeking and avoidance behavior indirectly. To better determine potential impact of acetylcholine receptor knockouts we have developed a set of short and robust stimulus-response behavioral tests for sensitivity to light, temperature, chemical agents, and mechanoreceptor stimulation using Daniovision (Noldus) and 48-well plates under standard conditions. In wild-type larval zebrafish, repeated mechanical stimulation (tapping) resulted in a quick attenuation that can partially recover within seconds. Bright light results in a partial freezing response and

significantly reduced swimming activity. Mustard oil but not capsaicin resulted in an increased movement activity and reduced attenuation to repeated tapping. Quick temperature changes triggered increased movement activity. Nicotine application (20 µM) caused significantly stronger behavioral responses. Attenuation in tapping experiments was enhanced and the recovery was stronger at 20 µM but not 1 µM nicotine application. Mecamylamine reduced nicotine-induced responses while the alpha3-beta4 nicotinic acetylcholine receptor antagonist SR16594 could weaken the attenuation in tapping experiments. Homozygous but not heterozygous chrna3 gene-knockout mutants showed a reduced attenuation in tapping experiments compared to wild-type larval zebrafish before nicotine application. Overall, the short tests are very well suited to probe the impact of acetylcholine receptor knockout mutations and pharmacotherapeutics on development, health, and behavioral responsiveness of larval zebrafish. Moreover, the tests could provide insight into the potential role of chrna3, chrna5 and chrnb4 subunits in the peripheral nervous system of larval zebrafish.

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Poster

PSTR420

Nicotine-Physiological, Behavioral, and Cognitive Effects

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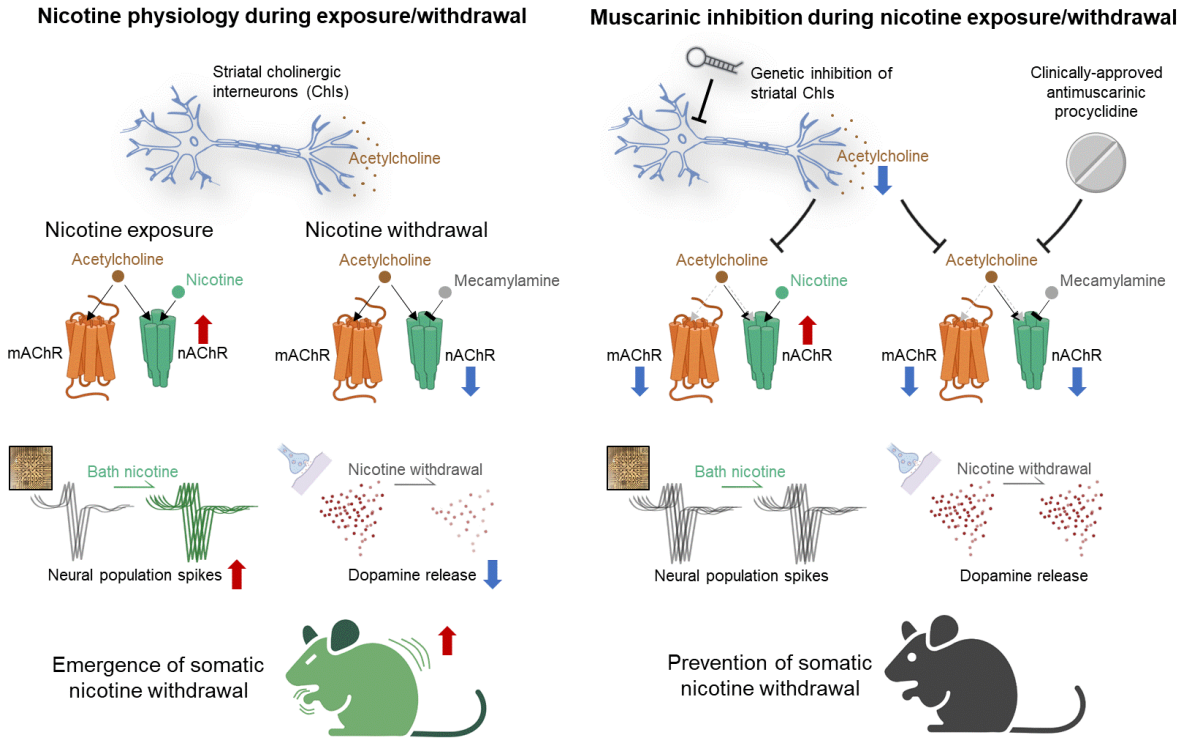
Title: Striatal cholinergic interneurons control physical nicotine withdrawal via muscarinic receptor signaling

Authors: *B. KIM, H.-I. IM;
Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of

Abstract: Striatal cholinergic interneurons (ChIs) provide acetylcholine tone to the striatum and essentially govern motor functions. Nicotine withdrawal elicits physical symptoms that dysregulate motor behavior. Here, we investigated the role of striatal ChIs in physical nicotine withdrawal. We first generated mice under RNAi-dependent genetic inhibition of striatal ChIs (ChI^{GI}) by suppressing the sodium channel subunit $Na_V1.1$. ChI^{GI} markedly reduced the somatic signs of nicotine withdrawal without affecting other nicotine-dependent or striatum-associated

behaviors. Multi-electrode array (MEA) recording revealed that ChI^{GI} reversed *ex vivo* nicotine-induced alterations in the number of neural population spikes in the dorsal striatum. Importantly, drug repurposing strategy revealed that a clinically-approved antimuscarinic drug, procyclidine, fully mimicked the therapeutic electrophysiological effects of ChI^{GI}. Furthermore, both ChI^{GI} and procyclidine prevented the nicotine withdrawal-induced reduction in striatal dopamine release *in vivo*. Lastly, therapeutic intervention with procyclidine dose-dependently diminished the physical signs of nicotine withdrawal. Our data demonstrated that the striatal ChIs are a critical substrate of physical nicotine withdrawal, and that muscarinic antagonism holds therapeutic potential against nicotine withdrawal.



Disclosures: B. Kim: None. **H. Im:** None.

Poster

PSTR420

Nicotine-Physiological, Behavioral, and Cognitive Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.05/O15

Topic: G.09. Drugs of Abuse and Addiction

Title: Nicotine use is associated with suboptimal behavior in a translational foraging task

Authors: *N. SCHMITZER-TORBERT, W. HORTON;
Psychology, Wabash Col., Crawfordsville, IN

Abstract: Nicotine use is associated with altered decision-making, such as increased discounting of future rewards and suboptimal foraging behavior, which may be important as risk factors for initiating nicotine use, and in encouraging nicotine cessation. We have previously found that while nicotine use was related to greater impulsivity (stronger discounting), no strong correlates with nicotine use were identified in the Movie Row, a translational foraging task based on the rodent Restaurant Row task. In the present study, we tested the impact of budget constraints (using time- or reward-based budgets) on the behavior of smokers and non-smokers.
Online participants (n = 280, 133 nicotine users) were recruited online through Prolific and completed a virtual navigation task (the Movie Row) in which they navigated around a virtual track presented, passing through four different reward zones. Rewards (4-sec video clips presented on movie screens) were offered in each of the reward zones (each with a different category of video), available after a variable delay. After an initial training phase, participants were allowed to forage under two conditions, either with a time budget (allowed to forage for a total of 30 minutes) or with a video budget (until they had received 40 rewards). Participants provided ratings of each video clip that was watched (1 to 5 stars) and after the experiment, also ranked the four video types (from favorite to least favorite), to assess stated preferences for each reward category.
Compared to time budgets, participants tested under video budgets were more sensitive to the delay of an offer, and shifted their behavior to be more willing to accept offers with shorter delays.
Overall, nicotine use was associated with suboptimal behavior in the Movie Row task, and especially for participants tested with video budgets. Smokers earned fewer rewards compared to non-smokers under both budgets, even though nicotine status was not related to enjoyment of the rewards (ratings of the videos watched were similar for smokers and non-smokers). Under the video budget, non-smokers strongly preferred offers with short delays, and avoided offers with longer delays, while smokers were more likely to accept offers with long delays. Also, after accepting an offer with a long delay, smokers were more likely to quit than non-smokers before completing the delay.
These data suggest that when foraging for actual rewards, nicotine status is associated with suboptimal behavior, where smoker accept disadvantageous offers and then correct their initial decision. Other parameters of decision-making (such as sunk-costs and deliberation) were not strongly related to nicotine status.

Disclosures: N. Schmitzer-Torbert: None. **W. Horton:** None.

Poster

PSTR420

Nicotine-Physiological, Behavioral, and Cognitive Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.06/O16

Topic: G.09. Drugs of Abuse and Addiction

Support: R01DA035942
R01DA040626

Title: Effects of the β2 nAChR on acquisition and motivation for nicotine self-administration

Authors: *N. WALKER[1], B. R. TUCKER[1], L. THOMAS[1], A. N. TAPP[1], R. M. DRENAN[2]; [1]Wake Forest Univ. Sch. of Med., Winston Salem, NC; [2]Physiol. & Pharmacol., Wake Forest Univ. Sch. of Med., Winston Salem, NC

Abstract: Despite nicotine's status as a weak reinforcer, cigarette smoking is the leading cause of preventable death worldwide highlighting a disparity between the 70% of smokers who desire to quit and the 8% of successful quit attempts persisting 6-12 months. The beta2 nicotinic acetylcholine receptor (nAChR), a subunit highly expressed in the mesolimbic dopamine pathway and throughout the CNS, has been shown to play a key role in several nicotine related processes including but not limited to nicotine-elicited firing of dopaminergic neurons, dopamine release in the nucleus accumbens, nicotine-stimulated locomotor activation, and maintenance of nicotine self-administration. In this study, we tested the hypothesis that ventral tegmental area (VTA) beta2 nAChR activation increases motivation to self-administer nicotine. We expressed a virus containing a hypersensitive mutation of the beta2 nAChR (β2L9S) which allows for selective activation of beta2* nAChRs using low doses of nicotine and have shown VTA beta2 activation to be sufficient for nicotine reinforcement in rats. However, the role of this subunit in motivation is unclear. Cohorts of rats self-administered different doses of nicotine each separated by a 2.7-fold increase ranging from 1.5 μg/kg to 30 μg/kg in Sprague-Dawley rats and from 0.08 μg/kg to 30 μg/kg in β2L9S rats. Rats then underwent 17 self-administration sessions on a fixed-ratio 1 (FR1) schedule followed by 5 progressive ratio (PR) sessions. These results, in addition to providing a behavioral dose-response curve for Sprague-Dawley and β2L9S rats, also provide novel insights on the role of VTA beta2 nAChR activation on motivation.

Disclosures: N. Walker: None. **B.R. Tucker:** None. **L. Thomas:** None. **A.N. Tapp:** None. **R.M. Drenan:** None.

Poster

PSTR420

Nicotine-Physiological, Behavioral, and Cognitive Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.07/O17

Topic: G.09. Drugs of Abuse and Addiction

Support: H.M. Kawade acknowledges 'Council of Scientific and Industrial Research, New Delhi, India' for providing research fellowship for Ph. D. program

Title: Neuropeptide S regulates nicotine-induced facilitation of intracranial self-stimulation in rats

Authors: *H. M. KAWADE[1], D. M. PANDHARE[1], N. K. SUBHEDAR[2], D. M. KOKARE[1]; [1]Dept. of Pharmaceut. Sci., Rashtrasant Tukadoji Maharaj Nagpur Univ., Nagpur, India; [2]Dept. of Biol., Indian Inst. of Sci. Educ. and Res., Pune, India

Abstract: Nicotine is a major reinforcing contributor of tobacco dependence, and identification of neurotransmitter systems that regulate rewarding actions of nicotine is clinically important. Emerging evidence has highlighted the potential of neuropeptide S (NPS) as a candidate neurotransmitter system for the treatment of drug addiction, but its role in nicotine reward has not yet been explored. Herein, we investigated the involvement of NPS/NPS receptor system in nicotine reward using intracranial self-stimulation (ICSS) protocol in the operant conditioning chamber. Adult male Wistar rats (n = 36) were implanted with bipolar electrodes targeted at lateral hypothalamus-medial forebrain bundle trained under a fixed-ratio 1 schedule for wide range of brain stimulation frequencies (165-33 Hz). The trained rats under control conditions showed frequency-dependent increase in lever press activity in the operant conditioning chamber. Nicotine injection (0.1-0.25 mg/kg, subcutaneous) facilitated ICSS behavior as compared to saline-injected rats. While intracerebroventricular (i.c.v.) NPS infusion (0.1-1 nmol) facilitated ICSS behavior and locomotor activity, NPS receptor antagonist SHA-68 (0.1-1 nmol) did not produce any effect. The increase in the lever press activity by NPS is not due to locomotor effect, because NPS injection was found to increase active lever but not inactive lever pressings. The pre-treatment of SHA-68 dose dependently blocked nicotine-induced facilitation of ICSS behavior. In addition, nicotine injection (0.25 mg/kg, subcutaneous) increased NPS expression (ELISA test) in the rewarding areas such as lateral hypothalamus and paraventricular thalamic nucleus but not in the amygdala and ventral tegmental area of operant conditioned rats. This data suggests that NPS/NPS receptors system may be involved in the nicotine reward and NPS receptors antagonist may serve as a candidate treatment for the abuse-related effects of the nicotine.

Disclosures: H.M. Kawade: None. **D.M. Pandhare:** None. **N.K. Subhedar:** None. **D.M. Kokare:** None.

Poster

PSTR420

Nicotine-Physiological, Behavioral, and Cognitive Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.08/O18

Topic: G.09. Drugs of Abuse and Addiction

Title: Effects of environmental enrichment on enhanced nicotine-seeking behavior following exposure to chronic variable stress

Authors: *J. CORTRIGHT, K. STEELE, S. STEELE;
Psychological Sci., Univ. of Wisconsin - River Falls, River Falls, WI

Abstract: Drug addiction is a major public health and serious economic concern in the Unites States costing taxpayers billions of dollars annually. Preclinical and clinical research indicates that individuals exposed to stress are more vulnerable to drug addiction (Koob, 2008; Ouimette et al., 2007). For example, acute stress exposure increases and reinstates nicotine-seeking behavior as measured by conditioned place preference (CPP) (Leão et al., 2009). Moreover, chronic unpredictable stress increases the response to nicotine-rewarding properties in the CPP test (Biala et al., 2018) and, more recently, we have shown the exposure to variable stress produces an enhanced, or sensitized, response to nicotine in an animal model of addiction (Cortright et al., in preparation). Contrary to the effects observed following exposure to variable stress, both running and environmental enrichment (EE) reduce CPP for cocaine (Mustroph et al., 2016). Based on the interactions observed between EE and chronic stress on learning and memory (Wright & Conrad, 2008), it is reasonable to postulate similar interactions on the rewarding properties of nicotine. To date, only one study (Biala et al., 2018) has assessed the effects of chronic stress on the rewarding effects of nicotine in the CPP model and none have measured the potential of EE in attenuating this effect. Therefore, this study will further investigate and determine whether EE can reverse, or abolish, the enhancement in the rewarding properties of nicotine following exposure to chronic variable stress. One group of animals will be exposed to 20 days of randomized stressors either during adolescence or early adulthood. Another group of animals will be exposed to four weeks of EE. An additional group will be exposed to 20 days of randomized stressors followed by four weeks of EE. Control animals will not receive any exposure treatment. Following exposure treatment animals will undergo place preference conditioning to nicotine (0.175 mg/kg, base, s.c.). To date, data indicate that animals exposed to chronic variable stress exhibit higher place preference for nicotine compared to controls, an effect that is attenuated by exposure to EE. Further data analysis will consist of comparisons between sex and adolescence versus early adulthood stress exposure. Therefore, in addition to providing evidence of the contributions of stress and environmental conditions to successful cessation of drug-seeking, the results obtained can have important implications for the use of environmental stimulation together with pharmacological and behavioral therapies in the treatment of addiction.

Disclosures: J. Cortright: None. **K. Steele:** None. **S. Steele:** None.

Poster

PSTR420

Nicotine-Physiological, Behavioral, and Cognitive Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.09/O19

Topic: G.09. Drugs of Abuse and Addiction

Title: Effects of low-dose nicotine treatment on methamphetamine-induced locomotor sensitization in Adolescent Sprague-Dawley rats

Authors: *Y. HAJY HEYDARY[1], S. LOTFIPOUR[2];
[1]Pharmaceut. Sci., Univ. of California, Irvine, Irvine, CA; [2]Emergency Med., Pharmacol., & Pathology, Univ. of California, Irvine, Irvine, CA

Abstract: Studies have reported that 70-80% of methamphetamine (METH) abusers smoke nicotine (NIC)/tobacco products. Given that NIC could act as a gateway for enhanced METH use, it is important to assess the interactive effects of these drugs. This is crucial, given the recent exponential rise of electronic nicotine vaping product use among youth. Our lab has previously shown sex- and age-dependent effects of NIC-enhancement on intravenous METH self-administration. Adolescent NIC-treated males have enhanced METH self-administration, while adult NIC-treated females showed enhanced discrimination between reinforced and nonreinforced responses for intravenous METH self-administration. The mechanisms impacting these relationships are unknown. Behavioral sensitization is a phenomenon where repeated exposure to a drug can lead to an enhanced response even after a period of withdrawal. In this study, we investigate locomotor sensitization in NIC/Sal-treated adolescent male and female Sprague-Dawley rats following repeated METH exposure to assess the combined effects of both drugs on locomotor activity. Based on our self-administration data, we hypothesize that NIC pretreated adolescent male rats will form a METH-induced sensitized locomotor response, which will not be observed in adolescent females. Adolescent male and female rats received sub-chronic intravenous (IV) injections of either saline or low-dose NIC (2*0.03 mg/kg/0.1 ml) for four consecutive days (postnatal day 28-32), followed by three days of METH (2*0.02 mg/kg/0.1 ml) or saline treatment, with locomotor activity measured in both a novel and habituated environment. After a withdrawal day, adolescent rats were injected with the same dose of METH or saline, and their locomotor activity was assessed. Our findings suggest adolescent NIC pretreatment enhances METH-induced locomotor sensitization in males but not females, with adolescent females showing reduced locomotor response to their saline-treated counterparts. Future studies aim to explore the findings in adult rats to further understand the age- and sex-dependent differences. This study highlights the importance of investigating drug co-exposure during adolescence in both sexes and provides implications on how adolescent NIC pretreatment could enhance the behavioral consequences of intravenous METH exposure.

Disclosures: Y. Hajy Heydary: None. **S. Lotfipour:** None.

Poster

PSTR420

Nicotine-Physiological, Behavioral, and Cognitive Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.10/O20

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA044242

Title: Fluorocitrate inhibition of astrocytes reduces nicotine self-administration and alters extracellular levels of glutamate and dopamine within the nucleus accumbens in Wistar rats

Authors: X. TAN, E. NESLUND, K. FENTIS, ***Z. DING**;
Penn State Col. of Med., Hershey, PA

Abstract: Emerging evidence suggests an important role of astrocytes in mediating effects of commonly misused drugs. Passive exposure to nicotine alters molecular, morphological, and functional properties of astrocytes. However, a potential involvement of astrocytes in nicotine reinforcement remains largely unexplored. The overall hypothesis tested in the current study is that astrocytes play a critical role in nicotine reinforcement. Protein levels of the astrocyte marker glial fibrillary acidic protein (GFAP) were examined in key mesocorticolimbic regions following chronic nicotine self-administration. Fluorocitrate, a metabolic inhibitor of astrocytes, was tested for its effects on behaviors related to nicotine reinforcement and relapse. Effects of fluorocitrate on extracellular neurotransmitter levels, including glutamate, GABA, and dopamine, were determined with microdialysis. Chronic nicotine self-administration increased GFAP expression in the nucleus accumbens core (NACcr), but not other key mesocorticolimbic regions, compared to saline self-administration. Both intra-ventricuclar and intra-NACcr microinjection of fluorocitrate decreased nicotine self-administration. Intra-NACcr fluorocitrate microinjection also inhibited cue-induced reinstatement of nicotine seeking. Local perfusion of fluorocitrate decreased extracellular glutamate levels, elevated extracellular dopamine levels, but did not alter extracellular GABA levels in the NACcr. Fluorocitrate did not alter basal locomotor activity. These results indicate that nicotine reinforcement alters molecular properties of NACcr astrocytes, metabolic inhibition of astrocytes attenuates nicotine reinforcement and relapse, and metabolic inhibition of astrocytes disrupts extracellular dopamine and glutamate transmission. Overall, these findings suggest that astrocytes play an important role in nicotine reinforcement and relapse, potentially through regulation of extracellular glutamate and dopamine neurotransmission.

Disclosures: X. Tan: None. **E. Neslund:** None. **K. Fentis:** None. **Z. Ding:** None.

Poster

PSTR420

Nicotine-Physiological, Behavioral, and Cognitive Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.11/O21

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA046411

Title: Sex differences in nicotine self-administration, somatic withdrawal signs, and nicotine seeking in adult Wistar rats

Authors: *A. BRUIJNZEEL, A. BEHNOOD-ROD, R. CHELLIAN;
Univ. of Florida, Gainesville, FL

Abstract: Tobacco use is highly addictive and the leading cause of premature mortality in the world. Long-access nicotine self-administration procedures in rats closely model human smoking and vaping. However, significant gaps remain in our understanding of sex differences in the self-administration of nicotine, the development of dependence, and relapse in adult rats. In the present study, we investigated operant responding for both nicotine and saline and the development of dependence in adult rats of both sexes. The rats had daily access to nicotine or saline for 6 hours per day, 7 days per week. Dependence was assessed by evaluating precipitated and spontaneous somatic withdrawal signs, measuring locomotor activity in the small open field test, and assessing anxiety-like behavior in the large open field and elevated plus maze test. The sucrose preference test was used to determine if cessation of nicotine intake leads to anhedonia. The study also investigated the effect of three weeks of forced abstinence on nicotine-seeking behavior. This study showed that nicotine intake is higher in females than males when given daily long access to nicotine. Although the females had a higher level of nicotine intake than the males, only the males displayed spontaneous and precipitated somatic withdrawal signs. While cessation of nicotine intake increased activity in both sexes in the small open field test, it did not increase anxiety-like behavior or cause anhedonia in either sex. Three weeks of forced abstinence increased both nicotine and saline-seeking behavior. The rats exhibited more nicotine than saline seeking, and the females displayed more nicotine seeking than the males. The present findings demonstrate that females self-administer more nicotine and display more nicotine-seeking behavior than males. In contrast, males display more somatic withdrawal signs than females. This work underscores the importance of considering sex differences across various aspects of addiction, including intake, withdrawal, and relapse, when developing novel treatments for tobacco and vaping addiction.

Disclosures: A. Bruijnzeel: None. **A. Behnood-Rod:** None. **R. Chellian:** None.

Poster

PSTR420

Nicotine-Physiological, Behavioral, and Cognitive Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.12/O22

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 5T34GM136481
NIH Grant T32GM146604

Title: Chronic nicotine increases NACHO in the ventral tegmental area

Authors: *S. POWELL[1], L.-B. SHINN[2], K. A. MARQUEZ[3], J. VIDES[4], Y. SHERAFAT[2]; [1]California State Univ. San Marcos, San Diego, CA; [2]California State Univ., San Marcos, San Marcos, CA; [3]Psychology, California State Univ. San Marcos, San Marcos, CA; [4]California State Univ. San Marcos San Marcos, CA ? Publ. ? 4-year, San Marcos, CA

Abstract: Nicotine addiction has been recognized as the leading cause of premature death in the United States, and current therapeutics are only modestly efficacious. Nicotine is rewarding because it activates the nicotinic receptors in the nucleus accumbens and ventral tegmental area, brain regions involved in natural rewards such as eating, drinking, and sleeping. Although we know how nicotine activates the nicotinic receptors, we do not know the full scope of the molecular mechanisms involved in this process. Nicotinic acetylcholine receptor chaperone (NACHO) is a chaperone protein that increases the function and expression of nicotinic receptors. Understanding how NACHO expression changes with the use of chronic nicotine allows us to understand the molecular mechanisms that are involved in nicotine use. Thus, the goal of our study was to examine NACHO in nicotine reinforcement pathways after chronic nicotine exposure. Our data found that chronic nicotine increases NACHO expression in the ventral tegmental area, which suggests that NACHO may play a role in nicotine reward. These findings may provide the basis for developing more efficacious treatments for nicotine addiction.

Disclosures: S. Powell: 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.; URISE NIH: 5T34GM136481. **L. Shinn:** None. **K.A. Marquez:** None. **J. Vides:** None. **Y. Sherafat:** None.

Poster

PSTR420

Nicotine-Physiological, Behavioral, and Cognitive Effects

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.13/O23

Topic: G.09. Drugs of Abuse and Addiction

Support: American Heart Association

Title: Insights from behavioral models into the autonomic modulation of nicotine's effects

Authors: *N. KUSI-BOADUM[1], R. A. SHETTY[2], N. SUMIEN[3], M. J. FORSTER[1]; [1]Pharmacol. and Neurosci., Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX; [2]Dept. of Pharmacol. and Neurosci., Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX; [3]Pharmacol. & Neurosci., Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX

Abstract: Nicotine, the primary psychostimulant in tobacco, drives high abuse liability predominantly by activating α4β2 nicotinic receptor subtype in the reward pathway. Despite treatment targeted for this subtype, smoking cessation failure rate is >90% and smoking remains

the leading cause of preventable death. The α3β4 subtype, being more abundant in the autonomic nervous system, has not been adequately explored as a potential target. Some studies have shown that α3β4 antagonists dose-dependently block nicotine self-administration. To determine whether this effect is mediated autonomical or central, we assessed nicotine monomethiodide (NicM), a brain-impermeable nicotinic receptor agonist, in the locomotor activity and drug discrimination models, in comparison with brain-permeable nicotine ditartrate (NicT). Swiss-webster mice were tested for locomotor activity in a standard apparatus to compare behaviorally active doses of NicT with equivalent doses of NicM over six hours. Discriminative stimulus (DS) of NicM was assessed in male Sprague-Dawley rats trained to discriminate NicT (0.1 mg/kg, 5-minute pretreatment) from saline. Finally, to assess the ability of NicM to potentiate the DS of NicT, a combination of submaximal doses of NicT and increasing doses of NicM were tested in the discrimination model. Percentage of drug lever responses and response rate were recorded and analyzed using repeated measures ANOVA. In the locomotor activity tests, NicT elicited a biphasic effect, producing an initial depressant effect within the first 30 minutes, and a later stimulant effect between 50 and 70 minutes at doses 1 and 3 mg/kg. NicM, although peripherally restricted, produced a stimulant effect between 30 and 50 minutes following 0.3 mg/kg. In the drug discrimination assay, NicM's stimulus (0.01 mg/kg- 1 mg/kg) failed to substitute for NicT's. The additive study showed high variability of responses (both potentiation and attenuation of DS) at varying dose combinations. Although NicM did not substitute for NicT's discriminative stimulus, its ability to reproduce the stimulant effect suggests that there is an autonomic component to the overall emotional state created by tobacco product use. Autonomic studies such as radiotelemetry and differential temperature measurements will be required to confirm the impact of nicotine's autonomic effects on the neurobiology of addiction.

Disclosures: N. Kusi-Boadum: None. **R.A. Shetty:** None. **N. Sumien:** None. **M.J. Forster:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.01/O24

Topic: H.01. Attention

Support: NICHD R01 HD108325-01A1

Title: The role of reward and familiarity in the self-face bias: insights from behavioral and neuroimaging measures

Authors: *T. M. MARCUS[1], A. WOOD[2], B. HUNTER[3], J. MARKANT[2];
[1]Tulane Brain Inst., [2]Psychology, Tulane Univ., New Orleans, LA; [3]UC Davis, Woodland, CA

Abstract: One's own face captures attention even when it is neither perceptually salient nor goal relevant, suggesting that this bias may be driven by factors beyond top-down and bottom-up attention. The self-face is rewarding, and motivationally salient stimuli such as social cues can drive selective attention biases (Anderson, 2016). However, the self-face is also highly familiar, and it is unclear whether the self-face bias reflects greater reward value or mere familiarity. Across two studies we used behavioral testing and neuroimaging to tease apart the roles of reward and familiarity in the self-face bias. In Study 1 we examined automatic attention capture to faces in a visual search task (preliminary N = 31; age= 20.05 +/- 2.42 years; 8 M, 21 F, 2 Other). Participants searched for a target in arrays of varying set sizes (3, 6 or 9 items). In some trials, the self-face, a familiar, or unfamiliar stranger face appeared as a distractor. Initial results indicated that target detection was more impaired in medium difficulty set sizes when the self-face appeared as a distractor compared to both familiar and stranger faces ($t(30)=-3.156$, p=.004; $t(30)=-3.468$, p=.002). Therefore, in some contexts the self-face may capture attention more than other familiar faces, suggesting that this bias may be driven by factors beyond mere familiarity. In Study 2 we used functional near-infrared spectroscopy (fNIRS) to examine if the magnitude of the self-face bias related to cortical reward activation in a subset of Study 1 participants (preliminary N = 15; age= 22.42 +/- 4.98 years; 4 M, 10 F, 1 Other). Participants passively viewed images of the self-face and an unfamiliar stranger face while we recorded cortical activity. We also included a happy versus neutral contrast because affect enhances effects of identity (Minagawa-Kawai et al., 2008). There were no main effects of face type or affect on activation in the OFC, however the magnitude of the self-face bias observed during the visual search task related to cortical reward activation. Specifically, participants who were more biased by their own face in the attention task showed stronger activation in the right OFC when viewing neutral faces, regardless of the identity ($r(15)=.560$, p= .030). This suggests that participants with increased reward sensitivity to faces may be more distracted by the self-face. Data collection is ongoing and continued analyses will further examine these effects.

Disclosures: T.M. Marcus: None. **A. Wood:** None. **B. Hunter:** None. **J. Markant:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.02/O25

Topic: H.01. Attention

Support: NIH grant MH117991
NSF grant BCS2318984

Title: From Preparatory Attention to Stimulus Selection: Neural Mechanisms Revealed by Multivariate Analysis of fMRI Data

Authors: *Q. YANG[1], S. MEYYAPPAN[2], M. DING[1], G. R. MANGUN[2];
[1]Univ. of Florida, Gainesville, FL; [2]Univ. of California, Davis, Davis, CA

Abstract: Preparatory attention is often studied using the cueing paradigm. According to the prevailing theory, following an attention-directing cue, top-down signals from the frontoparietal attention control regions propagate to visual cortex to bias sensory neurons to enable stimulus selection. Despite years of research, the underlying neural mechanisms remain to be better elucidated. We recorded fMRI data from participants performing a cued visual spatial attention task. At the beginning of each trial, participants were asked to covertly deploy attention to one of the two visual fields. Following a random cue-target period, a stimulus appeared either at the attended location or at the unattended location, and participants discriminated the stimulus appearing at the attended location and ignored the stimulus appearing at the unattended location. Applying MVPA to fMRI data, we reported the following findings: (1) attend-left vs attend-right can be decoded from the cue-evoked neural activity in all visual areas, (2) stimulus-left vs stimulus-right can be decoded from the target-evoked activity in all visual areas, (3) classifiers built on the cue-evoked data can decode stimulus-evoked activity in all visual areas and vice versa, and (4) the higher the cross-decoding accuracy, the better the behavioral performance. These results suggest that top-down control signals form neural patterns in the cue-target period that resemble the neural patterns evoked by the stimulus and these “attentional templates” enable stimulus selection and improve behavior.

Disclosures: Q. Yang: None. **S. Meyyappan:** None. **M. Ding:** None. **G.R. Mangun:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.03/O26

Topic: H.01. Attention

Support: Honda R&D Co., Ltd.

Title: Neural and eye-movement correlates of safe car driving: An fMRI study using an MR-compatible driving simulator

Authors: *M. CHO[1], S. MURAKAMI[1], D. MATSUYOSHI[2], Y. YOMOGIDA[2], R. KANAI[2], Y. AIZAWA[3], M. YAMADA[3];
[1]HONDA R&D CO., LTD., Saitama, Japan; [2]ARAYA Inc., Tokyo, Japan; [3]Natl. Inst. for Quantum Sci. and Technol., Chiba, Japan

Abstract: Honda has set a goal to achieve zero fatalities in traffic accidents by 2050. To realize this goal, it is necessary to elucidate and eradicate the causes of human errors by drivers. Previous studies have suggested that eye movements during driving reflect human error and are

related to driving safety. However, little is known about the cognitive and neural processes underlying drivers' visual behavior. To investigate this, we recorded fMRI and eye-tracking data while healthy adults (22-78 years old) operated a driving simulator. The driving simulator was constructed inside an MRI scanner and composed of a steering wheel with winker buttons, an accelerator pedal, and a brake pedal. Participants drove in target or other trials. In target trials, a road with risks, such as a car in the adjacent lane passing the participants' driving car, was presented, and participants were required to change lanes to the right safely. At the end of a trial, a traffic light in front of an intersection turned red, and participants had to put on a brake pedal to stop. One trial took about thirty seconds. In the other trials, participants drove on the road with a lane change to the left or without lane change or drove straightly on a road without any other traffic participants. We focused on the data of target trials and analyzed the relationship between the visual behavior to a risky car and the maximum deceleration, which becomes greater when drivers suddenly put on the brake pedal at the traffic light. The behavioral data showed that the number of fixations was negatively correlated, and the duration from the last fixation to the time when the risk was eliminated was positively correlated to the maximum deceleration. Next, we investigated what neural processes underlie these visual behaviors. The fMRI data showed that the duration from the last fixation to the time when the risk was eliminated modulated activations in the angular gyrus and supramarginal gyrus at the last fixation. In previous studies, it is known that the angular gyrus is related to theory of mind, and the supramarginal gyrus is related to integration of semantic and technical information. These results suggest that drivers who operate the brake pedal gently keep paying attention to other traffic participants until risks are eliminated to guess the mental state of other drivers, and then decide their own behavior. Based on this insight, it is possible that encouraging drivers to estimate the mental state of others can lead to more gentle driving operations.

Disclosures: M. Cho: A. Employment/Salary (full or part-time):; Honda R&D Co., Ltd. **S. Murakami:** A. Employment/Salary (full or part-time):; Honda R&D Co., Ltd. **D. Matsuyoshi:** A. Employment/Salary (full or part-time):; ARAYA Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ARAYA Inc.. F. Consulting Fees (e.g., advisory boards); Honda R&D Co., Ltd. **Y. Yomogida:** A. Employment/Salary (full or part-time):; ARAYA Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ARAYA Inc.. F. Consulting Fees (e.g., advisory boards); Honda R&D Co., Ltd. **R. Kanai:** A. Employment/Salary (full or part-time):; ARAYA Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ARAYA Inc.. F. Consulting Fees (e.g., advisory boards); Honda R&D Co., Ltd.. **Y. Aizawa:** None. **M. Yamada:** 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.; Honda R&D Co., Ltd..

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.04/O27

Topic: H.01. Attention

Support: OU Faculty Investment Program

Title: Utilization of Eye Blink Rate as a Proxy for Attentional Engagement in Emotion Processing

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Abstract: Previous research supports longer durations of eye blinks as an indicator of attention disengagement, following the concept that longer eye blinks reduce visual information gathering, whereas increased blink frequency may be associated with heightened emotional states via increased sympathetic tone. The aim of the study was to observe the relationship between blink dynamics (duration and frequency), the presence of negative emotional facial expression stimuli, and behavioral emotion regulation in children, to determine whether attentional engagement as indexed by eye blink predicted emotional behaviors and parent-child emotional regulation. Eye-tracking data was recorded during an emotional facial expression identification task in child and caregiver dyads (n=30 dyads). Blink rate and duration were quantified as biologically plausible data loss from both eyes during the task. Preliminary results from our analyses revealed a positive relationship between the number of times per trial children fixated on negative emotional stimuli and increased blink rate to negative emotional stimuli for their caregiver, sad (rho=.543, p=.001), fear (rho=.303, p=.047), angry (rho=.456, p=.032) which may indicate co-regulation in parent-child dyads toward increased processing of emotional states. Blink dynamics were not, however correlated with externalizing or internalizing emotional behaviors in the children. Additional analyses will examine quantification of overt attentional disengagement via looking away from the stimulus and its relationship to blink dynamics and behavior.

Disclosures: R. Rajala: None. **J.E. Norris:** None. **L. Ethridge:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

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National Research Foundation of Korea (2022H1D3A2A01092818)

Title: Compact and Explainable Decoding of Auditory Selective Attention in Normal Hearing and Cochlear Implant Listeners

Authors: *J. HAM[1], J. KIM[2], H. SHIM[3], K. LEE[4], I. CHOI[1,4];
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Abstract: Difficulty hearing in noise has been consistently reported by listeners with and without hearing loss, suggesting that peripheral amplification is not sufficient to mitigate this problem. Auditory selective attention (ASA) is thought to play a key role in speech-in-noise perception by enhancing the cortical representation of target speech over noise. Our group has previously developed a perceptual training protocol that provides feedback based on the strength of attentional modulation of cortical auditory evoked potentials, which improved speech perception in noise in normal hearing (NH) listeners. To improve and extend such neurofeedback training to a larger population, including cochlear implant (CI) listeners, we aimed to develop a new algorithm that can decode ASA from single-trial EEG. The attention decoder needed to be 1) adaptable to different speech stimuli and subjects, 2) compact enough to run in real-time on stimuli of a few seconds duration, and 3) explainable to reveal the neural process behind the attention decoding. We tested the combination of a temporal response function (TRF) and a template-matching algorithm for this purpose. 28 NH and 37 CI listeners were instructed to attend to one of two simultaneous speech streams: a female saying "up" 5 times and a male saying "down" 4 times within 3 seconds. We recorded 64-channel EEG during the experiment. The TRF, a linear model between the attended speech envelope and the single-trial EEG, was computed. By convolving the "up" and "down" speech envelope with the TRF, we generated an EEG template; an idealized EEG assuming perfect attentional modulation. We used the correlation coefficients between the template and the single-trial EEG for 64 channels as a feature set for binary classification. Classification performance was evaluated using leave-one-subject-out cross-validation with logistic regression and support vector machine classifiers. Using the TRF, we were able to generate a stimulus and subject general EEG template. With the template, decoding of ASA with a 3-s single-trial EEG achieved 60% accuracy for NH and 58% accuracy for CI listeners. The generated templates were well aligned with the grand-averaged event-related potentials, indicating that the temporal signature of the neural response seems to be critical for ASA decoding. We can use this compact and explicable attention decoding for neurofeedback training of ASA in NH and CI listeners. Fine-tuning the algorithm will improve the participants' engagement for better training. The interpretable process of the decoding scheme will verify that the proposed training targets the neural circuit of ASA.

Disclosures: J. Ham: None. **J. Kim:** None. **H. Shim:** None. **K. Lee:** None. **I. Choi:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

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Topic: H.01. Attention

Support: STI 2030–Major Projects (2021ZD0200500)
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Title: Visual selective attention and auditory change detection in children and adults

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Abstract: Even when our visual attention is dedicated to an important task, auditory change detection is essential for survival to turn our attention toward significant events. Our recent research revealed a positive correlation at the population level between the amplitudes of event-related potential indices associated with visual selective attention (posterior contralateral N2, N2pc) and auditory change detection (mismatch negativity, MMN) in adults. However, how visual selective attention, auditory change detection, and their relationship develop over time are still unresolved. In this study, we recorded electroencephalography signals from 42 healthy young adults and 76 typically developing children (7-13 years old) during a visual localization task and an auditory-embedded fixation task, separately. We aimed to investigate the development of visual selective attention, auditory change detection, and their association using both univariate ERP analysis and multivariate pattern machine learning analysis. Compared with adults, a smaller N2pc and an “adult-like” comparable MMN were found in children through univariate ERP analysis. Similarly, multivariate pattern decoding revealed lower decoding accuracy for visual target location in children compared to adults, while there was no significant difference in decoding accuracy for auditory stimulus type between the two groups. Furthermore, these neural markers associated with visual selective attention and auditory change detection were significantly correlated with age in children but not in adults, indicating larger ERP amplitudes and higher decoding accuracy with increased age during childhood. Most importantly, our results not only confirmed the close relationship between visual N2pc and auditory MMN observed in adults, but also revealed a positive correlation between visual decoding accuracy for target location and auditory decoding accuracy for stimuli type in adults. However, these correlations were all absent in children. Taken together, our findings provide neurophysiological evidence for the developmental trajectories of visual selective attention and auditory change detection, and highlight that the close relationship between individual differences in the two processes emerges alongside their respective maturation, becoming evident until in adulthood.

Disclosures: Y. Kong: None. **X. Yuan:** None. **Y. Nan:** None. **J. Guo:** None. **Y. Song:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.07/O30

Topic: H.01. Attention

Support: R01EY032071

Title: Limits in attentional enhancement of multiple features revealed by EEG decoding

Authors: *M. MENCELOGLU[1], S. SADIYA[2], S. M. RAVIZZA[1], T. LIU[1];
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Abstract: Attention to a feature enhances the sensory representation of that feature. Recent behavioral studies have shown that the effectiveness of attention diminishes when multiple features, instead of a single feature, are attended. Here, we studied the neural correlates of this limit and tested the effectiveness of attentional enhancement of one vs. two visual features using color. We recorded EEG while observers (N=28) completed a color-coherence detection task. On every trial, we presented a circular field of 240 dots drawn in five isoluminant colors. On target-present trials, a single color was overrepresented at threshold, while on target-absent trials, all five colors were equally represented. Observers were asked to indicate whether a target was absent or present on every trial. Before the onset of the dot field, we presented valid color cues, either one- or two-color cues about the target color. We found that observers were faster and more accurate on the one-cue than two-cue trials, indicating that observers could more effectively attend a single color at a time. Further, we were able to decode the target color on target-present trials using the EEG signals measured from the posterior electrodes. Notably, we found that decoding accuracy was greater on the one-cue than two-cue trials, indicating a relatively stronger color signal on the one-cue trials likely due to stronger attentional enhancement. Lastly, we observed a positive correlation between the improvement in the decoding accuracy and the behavioral accuracy from two-cue to one-cue trials, suggesting that the decoded neural signals functionally relate to behavior. These results provide behavioral and neural evidence pointing to a severe limit in attentional enhancement of multiple features. Overall, our study provides new perspectives on the debate about the number of attentional templates that can be deployed effectively.

Disclosures: M. Menceloglu: None. **S. sadiya:** None. **S.M. Ravizza:** None. **T. Liu:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

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Program #/Poster #: PSTR421.08/Web Only

Topic: H.01. Attention

Support: UNAM DGAPA PAPIITIG300121
CONAHCYT960289

Title: Event-related potentials during visual and verbal binding in working memory

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Abstract: Binding is the cognitive mechanism through which different objects are integrated and represented together in memory. In working memory, this representation has the quality of being retained for a brief period of time. The multicomponent model of working memory proposes that two components of the model, the visuospatial sketchpad and the phonological loop, are responsible for binding visual and verbal information, respectively. The aim of the study was to identify the event-related potential components that distinguish the binding between visual items from those between verbal items. Visual stimuli were abstract forms, and verbal items were triads of letters; both types of stimuli were not related to any known object to avoid the influence of long-term memory. ERPs were recorded in thirty adults while they were performing the working memory task. The task consisted of presenting two pairs of visual or verbal stimuli, followed by a single pair. Participants indicated whether the single pair was equal to one of the previous two pairs, even if the stimulus position was changed, or was not equal to any of them. The P100 and P300 showed greater amplitude during successful visual binding encoding, whereas verbal binding was characterized by greater P200 amplitude. During retrieval, a positive slow wave showed greater amplitude at frontal left derivations for visual binding and enhanced amplitude for verbal binding at frontal right sites. Processes such as attention, stimulus categorization, and response selection, indexed by the event-related potential components that were sensitive, underlie the binding of different stimulus modalities.DGAPA IG300204

Disclosures: J. Marcué-Arana: None. **S. Cansino:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

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Topic: H.01. Attention

Support: F32 EY025533
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R01 EY019691
T32 EY007136

Title: Voluntary temporal attention modulates shared fluctuations of stimulus information across cortical networks

Authors: *J. ZHU[1], M. CARRASCO[2], R. N. DENISON[1];
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Abstract: Motivation: Directing attention to a target at a specific moment in time improves perceptual performance. Although stimulus information must flow from the visual cortex to higher cortical areas to facilitate behavior, it remains unclear whether and how temporal attention modulates the communication of stimulus information across cortical networks. Here we measured "dynamic informational connectivity", or shared fluctuations in stimulus information across cortical areas, to investigate how voluntary temporal attention modulates stimulus-specific network dynamics during rapid sequential stimulus presentation. Methods: We recorded MEG in human observers performing a two-target temporal cueing task. On each trial, observers judged the tilt of one of two sequential grating targets (T1 and T2) independently oriented around the vertical or horizontal axes. Temporal attention was directed to one target on each trial via a 75% valid precue, and a response cue indicated which target to report. We tracked the flow of shared stimulus information across the cortex by constructing a dynamic informational connectivity network using source-reconstructed data. We measured the correlations of stimulus orientation decoding accuracy across all pairs of regions in sliding time windows and estimated a region's closeness centrality as its average inverse correlation distance to all other regions. Higher closeness centrality indicates the region has more informational connectivity with the rest of the network. We compared spatial patterns of closeness centrality using Kullback-Leibler divergence. Results: We found both early and late modulations of closeness centrality for the informational connectivity network that depended on temporal attention. First, temporal attention changed the spatial distribution of closeness centrality 110-180 ms after T1 onset for T1, and 85-160 ms after T2 onset for T2. Second, temporal attention increased closeness centrality for regions in the left occipital lobe 650-725 ms after T1 onset for T1, and in the left parietal lobe 680-830 ms after T2 onset for T2, before the response cue. Attention changed connectivity and decoding accuracy at different times, suggesting that connectivity enhancements were not merely due to enhanced stimulus information. Conclusion: Voluntary temporal attention modulates informational connectivity, changing how stimulus information is shared across cortex, at early and late times after target onset for both targets. This finding reveals how temporal attention dynamically mediates information flow through cortical networks during perception of sequential stimuli.

Disclosures: J. Zhu: None. **M. Carrasco:** None. **R.N. Denison:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.10/O32

Topic: H.01. Attention

Title: An eye-tracking-based Stroop test as a rapid screening tool for attention and frontal lobe impairment

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Abstract: Background People with dementia often show attention deficit and frontal lobe impairment. The assessment of these cognitive functions is clinically essential for the early detection and differential diagnosis of dementia. The Stroop test has been used to assess attention and frontal lobe functions, and its validity and reliability have been profoundly verified. Nevertheless, test-related burdens, such as a long administration time and the complexity of the administration and scoring procedures, have been a bottleneck for its widespread use in clinical settings. **Objective** We aimed to develop a novel Stroop test using an eye-tracking technology as a screening test for attention and frontal lobe impairment and to demonstrate its validity and utility in a clinical setting. **Design** The study cohort included 97 individuals with a mean age of 76.4 (SD 7.1) who were recruited from the memory clinic at Osaka University Hospital. All participants completed both a traditional pen-and-paper Stroop test and an eye-tracking-based Stroop test on the same day. The Mini-Mental State Exam (MMSE), Frontal Assessment Battery (FAB), and Trail Making Test (TMT)-A/B were performed to assess participants' global cognitive and frontal lobe functions. The eye-tracking-based Stroop test was composed of four distinct color and word tests, including both congruent and incongruent conditions, and took approximately 3 minutes to complete. **Results** The mean MMSE score of the participants was 24.1 (SD 4.4). The scores on the eye-tracking-based Stroop test were significantly correlated with the scores of the pen-and-paper Stroop test ($r = 0.633$, $p < 0.01$). In addition, the eye-tracking-based Stroop test scores correlated with scores of the FAB ($r = 0.503$, $p < 0.01$), TMT-A ($r = 0.431$, $p < 0.01$) and TMT-B ($r = 0.492$, $p < 0.01$). To detect individuals with frontal lobe impairment (defined as an FAB score of <11), the eye-tracking-based Stroop test archived an AUC-ROC curve of 0.716 (95% CI 0.60-0.81). **Conclusion** The eye-tracking-based Stroop test enables the rapid, quantitative, and objective assessment of attention and frontal lobe function and may be used as a screening tool for dementia.

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Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

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Program #/Poster #: PSTR421.11/O33

Topic: H.01. Attention

Title: Dual Attentional Modes Emerge from Frustrated Synchronization in the Human Connectome

Authors: *R. BAPAT, A. PATHAK, A. BANERJEE;
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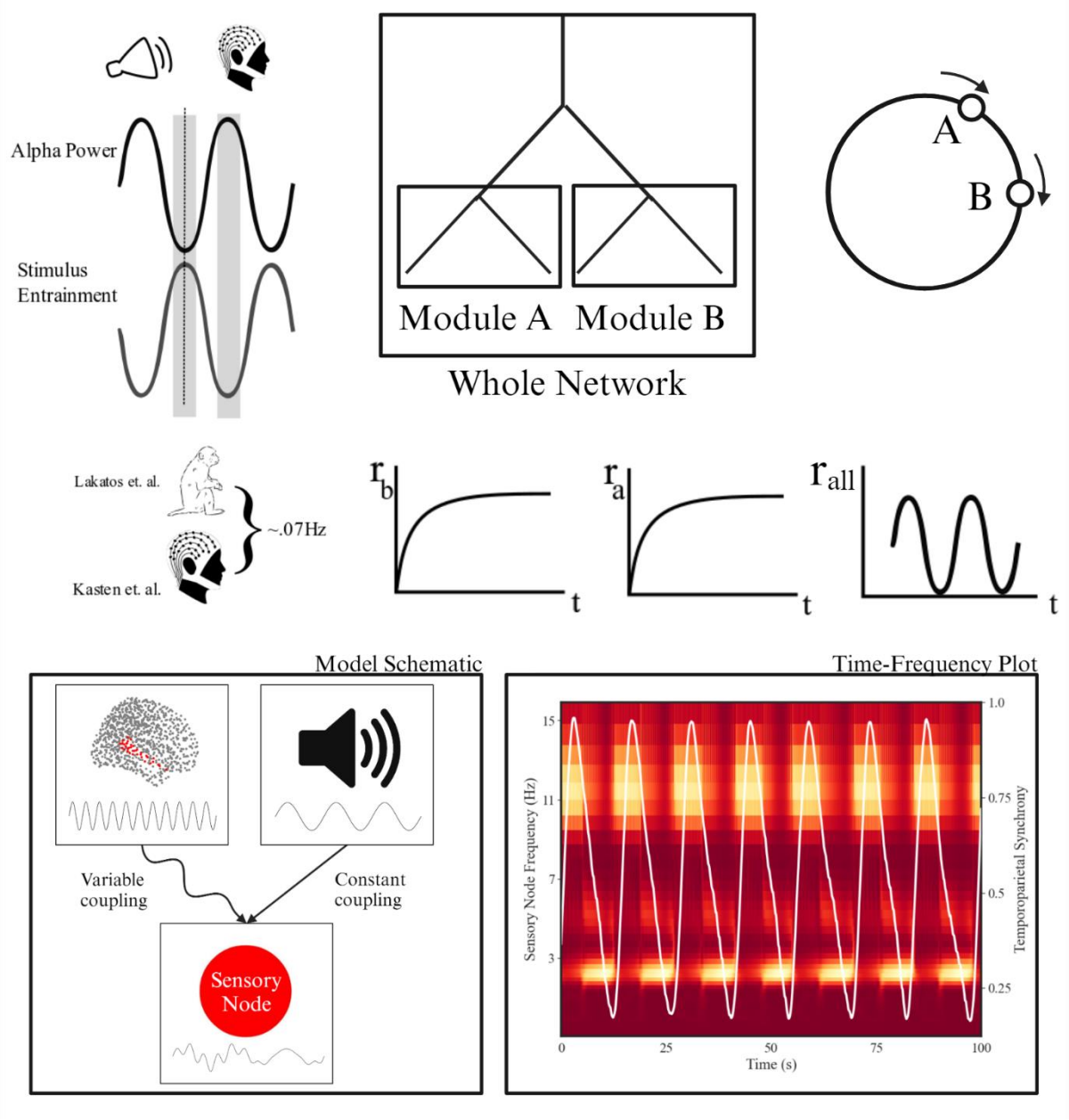
Abstract: Studies have shown that in tasks requiring sustained attention to a periodic stimulus, performance fluctuates periodically at frequencies around 0.05 Hz. These changes in performance are correlated with fluctuations in sensory entrainment and are anticorrelated with fluctuations in alpha power. Accordingly, researchers have posited that the brain alternates between dual attentional modes, one involving evidence accumulation and the other focusing on model updation for perceptual inference. From a dynamical systems lens, the brain is also known to balance integration and segregation for computational efficiency. We integrate these results and provide a dynamical mechanism for the emergence of dual attentional modes. Using a whole-brain model based on the human connectome, we show that a network of alpha oscillators can periodically synchronize and de-synchronize at frequencies matching those of attentional mode switching. We further demonstrate that these slow coherence oscillations (SCOs) can periodically suppress the entrainment of sensory cortices to external stimuli. Neuromodulatory gain is suggested as a possible mechanism to modulate SCOs as a function of task demands. Finally, through analytical arguments and numerical simulation we delineate the necessary conditions for SCOs in a system of coupled oscillators. The hierarchical and modular topology of brain organization emerges as a prerequisite that enables oscillatory coherence states by frustrating synchronization between sub-networks of the brain. These results comprehensively explain dual attentional modes by drawing parallels to dynamical systems neuroscience and provide fundamental insights into bimodal inference - the interaction between dual attentional modes and perceptual inference.



Disclosures: R. Bapat: A. Employment/Salary (full or part-time):; National Brain Research Centre. **A. Pathak:** A. Employment/Salary (full or part-time):; National Brain Research Centre. **A. Banerjee:** A. Employment/Salary (full or part-time):; National Brain Research Centre.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.12/O34

Topic: H.01. Attention

Title: Neural evidence of rhythmic attentional sampling during multi-location attention

Authors: *C. ZHUANG, F. FANG, Q. WANG, Y. SONG, X. GONG;
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Abstract: Neural evidence of rhythmic attentional sampling during multi-location attention
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Abstract: Recent research suggests a rhythmic sampling theory on attention. Instead of being stationary, attention samples items periodically. When attending to multiple stimuli, attention appears to engage in a sequential sampling process, alternating between stimuli in a rhythmic manner, typically at a theta rhythm when attending to two stimuli. However, direct neural evidence supporting this view is still lacking. In this study, we employed a machine learning technique (SVM, support vector machine) to decode attentional allocation process from MEG signals recorded when participants (N=16) simultaneously attended to two gratings appearing at the left and right side of the fixation. We defined neural templates for attentional states (attending to the left or the right) in a training task. Based on the templates established, we decoded the attentional allocation process in the multi-location attention task when participants attended to both locations simultaneously. Decoding results revealed attentional alternation between the left and right gratings in an anti-phase periodic relationship, occurring around 4 Hz. These findings provide solid evidence for the serial sampling hypothesis and are consistent with previous behavioral findings.
Keywords: attention; oscillation; rhythmic sampling; MEG; decoding

Disclosures: C. Zhuang: None. **F. Fang:** None. **Q. Wang:** None. **Y. Song:** None. **X. Gong:** None.

Poster

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Neural Mechanisms of Human Attention and Therapeutics

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Topic: H.01. Attention

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Title: Attention guides reward-based decision-making as measured by SSVEPs

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Abstract: Introduction: Visual attention or attention shift may infer and further modulate decisions. However, the attention-related decision mechanisms are not fully understood. In this study, frequency tagging is combined with EEG to investigate how attention, as measured by Steady-State Visual Evoked Potentials (SSVEPs), influences reward processing and guides subsequent behavioral adjustments. This approach allows us to extract attention-related components, both bottom-up and top-down, corresponding to each stimulus based on the flicker frequency, which is lacking in traditional ERP analysis, providing insights into the temporal dynamics of decision-making strategies. We measured attention states at two cards, which were options to choose for a gambling task. Methods: Thirteen healthy right-handed participants performed a two-choice gambling task with simultaneous EEG recordings. Stimuli were presented using Psychtoolbox. EEG data was recorded using a high-density BioSemi system at a sampling rate of 2048 Hz. Each trial started with a 2-second fixation period, followed by 2 seconds of flickering rectangles indicating the two gambling options. Subsequently, a 2-second decision phase was initiated by the color change of the fixation cross, and feedback with both cards was provided afterward and lasted for 2 seconds. We hypothesized that the SSVEPs before the decision-making phase could predict subsequent behavioral strategies that are also modulated by reward utility (e.g., gain or loss) and performance (e.g., correct or incorrect) related information. Results: We first investigated the impact of feedback on participants' decision strategies by analyzing the frequency of choosing the same card in the subsequent trial. The staying/switching frequency can index the behavioral strategies. We found that participants were more likely to stick with the same option following correct trials than incorrect trials (two-tailed paired *t*-test: $t(12) = 3.51$, $P < 0.01$, Cohen's $d = 0.97$). However, there was no significant difference between win and loss trials ($t(12) = 0.51$, $P = 0.6197$, $d = 0.14$). Neuronally, we found that SSVEP amplitude prior to the decision phase predicted subsequent choice within the same trial. Conclusions: These findings highlight the critical role of reward-related self-initiated attention in feedback-based decision-making, with SSVEP demonstrating potential as a predictive measure for subsequent choices. This research contributes to our understanding of how attention influences and can be used to predict adaptive behavior in response to feedback and rewards.

Disclosures: D. Hou: None. **S. Sun:** None. **S. Shioiri:** None.

Poster

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Title: 40 hz audio and visual stimulation improves attention and modulates neural activity outside of gamma range

Authors: *M. ATTOKAREN[1], L. ZHANG[2], S. METTUPALLI[3], S. LYU[3], A. C. SINGER[4]; [1]Georgia Inst. of Technol. & Emory Univ., Atlanta, GA; [2]Biomed. Engin., Natl. Inst. of Hlth., Rockville, MD; [4]Coulter Dept. of Biomed. Engin., [3]Georgia Inst. of Technol., Atlanta, GA

Abstract: Gamma oscillations (30-100Hz) have long been theorized to play a key role in sensory processing and attention by coordinating neural firing across distributed neurons. While gamma oscillations observed during attention are internally generated by neural circuits, gamma frequency activity also results from exogenous stimuli that turn on and off at gamma frequencies. However, it remains unknown if driving gamma activity via exogenous sensory stimulation affects attention. We tested the hypothesis that non-invasive sensory stimulation in the form of flashing lights and sounds at 40Hz improved attention and modulated neural oscillations during an attention task. We exposed 61 healthy young adults (aged 18-40) to sensory stimulation while performing an attention task and recording scalp EEGs. Participants were split into three stimulation groups, one that received 40 Hz visual and auditory flicker stimulation and two control groups. One control group received a random non-periodic flicker stimulation that had the same average on-time as the 40 Hz group to control for the effects of a flashing stimulus. The other control group was exposed to constant light that was measured to be the same brightness as the 40 Hz stimulation. Over 1 hour, subjects were exposed to their respective stimuli while performing a task to assess their attention. In this task, participants were asked to look at a dot on a computer screen and press a key whenever the dot briefly changed colors. We found that participants that were exposed to 1-hour of 40 Hz flicker stimulation on average had better accuracy and faster reaction times compared to participants in the control groups. Scalp EEG recordings of participants in the 40 Hz group showed increased 40 Hz activity compared to the control groups in agreement with previous studies. Surprisingly, our EEG data also showed that 40 Hz subjects had significantly greater power in specific neural frequency bands outside the gamma range compared to the control groups. We found a strong correlation between power outside the gamma range and accuracy on the attention task. These results provide insights into the cortical mechanisms of gamma stimulation and highlight the potential implications for therapeutic interventions for attention disorders and cognitive enhancement in healthy individuals.

Disclosures: M. Attokaren: A. Employment/Salary (full or part-time):; Georgia Institute of Technology. **L. Zhang:** A. Employment/Salary (full or part-time):; NIH. **S. Mettupalli:** None. **S. Lyu:** None. **A.C. Singer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cognito Therapeutics.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.15/O37

Topic: H.01. Attention

Support: NS114191

Title: Effects of distracter location and predictability on tactile distracter suppression

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Abstract: When searching for or manipulating objects with the hand, it is critical to allocate resources to the neural areas that process the information from the fingers that grasp the object. Selective attention plays a key role in this process by enhancing neural signals encoding relevant tactile information while discarding irrelevant information through a process called distracter suppression. Several studies, mainly in vision, have shown that suppression can be proactively deployed to reduce distracter interference using a statistical learning mechanism, meaning that distracters are more efficiently ignored when occurring at high-probability or repeated locations. However, the underlying properties of how active suppression is deployed in the tactile modality are relatively unknown. In particular, it is unclear whether tactile distracter suppression can be flexibly allocated to non-contiguous areas within the hand, and operates using an expectation-dependent mechanism. Through a series of human psychophysical studies, we test if suppression can be deployed to different parts of the hand simultaneously, and whether tactile suppression arises from the predictability of distracter location. Participants were cued to different parts of the hand using virtual reality (VR) where attended or distracter stimuli would be presented. Vibrotactile stimuli were of 200 Hz frequency and 1000 ms duration, but distracters were at half the intensity of the attended stimuli. Distracters were provided in flanking (located on both sides of the attended stimulus on the same axis) or non-flanking spatial arrangements. Participants reported whether they felt an increase or decrease in vibration intensity at the attended location only. The data showed that the presence of distracters impairs behavior and causes a shift in sensitivity. We also observed a decrease in performance and increased reaction time for flanking vs. non-flanking distracter arrangements. Furthermore, distracters occurring at fixed locations

cause a decrease in reaction time, suggesting that tactile suppression is more efficiently deployed when distracter location is anticipated. These findings further our understanding of how distracter suppression is spatially deployed in the somatosensory system, and the role that active suppression mechanisms play in the integration of information across the hand during object manipulation.

Disclosures: A. Ignaco: None. **N. Serino:** None. **A. Trzpis:** None. **M. Gomez-Ramirez:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.16/P1

Topic: H.01. Attention

Title: Quality sleep and attentional processes in medicine students

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Abstract: Sleep: period where there is a decrease in consciousness, with the ability to react to external stimuli, reversible. Phases of sleep: NON-REM Phase: (Non Rapid Eye Movements), state from wakefulness to sleep. Developed in Stage I, II, III and IV. Approx. time 1 hour. Maintenance of body energy, restoration of the nervous system. REM Phase (Rapid Eye Movements): Approx. time. 5-30 minutes. Regular brain activation, improved memory and emotional control. Sleep deprivation: decreases visual and executive attention capacity, speed and reaction capacity, working memory and visual memory, verbal fluency, executive functions, creative thinking, cognitive performance and motor function. Sleep quality level is assessed using the Pittsburgh Sleep Quality Index (PSQI). Attention: brain mechanism that allows us to process relevant stimuli, thoughts or actions and ignore irrelevant or distracting ones. Types of attention: Sustained, Selective and Divided Attention. Evaluations and tests for attention level: Trail Making Test (TMT), BTA (The Brief Test of Attention/ Test Breve de Attention).Identify the association between sleep quality and attentional processes, of Medical Students, from the first to third semester, of the Tepeaca Higher Studies Center. The study was carried out at the Center for Higher Studies of Tepeaca, Bachelor of Surgeon and Midwife in the groups from 1st to 3rd semester. The study was Observational, Prospective, Cross-sectional, Homodemic, Single-center, non-probabilistic sample type. With the authorization of the authorities of the institution, I can have contact with the students.The total number of participants was 256 students, with greater participation of women with 55.47% and men 44.53%. For the overall Sleep Quality

Index test score with good sleep 26.56% and bad sleep 73.44%. TMT exceeded 55.86%, not exceeded 44.14%. BTA Without Deficiency 6.64%, With Deficiency 93.36%.

Disclosures: I. Pliego-pliego: None. **J. Sanchez-Flores:** None. **A.V. Flores Pliego:** None. **D. Tizo-Daniel:** None. **A. Osorio-Espinoza:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.17/P2

Topic: H.01. Attention

Support: Netherlands Organization for Scientific Research (grant No. VICI 181.032)

Title: Strength of low-frequency EEG phase entrainment to external stimuli is associated with fluctuations in the brain's internal state

Authors: *V. EXPOSITO[1], A. FELEA[2], S. NIEUWENHUIS[3];
[1]Oslo Univ. Hosp., Oslo, Norway; [2]Leiden Univ., Leiden, Netherlands; [3]Leiden University, The Netherlands, Leiden, Netherlands

Abstract: Rhythmical sensory stimulation triggers synchronized oscillatory rhythms throughout the brain. The coupling of brain activity with environmental rhythms, known as phase entrainment, facilitates sampling the external world and tunes the brain to incoming information. This mechanism is found across modalities, including auditory, tactile and visual, and may be a key aspect in how the brain perceives and processes relevant information. When faced with two oscillatory stimuli of different modalities, phase entrainment develops at the frequency of the modality that is attended. However, the strength of entrainment fluctuates, and the factors underlying these fluctuations remain largely unknown. In the present study we examined if the strength of low-frequency EEG phase entrainment to rhythmic stimulation varied with pupil size and alpha-band power. These signatures are thought to reflect arousal level and excitability of posterior cortical brain areas, respectively. We measured entrainment to the attended and the unattended modality during simultaneous visual and auditory rhythmic stimulation using EEG. Concurrently, we measured pupil size, a signature of arousal level. Our results show specific relations between pupil size, alpha power and phase entrainment, and provide insight into the endogenous sources that contribute to the fluctuations of EEG phase entrainment.

Disclosures: V. Exposito: None. **A. Felea:** A. Employment/Salary (full or part-time):; Brain Products. **S. Nieuwenhuis:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.18/P3

Topic: H.01. Attention

Support: Partial support by Brainwave Science, Inc.

Title: Understanding Longitudinal Effects of Mantra Meditation and Breath-focused Meditation using EEG

Authors: *A. LI[1], P. PRADHAN[1], K. IKA[2], M. ZHANG[1], B. H. COHEN[3], S. RAVISHANKAR[1,4];
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Abstract: Previous research on meditation has demonstrated its potential to enhance attentional focus. However, previous studies largely focused on comparing expert meditators to novices. What remains unknown is how attentional focus evolves over meditation practice and what underlying neural dynamic mechanisms support that. Hence, we conducted a pilot longitudinal study to examine the effects of specific meditation methods on attention and brain dynamics during a few months of regular practice. Considering the many meditation techniques available, including mantra-based meditation and breath-focused meditation, our objective is to compare these methods to understand their long-term cognitive and neural dynamic effects.In this pilot study, 12 participants were randomly assigned to 3 groups. Two groups practiced Hare Krishna and SATANAMA mantra meditation (focusing on specific sounds or words), while the third group did breath-focused meditation. These participants, all novices to meditation, received initial training. Over two months of regular meditation practice, we collected EEG data at baseline, one month, and two months after the start of practice. Utilizing the P300 speller test (a widely used measurement to reflect attention), we compared the effects of meditation on attention across time points. Data were gathered using iCognative technology from Brainwave Science, Inc. Participants' stress levels and mindfulness were assessed through Perceived Stress Scale and Five Facet Mindfulness questionnaires before and after meditation practice. Additionally, a 64-channel EEG device was used to collect data from one participant in each group during before and after 2 months of practice. The empirical results showed a numeric decrease in mean latency (effect-size r = 0.224 using means and std.)—an evaluation index for the P300 test—across the groups compared to their baseline measurements before starting meditation. Among the groups, the Hare Krishna group exhibited the greatest numeric reduction in latency. Moreover, comparing the two mantra groups, the reduction was significant for Hare Krishna (p value < 0.05). Questionnaire assessments indicated a numeric decrease in stress levels after two months of meditation. For the 64-channel EEG data recorded during meditation, the power in the alpha band became more sustained after 2 months of practice for both types of

mantra meditation.To conclude, our preliminary results suggest that regular meditation practice may influence P300 latencies and stress levels, underscoring the feasibility of further study. Future research will consider larger sample sizes, and longer duration of meditation practice.

Disclosures: A. Li: None. **P. Pradhan:** None. **K. Ika:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Research funded by Brainwave Science, Inc., whose EEG headset and ERP software were used within the study. **M. Zhang:** None. **B.H. Cohen:** None. **S. Ravishankar:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.19/Web Only

Topic: H.01. Attention

Title: The impact of stress on brain load in combatants: EEG analysis

Authors: O. KORBUSH[1], N. KRUSHYNSKA[2], I. KOHUT[2], *S. FEDORCHUK[2], S. TUKAIEV[3];
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Abstract: With the onset of full-scale invasion in Ukraine, the number of combatants experiencing significant stress has greatly increased. This situation necessitates careful analysis of the psychological reactions of participants to identify and prevent potential health problems. One aspect requiring attention is the study of the impact of stress on the functional organization of the central nervous system. Against this backdrop, a study was conducted to assess the influence of stress on combatants through the analysis of electroencephalography (EEG) parameters, which holds importance for developing rehabilitation and stress management techniques. The research was conducted at the Scientific Research Center of the National University of Physical Education and Sport of Ukraine. Ten volunteer combatants aged 28.1±5.3 years participated in the study. A mobile EEG system, SMARTING, was used for recording brain electrical activity. To simulate stressful conditions, the "Landolt Rings" test from "BOS-Test-Professional" was employed with a time limit of 5 minutes. The brain load index was determined as the ratio of spectral power in the theta range at the frontal-central site (Fz) to spectral power in the alpha rhythm at the temporal-central site (Pz). Changes in this index indicate the level of stress and cognitive control. The research results revealed an average level of brain load, which decreased towards the end of the testing period. The highest level was observed at the 3rd minute of testing, followed by a subsequent decrease until the end of the test, indicating adaptation to the stressor during task undertaking. The findings demonstrate a

connection between psychophysiological indicators and task performance success, which may be crucial for developing rehabilitation and stress management methodologies.

Disclosures: O. Korbush: None. **N. Krushynska:** None. **I. Kohut:** None. **S. Fedorchuk:** None. **S. Tukaiev:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.20/P4

Topic: H.01. Attention

Title: Comparing subjective meditation experiences and EEG correlates in mantra meditation experts and novices as meditation duration increases

Authors: *C. SRIVASTAVA[1], R. GUPTA[2], J. O'REILLY[3];
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Abstract: Previous research using frequent and random experience sampling probes indicates that individuals trained in focused attention meditation show reduced distraction during meditation. However, frequent and random sampling methods may not adequately engage participants in meditation, and random durations make it challenging to compare subjects within specific timeframes. This study implements a sequentially increasing probe duration approach, which is ideal for assessing fluctuations in mind wandering and meditation depth over extended meditation sessions. We employ mantra meditation, a focused attention meditation practice, to explore how subjective meditation experiences and electroencephalography (EEG) correlates differ between mantra meditation experts and novices as the duration of meditation increases. Both experts and novices engaged in meditation sessions for a specified duration (15 min) divided into five blocks of increasing duration (1, 2, 3, 4, and 5 min) with probes at the end of each block to evaluate subjective reports on the average attentional state preceding each probe using a graded 5-level scale. Continuous EEG was concurrently recorded from 30 scalp electrodes. In mantra meditation, practitioners typically count mantras on rosary beads. To simulate the natural setting of mantra meditation while minimizing EEG artifacts from hand movements, participants gently pressed a key after each mantra instead of counting beads. We analyzed inter-keypress-interval variance to investigate correlations with mind wandering ratings. Experts consistently reported deeper meditation, reduced mind wandering, and less fatigue across meditation blocks compared to novices. Consistent with these findings, experts showed higher mean gamma band power compared to novices, suggesting enhanced attention and neural activation. Increased meditation depth and absorption were reported with each successive meditation block across all subjects. Additionally, we observed a weak correlation

between variability in button presses and reports of mind wandering, likely due to the lower sensitivity of button press variance over extended durations in reflecting mind wandering. In summary, as meditation session duration increased, both experts and novices reported similar levels of mind wandering intensity or depth across sequential meditation blocks. However, overall, experts consistently reported higher levels of absorption or calmness and lower levels of mind wandering during meditation compared to novices when averaged across meditation blocks, as reflected in the EEG gamma band power.

Disclosures: C. Srivastava: None. **R. Gupta:** None. **J. O'Reilly:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

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Program #/Poster #: PSTR421.21/P5

Topic: H.01. Attention

Support: JST Moonshot R&D JPMJMS2012

Title: The effect of publicly available non-invasive brain stimulation on attention functions in healthy adults: A systematic review

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Abstract: Noninvasive brain stimulation (NIBS), including transcranial direct current stimulation (tDCS) and transcranial static magnetic stimulation (tSMS), is becoming available to the general public, who are non-experts in brain science. Several studies imply that these techniques may improve attention function. However, the effects of those techniques on attention functions are controversial. Therefore, we conducted a systematic review of studies that used those techniques to investigate their effects on attention functions (preregistered in PROSPERO as CRD42023487035). We searched databases including Pubmed, Web of Science, Scopus, and PsycINFO, identifying 2634 relevant studies, while one study was identified through another source. We screened these studies based on the following criteria. Inclusion criteria: Parallel randomized controlled trials aiming to improve attention functions by using NIBS for healthy human adults. Exclusion criteria: Participants aged under 18 or over 65 and interventions by transcranial magnetic stimulation and transcranial ultrasound stimulation because these are not accessible to the general public. Control conditions included sham stimulation, attention training without NIBS, and no intervention. The screening was conducted independently by two reviewers, and studies with disagreements between them were discussed and re-screened with

the rest of the authors. In the first screening based on the title and abstract alone, 2177 studies were rejected and 458 met the criteria. In the second screening based on the full-text examination, 398 studies were rejected and 59 met our criteria. As a result, we found 53 tDCS studies, 2 transcranial alternating current stimulation (tACS), 2 transcranial random noise stimulation (tRNS), and 1 transcranial pulsed current stimulation (tPCS) study. One study dealt with both tDCS and tACS. No studies using tSMS met our criteria. There were insufficient studies using tACS, tRNS, tPCS, and tSMS to test the effects by meta-analyses. More rigorous research is needed to explore the effects of NIBS in detail.

Disclosures: T. Takahashi: None. **S. Vafaei:** None. **I. Kimura:** None. **J.I. Kawahara:** None. **T. Yanagisawa:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

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Program #/Poster #: PSTR421.22/Web Only

Topic: H.01. Attention

Support: MFIRP (IITD and IIITD) # 222
Centre For Design and New Media, IIITD
IIITD

Title: Gray matter volume correlates of the spatial distribution of visual attention in high trait anxiety

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Abstract: Flexible allocation of attentional resources to different points in space is crucial to meet the demands of a dynamically changing environment which we navigate in daily life. The inability to adaptively redistribute spatial attention may lead to constricted or tunnel vision, which may compromise sampling the span of the visual field towards optimizing visually guided behaviour. This is of interest in anxious individuals, known to have biases in visual processing. We used an affect-primed, visual-spatial behavioural attention task with structural magnetic resonance imaging (MRI) in a sample of healthy young adults with relatively high trait (dispositional) anxiety (n = 50, age = 22.8 ± 3.8, 18 females; trait anxiety >= 46 in 82% of the sample). Using objective measures from the behavioural task and MRI, we explored if a) fear and neutral affect from image primes differed relative to no affect (scrambled image prime) in modulating the distribution of visual spatial attention; b) there was an association between the

gray matter volume (GMV) of any particular region(s) of the whole brain and a measure of the spatial distribution of attention by individual valences of affect and overall. We calculated the spatial gradient of visual attention as a metric for tunnel vision using measures of attentional efficiency at spatial loci near the central fixation (1.5 degrees; close to the presentation of the image prime) minus far from it (6 degrees). Thus, greater positive gradient values would reflect better attentional efficiency at the near relative to farther spatial loci or higher degrees of tunnel vision. The average gradient when submitted to a multiple regression analysis, correlated negatively with the GMV of right cerebellum lobule VI, after controlling for the participants' total intracranial volume and age (height threshold $p = 0.001$, cluster extent threshold = 1250 voxels, cluster-level p corrected for Family-Wise Error = 0.003; MNI coordinates: x = 32, y = -57, z = -27). Further, the strength of this negative correlation (beta estimates) was significantly lesser in females than males (Mann Whitney U Test, z = - 4.09, $p < 0.001$). The interim results suggest that lesser the GMV of right cerebellar lobule VI, greater the individual severity of tunnel vision. The results point at the possible role of the right cerebellar lobule VI in tuning fine grained spatial attention, in the sampled cohort. Our results add to the emerging evidence of relatively less known roles of cerebellum in visual attention. The evidence enables better understanding of the brain-basis of spatial deployment of visual attention in humans and is also relevant for affective traits and disorders.

Disclosures: M. Chakrabarty: None. **S.R. Mir:** None. **V. Singh:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.23/P7

Topic: H.01. Attention

Title: Aging increases the attentional cost of proprioceptive processing in passive and active matching tasks in sedentary seniors

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Abstract: Ankle proprioception and attentional resources are crucial for postural stability and safe driving. Evidence suggests that ankle proprioceptive processing is more attentionally demanding in aging. However, factors that affects the recruitment of attentional resources for proprioceptive processing are underexplored. We examine how different proprioceptive-attentional conditions influence the proprioceptive and cognitive performances of seniors.

Sedentary old (n=10) and young (n=7) adults were tested in two dual-task paradigms involving either a passive or an active ipsilateral ankle proprioceptive-matching task with a long target encoding time (12 s). Both paradigms also involve a cognitive-attentional subtraction task (n-3). These tasks were assessed separately (single) and concurrently (dual). In the proprioceptive tasks, participants reproduced two target angles in dorsiflexion (20% and 30% of maximal range of motion) in absence of vision. A motion analysis system (Polhemus, *Innovation in Motion*^{TM}) combined with an automatized software allowed the presentation of target angles as well as the recording of the matching performance. Our preliminary findings revealed that old and young adults performed the cognitive-subtraction task at an optimal level in both attentional conditions. Conversely, seniors exhibited greater absolute and variable matching errors than younger adults in all proprioceptive-attentional conditions ($p<0.01$). Hence, in striking contrast to young adults, absolute errors of seniors substantially increased when concurrently performing the ankle-matching and cognitive-subtraction tasks in both the passive and active proprioceptive conditions ($p<0.01$). This observation suggests that the additional proprioceptive efferent motor signals involved in active matching do not reduce the contribution of attentional resources directed to proprioception in sedentary seniors. Interestingly, as opposed to young adults, absolute errors made by seniors were significantly smaller when matching the target located farther from the starting ankle position (30%). Hence, this effect was more pronounced when dual-tasking ($p<0.01$). Such finding might reflect the intervention of a compensatory mechanism operating to correct proprioceptive errors of seniors during the ongoing movement. Notably, this correction mechanism appears effective in reducing the amount of attentional resources needed for the proprioceptive monitoring of matching movements. Understanding the interplay between ankle proprioception and attentional resources in various contexts is crucial to prevent falls and driving accidents in seniors.

Disclosures: E. Paré: None. **M. Vermette:** None. **L. Holdrinet:** None. **A. Morin:** None. **B. Pageaux:** None. **F. Prince:** None. **J.Y. Messier:** None.

Poster

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Neural Mechanisms of Human Attention and Therapeutics

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Topic: H.01. Attention

Support: CONACYT-SEP-220973
SEP-UDG-CGA/CIP/0477/2014

Title: Attentional electrophysiological components and sleep habits in preschooled children

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Abstract: Attentional performance in school is critical for children's academic achievement. It has been reported that no getting enough sleep both in quantity and quality can directly impact attention performance. One of the main reasons for the sharp decrease in sleeping hours are the everyday night habits at home. The aim of this study is to determine if sleep habits in have an impact on the performance of any of the attentional components (alert, orientation and executive control) analyzed during the Attentional Network Task (ANT) changing the morphology of the associated event-related potentials (ERPs). The sample consists of 30 Mexican children aged 4 to 6 years old attending preschool whose parents answered the Children Sleep Habit Questionnaire (CSHQ) to evaluate their sleep habits. Attentional components are being assessed by a child-friendly version the ANT, the ANTI-birds. Experimental session included 144 trials divided into three blocks of 48, each beginning with a fixation point screen (1000-1600 ms), which was followed by a no warning/warning sound signal (200 ms), another fixation (350 ms), a cue/no cue screen (200 ms), an ISI (200 ms) and a target picture to respond to a congruous/incongruous image. ERPs N100, N200 and P300 are extracted from the electrical activity recorded while the children performed the task. It is expected that the children sleep habits will exert an influence on the attentional components and the ERPs corresponding to each type of processing.

Disclosures: E.E.M.A. Garcia: None. **F.A. Robles:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

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Program #/Poster #: PSTR421.25/P9

Topic: G.05. Mood Disorders

Title: Adhd mr image classification using radiomic and neural networks techniques

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Abstract: Introduction: Attention Deficit Hyperactivity Disorder (ADHD) is one of the most common neurodevelopmental disorders in childhood. This developmental disorder usually has associated comorbidities of the personality or cognitive impairment that will affect quality of life of patients when reaching adulthood. Given the consequences that a late diagnose and treatment can have in adulthood, it is necessary to be able to diagnose child populations in an effective way as soon as possible.**Aim:** The aim of this work was to build classifiers capable of distinguishing ADHD from Control patients using radiomic techniques applied on MRI images of the brain and

combining these with the use of neural networks (NN). **Methods:** Volunteer information was obtained from the *New York University Child Study Center* (NYU) database. MRI structural images were pre-processed for this study using *The Athena Pipeline for IRMe*, as indicated in (27) and performed with FSL applications (FSL - FslWiki (ox.ac.uk). A total of 107 radiomic features were extracted from each subject using the Pyradiomics library in Python. Two NN models were proposed for the classification of ADHD volunteers. One used only radiomic data as input and will be named CRF, the other used radiomic data plus two-dimensional sections of the MR images as inputs (CRF+VGG16). Once the NN models were built, their performance with different subsets of the data was assessed: *Unbalanced classes (NB), balanced classes (B),* S*emi-balanced classes (SB).* For all subsets, 70% of the data was taken for training and the remaining 30% for validation. **Results:** Only 8 radiomic features presented differences between classes, four of these features belonging to the 3D shape analysis approach. No large differences in accuracy results were found between the two NN studied. The best model was the CRF, that when applied to the SB subset, recorded an accuracy of 75% (0.7491 ± 0.0142). This value is similar to those reported in literature using the similar technical approaches. **Conclusions:** Accuracy results presented here are in line with those reported in the literature for radiomic applications. The combination of Radiomic features with MR images did not improve classifiers. Radiomic features related to shape and size of the brain, were the ones relevant for the classification task.

Disclosures: B. De Celis Alonso: None. **J. Osuna González:** None. **J. Suárez-García:** None. **S. Hidalgo-Tobon:** None. **J. Hernandez-Lopez:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.26/P10

Topic: F.07. Biological Rhythms and Sleep

Title: Peri-somatic Modulation of the Diffraction of Light by Conscious Brain Activity

Authors: *S. HELEKAR[1,2,3], B. JOHN[4], L. NGUYEN[4], S. HAMBARDE[5], A. PANDEY[5], A. SALDON[4];
[1]Psychiatry and Behavioral Hlth., Houston Methodist Res. Inst., Houston, TX; [2]Neuroscience, Weill Cornell Medicine, New York, NY; [3]Translational Biomagnetics and Neurometry Program, Houston Methodist Research Institute, Houston, TX; [4]Translational Biomagnetics and Neurometry Program, Houston Methodist Res. Inst., Houston, TX; [5]Neurosurg., Houston Methodist Res. Inst., Houston, TX

Abstract: Neurophysiological and neuroimaging methods have led to significant advances in understanding mechanisms of sensory processing, representation of the body and external

objects, interoception and proprioception, storage and recall of memories, voluntary action, and processing of emotions. These methods depend upon recording of neuronal electrical activity or changes in cerebral blood flow or metabolism associated with this activity. We have recently discovered a new type of biophysical activity that can be recorded in the peri-cranial space with a noninvasive, non-contact device called a Sentiometer, developed in our laboratory. This effect (sentiometric response or SR), recorded in a double-blinded manner, involves a marked decrease in the amount of diffracted light produced by proximity of the Sentiometer sensor module to the head of a conscious person. The diffracted light is generated by a low power laser light emitting diode enclosed within the sensor module. The amplitude of the SR, measurable above the baseline, declines with the distance between the head and the sensor module. SR like that in humans is observed in mice, but in some invertebrate species studied by us so far, it is inverted. The effect appears to spread to the whole body and can be detected in the peri-somatic space as well. Control experiments indicate that it cannot be accounted for by a change in temperature due to body heat, and by expired air, sound, pressure waves, device power fluctuations, electromagnetic interference or recording artifacts. It is not blocked or attenuated in vacuum, by a magnetic shield, grounded metal barrier, or Faraday enclosure.
The sentiometric effect appears to be related to consciousness for the following reasons. In mice, general anesthesia administered by an inhalational (isoflurane) or injectable (pentobarbital or ketamine/xylazine) anesthetic causes a significant and substantial reduction of the SR amplitude and rise time. Recordings during sleep in humans show oscillations of the sentiometric trace with periods ranging from ~60 to 80 min. The peri-somatic effect in mice disappears completely ~3 h after euthanasia. However, a decapitated mouse head and excised brain produce an initial biphasic SR followed by an inverted SR at ~3 h. Taken together these findings point to a hitherto unrecognized biophysical effect of neural origin, which might have a causal connection with consciousness. Furthermore, the sentiometric recording method may provide a new approach to assessing the activity and function of the brain.

Disclosures: S. Helekar: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventorship on a patent application, Houston Methodist Hospital. **B. John:** None. **L. Nguyen:** None. **S. Hambarde:** None. **A. Pandey:** None. **A. Saldon:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.27/P11

Topic: H.01. Attention

Support: University of Birmingham

Title: Exploring the effect of transcranial direct current stimulation (tDCS) of the parietal lobe on spatial attention.

Authors: *I. GIANNAKOU, A. FREW, E. VENN, N. JENKINSON, D. PUNT;
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Abstract: Lesions in specific brain regions, particularly the right posterior parietal cortex (PPC), can lead to deficits in detecting a contralateral stimulus when presented alone (unilateral spatial neglect) or with a competing ipsilateral stimulus (spatial extinction). The role of PPC in distinguishing between single and competing stimuli remains a topic of ongoing research debate. This study aimed to explore the effect of transcranial direct current stimulation (tDCS) of the right PPC on single and competing visual stimuli, partly replicating an approach taken in a previous study by Filmer et al (2015). Their study found anodal tDCS disrupted single contralateral stimuli detection, while both anodal and cathodal tDCS disrupted bilateral stimulus detection. We recruited 63 neurologically healthy adult participants and divided them into three groups who received either Anodal, Cathodal or Sham tDCS stimulation of the right PPC. Consistent with Filmer et al. (2015), participants completed a Staircase detection task of target gratings appearing among noise patches, to establish a 70% accuracy threshold prior to commencing three phases of the Main task (pre-stimulation, immediate post-stimulation, and 20 minutes post-stimulation), where we gathered accuracy data (%). Analysis of these data as a function of Phase (Before and Immediately After tDCS), Stimulus Location (Ipsilateral, Contralateral, Bilateral) and Stimulation Type (Anodal, Cathodal, Sham) revealed significant effects of Stimulus Location ($F(2, 108) = 20.257$, $p < 0.001$), with higher accuracy for single than bilateral stimuli. There was also a Phase effect ($F(1, 54) = 5.933$, $p = 0.018$), indicating enhanced accuracy before stimulation. However, there were no significant effects of Stimulation Type or any interactions. In contrast to Filmer et al. (2015), we did not observe any general or selective effects of tDCS to the right PPC. Our study failed to show any evidence that tDCS of the right PPC modulates spatial attention, a negative but important finding given recent interest in the field.

Disclosures: I. Giannakou: None. **A. Frew:** None. **E. Venn:** None. **N. Jenkinson:** None. **D. Punt:** None.

Poster

PSTR421

Neural Mechanisms of Human Attention and Therapeutics

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.28/P12

Topic: H.03. Decision Making

Support: U24AA029970

Title: Greater strategy use is associated with low cognitive flexibility in monkey attentional set shifting

Authors: *C. E. MCGONIGLE[1], S. GONZALES[2], K. GRANT[2], C. C. LAPISH[1];
[1]Anatomy, Cell Biol. and Physiol., Indiana Univ., Indianapolis, IN; [2]Div. of Neurosci., Oregon Natl. Primate Res. Ctr., Beaverton, OR

Abstract: Deficits in decision making are commonly observed in Alcohol Use Disorder. Cognitive inflexibility results in deficits in directing behavior based on current environmental information and has been demonstrated to be a risk factor for problematic alcohol use. The attentional set shifting task has been used to assess cognitive flexibility and requires the ability to use different rules to guide decision-making. Each phase of the task requires that an animal determine which feature of a complex stimulus is being rewarded and adjust their behavior accordingly. Strategy, or planned choices, can be identified through consistent selection of a particular stimulus feature and can provide insight into the ways in which the animal searches for the currently rewarded feature. The present study used male and female rhesus monkey data to assess the manner in which strategies were used in attentional set shifting, as it relates to cognitive flexibility and future drinking. Bayesian inference was used to detect the presence of spatial, shape-, and color-based strategies. This analysis revealed that high performing monkeys tend not to search with firm strategies until they have accumulated sufficient evidence about which feature is rewarded. On the other hand, low performers tend to deploy strategies more strongly, indicating either a tendency to perseverate on strategies that are not rewarded, or a tendency to sequentially employ potential strategies. This difference in how monkeys searched for the correct feature of the stimulus may indicate a difference in the tendency to use model-based versus model-free exploration. Model-based actions are those guided by expectations of reward contingencies built upon a learned model of the environment. Model-based exploration is often considered more efficient than model-free, which involves evidence accumulation through trial and error. The current data suggest that higher cognitive flexibility may be counterintuitively associated with model-free exploration or may involve monitoring multiple strategies at once, although we cannot yet disambiguate these two approaches. Lower cognitive flexibility is also associated with higher levels of future drinking, and future work will focus on the relationship between strategy evolution and drinking risk. This work is part of the INIA Stress Consortium’s goal to promote cross-species translation in the assessment of cognitive risk factors for alcohol use disorder.

Disclosures: C.E. McGonigle: None. **S. Gonzales:** None. **K. Grant:** None. **C.C. Lapish:** None.

Poster

PSTR422

Functional Networks in the Human Brain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.01/Q1

Topic: H.04. Executive Functions

Support: NINDS Grant R01NS124738

Title: Functional brain network stability in Parkinson's Disease

Authors: *J. CHERNICKY[1], A. DWORETSKY[1], S. GROSSEN[2], E. C. CARR[2], A. EID[2], S. A. NORRIS[3], M. C. CAMPBELL[4,3], C. GRATTON[1,5,6,7,8];
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Abstract: Parkinson's Disease (PD) is a prevalent neurodegenerative disorder characterized by the progressive accumulation of alpha-synuclein aggregates that start in the brainstem and spread throughout the brain. This pathology manifests in a spectrum of motor, cognitive, and psychiatric deficits that vary across people, and are only partially ameliorated by current treatment options. Thus, there is a need for methods that can measure reliable differences in brain function related to these deficits at the individual level. Complex behavioral deficits in PD have been linked to the interactions between brain regions that form large-scale brain networks. These networks can be measured non-invasively using resting-state functional connectivity (RSFC) measures derived from functional MRI data. Conventional RSFC methodologies encounter limitations in precision at the individual level due to brief (<10 min.) acquisition periods, reliance on group-level network definitions, and poor signal-to-noise ratios. Emerging "precision" RSFC techniques, characterized by extended (>40 min.) multi-session within-subject data collection, offer potential in overcoming these challenges. However, their application in PD remains untested, limiting their clinical utility for prognosis and treatment optimization. To assess the feasibility and reliability of precision RSFC in PD, individuals with PD (N=15) and healthy controls (N=4) completed 3-5 fMRI sessions up to six months apart. All participants demonstrated the capacity to generate high-fidelity data with minimal motion artifact (>75% of frames retained). To quantify the coherence of functional networks across sessions and substantiate the efficacy of precision RSFC, we computed the mean session-to-session correlation within subjects with increasing scan time. Results revealed improved intersession similarity with prolonged scan durations in the whole brain, thalamus, basal ganglia, and cerebellum, with marked differences in reliability between 5 min and 40 min of data collection. These results underscore the methodological advantages of precision RSFC in individuals with PD. These observations not only advocate for the broader adoption of precision RSFC techniques in PD research, but also emphasize their potential for facilitating personalized therapeutic interventions via the identification of robust individual-level biomarkers.

Disclosures: J. Chernicky: None. **A. Dworetsky:** None. **S. Grossen:** None. **E.C. Carr:** None. **A. Eid:** None. **S.A. Norris:** None. **M.C. Campbell:** None. **C. Gratton:** None.

Poster

PSTR422

Functional Networks in the Human Brain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.02/Q2

Topic: H.04. Executive Functions

Support: NSF CAREER BCS-2048066

Title: The subcortical connectivity of prefrontal visual and auditory-biased regions

Authors: *E. HOUSTEAU[1], Z. LADWIG[2], C. GRATTON[3,4];
[1]Northwestern Univ., Evanston, IL; [2]Interdepartmental Neurosci. Program, Northwestern Univ., Chicago, IL; [3]Psychology, Northwestern Univ., Chicago, IL; [4]Psychology, Florida State University, Tallahassee, FL

Abstract: The role of the prefrontal cortex (PFC) has traditionally been ascribed to amodal higher-level processing; however, recent evidence has suggested that it also contains regions that are biased to visual or auditory domains in attention and working memory. In total, four bilateral sensory-biased regions have been identified in the PFC using task-evoked fMRI contrasts and individual-level data analysis; the regions alternate between visual and auditory in a tightly interdigitated pattern along the precentral and inferior frontal sulci that can be obscured in more standard group-average approaches. One way in which these regions can be further characterized is by their functional connectivity (FC) profiles. FC is a measure of correlated neural activity between two distinct areas of the brain and can be measured even during rest. Prior work on cortical FC patterns suggests that these PFC sensory-biased regions correlate with visual and auditory cortical networks. But, no work has yet investigated the subcortical FC of these regions, despite knowledge of structural and functional connections between the PFC and cerebellum. Here, in a new dataset of ten highly-sampled individuals (F = 5; age = 19-33), we first replicated the finding of interdigitated sensory-biased regions in the PFC using visual-auditory fMRI task contrasts. Then, using these regions as seeds, we calculated the FC to every cerebellar voxel, and the strongest five percent of correlations were isolated to give a set of cerebellar visual PFC-connected and auditory PFC-connected regions. The analysis was performed on the individual level, but subjects displayed consistency in the locations of visual and auditory PFC-connected regions. Visual PFC-connected regions were consistently seen in the vermis VI, lobules VIIB/VIIa, and lobules VIIIb/IX while auditory PFC-connected regions were identified in lateral crus I, medial crus I/II, and lobules VIIb/VIIIa across subjects. Strikingly, while these regions were adjacent, within each subject there was minimal overlap between the cerebellar visual and auditory PFC-connected regions, mirroring the distinctive interdigitated pattern seen in the PFC. But, when all subjects' results were displayed on an overlap map, the clean separation between visual and auditory PFC-connected regions was lost, highlighting the importance of an individual-level analysis. Future steps of this analysis include evaluating these new cerebellar regions in terms of their task responses and functional network identities. This work suggests that there are modality-specific higher-level processing areas in the cerebellum that are related to those seen in the PFC.

Disclosures: E. Housteau: None. **Z. Ladwig:** None. **C. Gratton:** None.

Poster

PSTR422

Functional Networks in the Human Brain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.03/Q3

Topic: H.04. Executive Functions

Title: The Low Identifiability of Psychopathology Measures Hinders Clinical Neuroscience Research

Authors: *B. KRAUS[1], A. PORTER[2], I. RISTANOVIC[1], Z. ANDERSON[1], K. DAMME[3], R. ZINBARG[1], V. MITTAL[1], R. NUSSLOCK[1], M. CRASKE[4], C. GRATTON[5];
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Abstract: One of the main goals of clinical neuroscience is to identify features of neural activity that correlate with behavioral measures of psychopathology. A common study design in clinical neuroscience is the longitudinal design, which allows for direct within-person comparisons of changes in psychopathology symptoms to changes in brain activity. For example, longitudinal designs are commonly used to quantify why participants who look alike on some measure at timepoint 1 later diverge at timepoint 2. However, for the logic of this study design to hold, it would have to be the case that enough information was collected about each individual at each timepoint to accurately track them over time. Otherwise, if it is not possible to track the identity of individuals over time using their psychopathology or fMRI data, then we will not be able to accurately predict why individuals' outcomes differ.
To empirically test identifiability, we used a variant of connectome fingerprinting to test the longitudinal identifiability of psychopathology data versus fMRI data. We used the publicly available ABCD dataset as well as a second dataset known as BrainMAPD. The BrainMAPD dataset contains 274 adolescents from 18-19 years old who are followed longitudinally for 3 years. In both datasets, participants completed annual psychopathology assessments and underwent fMRI scanning baseline and again either 2 years (ABCD) or 3 years (BrainMAPD) post-baseline. For fMRI data in ABCD, the publicly available processed Gordon 333 network-to-network connectivity was used while in BrainMAPD the full Gordon 333 connectivity matrix was used for calculating identifiability. In ABCD, identifiability was calculated by randomly selecting 200 individuals 1000 times and compared their baseline measures to their 2-year follow-up, while in BrainMAPD the full sample was used and compared their baseline measures to their 3-year follow-up. For these analyses, ABCD had in total 34 psychopathology scales, 696 psychopathology items, and 749 fMRI features, while BrainMAPD had 32 psychopathology scales, 153 psychopathology items, and 55,278 fMRI features. In both datasets, longitudinal fMRI identifiability was the highest (ABCD: ~66%, BrainMAPD: ~97%), followed by psychopathology item identifiability (ABCD: ~25%, BrainMAPD: ~52%), and lastly

psychopathology scale identifiability (ABCD: ~18%, BrainMAPD: ~30%).
These findings demonstrate that fMRI data is much more identifiable across time than psychopathology data. The lack of reliable information in psychopathology data over time makes it unlikely that typically used measures will be sufficient to identify reliable neural correlates.

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Poster

PSTR422

Functional Networks in the Human Brain

Location: MCP Hall A

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Program #/Poster #: PSTR422.04/Q4

Topic: H.04. Executive Functions

Support: NIH Grant T32 AG020506
NSF CAREER BCS-2048066

Title: Evaluating fine scale functional organization in the human lateral prefrontal cortex using precision fMRI

Authors: *Z. LADWIG[1], N. R. LABORA[2], M. DORN[3], J. HERNANDEZ[4], E. HOUSTEAU[5], R. M. BRAGA[6], C. GRATTON[7];
[1]Northwestern Univ. Interdepartmental Neurosci. Program, Chicago, IL; [2]Florida State Univ., Tallahassee, FL; [3]Radiology, Northwestern Univ., Chicago, IL; [4]Northwestern Univ., Chicago, IL; [5]Northwestern Univ., Evanston, IL; [6]Neurol., Northwestern Univ., Chicago, IL; [7]Psychology, Florida State Univ., Tallahassee, FL

Abstract: There is an ongoing debate as to how the human lateral prefrontal cortex (LPFC) is functionally organized, with evidence suggesting the existence of both "multiple demand" and functionally specialized regions. Recent work using precision fMRI has suggested that both types of LPFC regions may coexist at fine-scales not previously visible in group or meta analyses (Fedorenko et al., 2011, DiNicola et al., 2020, Noyce et al., 2017, Assem et al., 2020). An open question is how these regions are arranged relative to each other. To address this question, we collected a preliminary data set of five deeply sampled subjects (4F 1M), each with 8-15 hours of task fMRI. Tasks encompassed various cognitive domains, including language, episodic projection, social cognition, and a wide variety of cognitive control demands. Data collection involved 30-60 minutes per task using block design paradigms. In the two most densely sampled subjects, we completed split-half reliability analyses, finding that 30 minutes of data per task was sufficient to reliably identify fine-scale details within each subject. Across all five subjects, we found that task contrasts targeting specialized cognitive functions (language, episodic projection, and theory of mind) activated distributed sets of distinct but often adjacent

regions in the lateral prefrontal cortex. This was shown using split-half analysis to define task-specific ROIs in one half of the data and quantifying relative activation to various cognitive demands in the second left out half. In all five subjects, we saw separation between cognitive domains in the LPFC. In addition, we found in all five subjects that a wide variety of cognitive control contrasts activated a distributed set of "general control" regions nearby, yet non-overlapping the specialized regions. These results support a model in which high-level cognitive functions are supported by distributed sets of specialized regions which are often adjacent to "multiple demand" regions active during a diverse set of tasks. Additionally, this work supports the idea that there is fine-scale functional organization which is reliable at single subject level and enables a more detailed characterization of brain organization than is often possible in group or meta analyses which optimize for identifying central tendencies.

Disclosures: Z. Ladwig: None. **N.R. Labora:** None. **M. Dorn:** None. **J. Hernandez:** None. **E. Housteau:** None. **R.M. Braga:** None. **C. Gratton:** None.

Poster

PSTR422

Functional Networks in the Human Brain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.05/Q5

Topic: H.04. Executive Functions

Support: NIH Grant R00MH126161

Title: Frequency mapping of large-scale networks using multiple cognitive tasks

Authors: *S. DHULASHIA[1], J. RIDDLE[2];
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Abstract: Higher-order cognition requires the coordination of regions distributed across large-scale brain networks. Prominent functional networks, such as the central executive network (CEN) and default mode network (DMN), display temporally coordinated functional magnetic resonance imaging (MRI) activation which are found to be engaged by specific cognitive processes such as cognitive control and long-term memory. While neural oscillations are proposed to be the substrate for network-scale brain activity, systematic investigation into the frequency-specific network organization that supports higher-order cognition has yet to be performed. Here, we collected high-density electroencephalography (EEG) in multiple tasks known to recruit diverse regions of association cortex and the primary hubs of the CEN and DMN. To maximally drive the CEN, we administered a hierarchical cognitive control task that was shown to activate the anterior middle frontal gyrus and superior parietal lobule, the core hubs of the CEN. For the DMN, we administered an associative long-term memory task that was shown to recruit the medial prefrontal cortex, precuneus, and lateral temporal association region,

hubs of the DMN. As a control, we administered motor and visual localizer tasks that provide a means for dissociating activity in the sensorimotor network and visual network. We estimated source-localized and frequency-separated signals in regions across the cerebral cortex using the center of gravity from an atlas derived from functional MRI connectivity, and investigated the presence of stable networks that were altered as a function of cognitive demands. We found frequency-specific brain networks that approximated those observed in functional MRI. In particular, low-frequency oscillations played a key role in dissociating distinct frontal-parietal networks. These analyses and dataset provide an analog to the approach frequently utilized in functional MRI and might serve to complement existing network models of the brain by including frequency-specificity.

Disclosures: S. Dhulashia: A. Employment/Salary (full or part-time):; Florida State University.
J. Riddle: A. Employment/Salary (full or part-time):; Florida State University.

Poster

PSTR422

Functional Networks in the Human Brain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.06/Q6

Topic: H.04. Executive Functions

Support: NIH

Title: The role of the thalamus in dynamic decision-making

Authors: *C. CALDINELLI[1,2], J.-J. LI[2], M. J. ARCARO[3], A. G. COLLINS[4];
[1]Univ. of California, Berkeley, Berkeley, CA; [2]UC Berkeley, Berkeley, CA; [3]Psychology, Univ. of Pennsylvania, Philadelphia, PA; [4]UC Berkeley, Psychology Dept., UC Berkeley, Berkeley, CA

Abstract: The thalamus lies in a pivotal position as an integrative hub for areas involved in high-level cognition. Despite its important theoretical role in many computational models and its involvement in psychiatric disorders, the human thalamus has not been studied much in the context of higher level cognition and cognitive flexibility. However, studies with non-human animals show involvement of the mediodorsal nucleus (MD) and the prefrontal cortex (Rikhye et al., 2018) during rule representation and switching, and a dysfunction of hub regions is associated with behavioral and cognitive impairment (Bassett et al., 2009, van den Heuvel et al., 2013). Executive function processes require dynamic and flexible coordination of information across brain-wide cortical networks, and the thalamus appears well positioned to perform such integrative processes through their extensive and convergent connections with the prefrontal cortex (Fiebelkorn et al., 2019, Saalmann et al., 2012). We developed a novel behavioral task aimed at eliciting frequent switches between rule exploitation and rule exploration (Donoso et al, 2014). We are adapting this task for use with fMRI to measure responses in the thalamus and

prefrontal cortex. Behavioral and computational modeling results show that during this task participants integrate reward uncertainty together with higher order task structure knowledge to efficiently explore the rule space. We predict that the MD will be involved in the neural underpinnings needed to perform the task successfully. In particular, our prediction is that the interplay between MD and the prefrontal cortex will support rule switching and rule exploration. This task will inform us on the role of thalamic nuclei and will help clarify the extent to which they are involved in human learning and higher cognition. If the thalamus shows to be involved in a rule switching task, it would suggest a primary role in enabling the integration of information in dynamically changing and ambiguous environments such as solving a problem in the presence of uncertainty and updating the rules required to achieve a goal thanks to the extensive and reciprocal connections between the frontal and parietal cortices.

Disclosures: C. Caldinelli: None. **J. Li:** None. **M.J. Arcaro:** None. **A.G. Collins:** None.

Poster

PSTR422

Functional Networks in the Human Brain

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.07/R1

Topic: H.04. Executive Functions

Support: Baden Wuerttemberg Foundation, German Research Foundation
Emmy Noether Program (DFG HE8329/2-1)
Jung Foundation for Science and Research

Title: Neural variability as an organizing principle of the cortical hierarchy that enables cognitive flexibility

Authors: *J. MARTINI, J. TERLAU, R. F. HELFRICH;
Hertie-Institute for Clin. Brain Res., Tuebingen, Germany

Abstract: Neural variability in response to repeated presentations of the same sensory stimulus spans various spatial scales within the nervous system. At the level of single neurons, this variability exhibits a clear spatial organization with increasing variability along the visual hierarchy. To date, it remains unknown whether this spatial organization also reflects a central organizing principle at the level of neural populations. Moreover, while prior work has primarily focused on understanding trial-to-trial variability in early sensory areas, much less is known about variability states and their dynamic adaptation to cognitive demands and arousal states in higher-order association areas. Here, we used a multi-task design combined with direct intracranial recordings in humans to address these questions. In line with prior work, we demonstrate that neural variability was strongly reduced in response to visual input but rapidly increased after stimulus offset, when sensory information was absent, but had to be maintained. Critically, these modulations of local neural variability were highly robust across different tasks,

suggesting that neural variability is structured by network properties and anchored in robust temporal motifs. Furthermore, neural variability gradually increased along a cortical sensorimotor-association gradient, critically extending the spatial organization previously observed in animal models to higher-order association areas in humans. Importantly, the increased variability in association areas varied according to task demands and arousal levels, suggesting that neural variability in association areas does not reflect noise, but instead supports cognitive flexibility in a task-dependent manner. In sum, these results replicate and critically extend previous observations at the level of single neurons to the level of neural populations thereby providing new insights into the functional organization of neural variability across the human brain.

Disclosures: J. Martini: None. **J. Terlau:** None. **R.F. Helfrich:** None.

Poster

PSTR422

Functional Networks in the Human Brain

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Program #/Poster #: PSTR422.08/R2

Topic: H.04. Executive Functions

Support: Varela Grant, Mind and Life Institute
Bial Foundation
International Association for the Study of Dreams & Dream Science Foundation

Title: Neural Correlates of Contemplative Sleep Practices

Authors: *S. G. TORRES PLATAS[1], D. MORRIS[1], K. R. KONKOLY[1], E. DEMSAR[2], M. SHEEHY[3], D. GERMANO[4], K. A. PALLER[5];
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Abstract: Recent neuroscientific advances have provided new ways to investigate dream experiences, both to shed light on dreaming and to seek useful applications of this knowledge, such as in treatments for nightmare disorders. For centuries, Buddhist traditions have described advanced forms of contemplative practice during sleep. Tibetan Dream Yoga is a suite of such practices that includes waking-imagination exercises and strategies to induce lucid dreaming—awareness of the dream state while one is still in the dream. Autobiographical evidence suggests that these practices can produce a virtual environment for training with various dream scenarios that results in adaptive insights for one's waking life. In this pilot study, we assessed the feasibility of studying highly trained dream-yoga practitioners in a laboratory setting. Individuals were interviewed to document their prior contemplative training before visiting a sleep

laboratory for overnight sessions with standard polysomnographic recordings. Before sleep, participants were trained to associate specific sounds with a small set of lucid-dream experiences. Then, during Rapid-Eye Movement (REM) sleep, these sounds were presented with the goal of impacting dream experiences and functioning to help individuals achieve lucid dreaming and perform specific tasks. Before sleep, participants were instructed to use combinations of distinctive eye-movement and respiratory signals to indicate their experience of a lucid dream and progress in their dream tasks. We focused on instances when instructions were communicated to dreamers and they successfully signalled back about their experiences. These signals thus provided a time-stamp for their dream experiences in polysomnographic data. After waking, participants completed a micro-phenomenological interview intended to document recall of some of their dream experiences in fine-grained detail. We also included a battery of questionnaires and cognitive testing. Our results demonstrate the feasibility of a multidisciplinary approach to studying neural correlates and the phenomenology of contemplative sleep practices. By studying experienced practitioners in the sleep lab, combined with first-person data and humanistic methods that contextualize these practices philosophically, socially, and culturally, we aim to produce a comprehensive understanding of these contemplative sleep practices and neural correlates to gain insights into how individuals can use their sleep to produce various waking benefits.

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Poster

PSTR422

Functional Networks in the Human Brain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.09/R3

Topic: H.04. Executive Functions

Support: CONAHCYT 191975

Title: Examining the Impact of Lead Exposure on Resting-State Alpha and Gamma Absolute Power and Working Memory in Children Residing near Mine Tailings in South-Central Mexico

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Abstract: Lead (Pb) is a heavy metal known to disrupt nervous system function, acting as a non-competitive inhibitor of NMDA receptors, and interfering with intracellular calcium regulation. These disruptions affect plasticity, altering cellular excitability and inhibitory dynamics. Such physiological effects can extend to impairing higher cognitive functions mediated by prefrontal cortex circuits. Despite this well-established understanding, the impact of Pb on quantitative brain electrical activity during childhood remains largely unexplored. Huautla is a region of

south-central Mexico with a great anthropogenic impact derived from mining activity, which has left an estimated 780 thousand tons of tailings with high concentrations of Pb. Accordingly, we aimed to evaluate the association between Pb and cerebral neocortex dynamics and function. To this end, we grant the participation of 57 children (29 girls and 28 boys) between 5 and 11 years old. We obtained a hair sample to determine Pb levels, a resting-state EEG recording in open (OE) and closed eyes (CE), and an evaluation of working memory, planning, cognitive flexibility, and behavioral control. In this study sample, Pb showed a mean value of 5.36 μg/g (± 4.1 μg/g), 3.36 μg/g greater than the most conservative allowed value reference (Mutap et al., 2016). Brain dynamics in terms of absolute power showed a negative association with Pb concentrations in the alpha frequency in EO ($r = 0.47$, $p = 0.03$), as well as alpha ($r = 0.46$, $p = 0.03$) and gamma ($r = 0.42$, $p = 0.05$) frequency in EC. All changes occurred in the frontal channels. Furthermore, we observed increased interhemispheric asymmetry between power bands in frontal regions in OE and CE. We also find a negative association between Pb concentrations and duration for working memory tasks. Alpha and delta power and symmetry play a role in modulating several cognitive processes in the prefrontal cortices. These data show an association between brain dynamics, executive functions, and exposure to Pb, which we must continue to explore to determine the effects of lead on brain dynamics and if this activity is associated in simultaneous and real-time with cognitive dissipation (using, for example, ERP).
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Disclosures: C.G. Curiel-Guerrero: None. **A. Elizondo:** None. **I. Santoyo:** None.

Poster

PSTR422

Functional Networks in the Human Brain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.10/R4

Topic: H.04. Executive Functions

Support: JSPS KAKENHI Grant Number JP22K11614

Title: Afferent input associated with muscle activity facilitates response execution during moderate acute exercise

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Abstract: It is well known that various types of chronic exercise improve cognitive function. However, the temporary effects of acute exercise on cognitive functions remain unclear. Our

previous study using a stop-signal task showed that executive function, rather than response inhibition, is promoted by acute exercise. In the present study, we investigated the factors, lower limb muscle activity and respiratory response, that influence executive function during acute exercise. Nine healthy adults participated in the study. A simple visual response task was conducted in two blocks of 50 trials, each in the control and exercise conditions. The participants responded by pressing a switch with the index finger of their dominant hand. Under the exercise conditions, the ergometer was manually operated such that the exercise intensity was moderate (50% heart rate reserve). Electroencephalography signals were recorded from the scalp during the task to obtain the event-related potential (ERP). Electromyography was recorded from the vastus medialis on both lower limbs, and reaction times were compared for each phase of lower limb muscle activity (contralateral, ipsilateral, and blank of the dominant hand). Ventilatory parameters such as oxygen uptake (VO_2), tidal volume (Tv), ventilation equivalent (Ve), and respiratory frequency (Rf) were recorded during exercise, and time-series changes over two blocks were examined. The results showed that the reaction time was shorter during the exercise condition than during the control condition, and it was the shortest during the ipsilateral phase. The peak latency of the P3 component of the ERP tended to shorten during exercise, but the difference was not significant. Although Tv, Ve, and Rf increased during the second half of the exercise period compared with the first half, there was no difference in the reaction time and peak latency of the P3 component. These results suggest that ventilatory parameters have no effect on executive function under moderate acute exercise loads, and that afferent inputs related to muscle activity may promote executive function.

Disclosures: M. Takayose: None. **R. Koshizawa:** None. **K. Oki:** None.

Poster

PSTR422

Functional Networks in the Human Brain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.11/R5

Topic: H.04. Executive Functions

Support: NIMH Grant P50MH132642

Title: Functional coupling with cognitive control networks reveals shared and distinct organizational principles of pulvinar and mediodorsal nucleus.

Authors: *X. LIU, M. J. ARCARO;
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Abstract: Traditionally viewed as a sensory relay, the thalamus has been increasingly recognized for its role in perceptual and cognitive processes, facilitated by its widespread reciprocal connectivity with association cortices. In particular, the mediodorsal (MD) nucleus and pulvinar (PUL) are thought to be crucial in coordinating information flow across large-scale

cortical networks. However, the functional organization within these structures is difficult to resolve due to their small size, diverse connectivity, and challenges associated with recording activity from midbrain structures. Here, we aim to elucidate the functional organization within the PUL and MD by deriving fingerprint functional connectivity (fFC) profiles that characterize each region's functional connections with various perceptual and cognitive control cortical regions. Our analysis revealed a shared, primary sensory-to-associative (SA) axis of cortical connectivity across both nuclei, extending from ventrolateral to dorsomedial in the PUL and from posterolateral to anteromedial in the MD. The PUL and MD further differentiated based on distinct secondary axes: PUL's spans from the visual (posteroventral) to cognitive control networks (anterodorsal), while MD's from auditory and language (ventromedial) to broader cognitive control and attention networks (dorsolateral). Notably, the spatial arrangement of the first three principal axes of thalamocortical fFC within both nuclei was nearly orthogonal, suggesting an underlying spatial organization principle that may guide functional specialization in 3D subcortical brain structures. We also evaluated PUL and MD engagement during task-based fMRI to determine if fFC profiles during "rest" reflect task-specific activations and how thalamocortical functional motifs are reconfigured under cognitive demands. We found that fFC profiles during "rest" captured the spatial states of thalamic activation across various perceptual and cognitive tasks. While the SA axis dominated the spatiotemporal signal during rest, the minor axes more accurately reflected spatial patterns of task-evoked activity, demonstrating a dissociation between the "variance explained" in the spatial organization of resting networks and task-relevant organization. These findings suggest that the SA axis reflects a general organizational architecture, while specific cognitive functions are likely instantiated in more nuanced features of thalamocortical connectivity. Collectively, our results enhance our understanding of the relationship between higher-order thalamic regions and brain-wide networks supporting cognition.

Disclosures: X. Liu: None. **M.J. Arcaro:** None.

Poster

PSTR422

Functional Networks in the Human Brain

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Program #/Poster #: PSTR422.12/R6

Topic: H.04. Executive Functions

Support: R01MH120118
P50MH132642
R01MH134466
R01MH122613

Title: Mediodorsal thalamic engagement and enhanced prefrontal functional connectivity during context switching in humans

Authors: *L. MENGXING[1], N. H. LAM[1], S. LEACH[2], J. JIANG[2], M. HALASSA[1], K. HWANG[2];
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Abstract: The prefrontal cortex (PFC) is critical for cognitive control, the ability to regulate thoughts and action plans based on an internal model of the world. Recent animal studies have suggested that the PFC requires interactions with the mediodorsal (MD) thalamus for optimal function, particularly with increased cognitive demands. For example, in both non-human primates and rodents, context (cue-to-rule mapping) switching is delayed when the MD thalamus is lesioned or optogenetically suppressed. Neural recordings indicate that MD neurons encode the context as well as context-prediction errors that together regulate the ability of the PFC to switch its executive representations in the service of behavioral adaptation. However, the extent to which these findings can be generalized to the human brain is unknown. To address this question, we collected behavior and fMRI data from human participants performing hierarchical decision making tasks in which the context can abruptly change. In the most complex version of the task, participants had to adjust their behavior following abrupt context changes (between four) based on feedback. Using a computational model-derived metric that predicted context changes as a regressor for BOLD signals, we identified a network of brain regions with enhanced activity for high switching probability. These 'switch-related' regions included dorsal PFC regions (dorsomedial and dorsolateral PFC), the anterior cingulate cortex, and the lateral portion of the MD thalamus. Strikingly, functional connectivity analysis indicated enhanced connectivity among these prefrontal areas associated with context switching. Given that the MD is a target for midbrain dopaminergic and basal ganglia output, structures known to be engaged in prediction error signaling, as well as its massive corticofugal input from PFC, we wondered whether it is a locus for integrating such signals and generate high level context representation to facilitate context switching. Indeed, in a simper task where subjects performed context reversals (between two) across blocks, we found the lateral MD activity pattern encoded the high level context representations regardless of the cue and rule. Overall, to our knowledge, our data is the first to show a possible association between MD thalamic activation and changes in PFC functional connectivity in the human brain during context switching. These results highlighted the critical contribution of MD thalamus in cognitive flexibility in human.

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Poster

PSTR422

Functional Networks in the Human Brain

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.13/Web Only

Topic: H.04. Executive Functions

Support: TUBITAK 120K924

Title: The 'Task' of Mind-Wandering Reveals Functional Distinctions within both the Multiple Demand and the Default Mode Regions

Authors: *I. GIRAY[1,3], A. A. FAROOQUI[2];
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Abstract: Neuroimaging tasks are well-known to deactivate Default Mode (DMN) and activate Multiple Demand (MD) regions. However, the cause of these deactivations and activations is unclear. Popular views link DMN deactivation to decreased mind-wandering and/or internal cognitions (e.g., autobiographical reflection) and MD activation to focused and convergent thought and/or external tasks. In contrast, we have previously suggested that any period of purposive cognition, or *any* task, deactivates DMN and activates MD regions, regardless of whether it involves internal or external attention, convergent or divergent thinking, focussed or wandering mind. We pitted our account against other existing views by creating a *task* of mind-wandering. Participants were asked to wander their minds and think as many diverse thoughts as possible during the task periods and rest during the rest periods. Task periods thus involved a more wandering mind than the rest periods and required no external attention and no focused and convergent thinking.If the DMN deactivation typically seen during tasks is due to decreased mind-wandering and internal cognitions, and if MD activation is due to external attention or focused and convergent thought, then there should be no DMN deactivation and MD activation during this task.We found a split amongst both DMN and MD regions. Some DMN regions, notably the temporoparietal junctions, deactivated, while others, like the posterior cingulate and anteromedial prefrontal cortex, activated during the task blocks compared to rest. Likewise, some MD regions – the intra-parietal sulcus and right anterior prefrontal cortex – deactivated, while others – presupplementary motor areas, regions along the posterior inferior frontal sulcus, anterior insula, and frontal eye fields – activated during these task blocks. This double dissociation within DMN and MD regions suggested functional distinctions within them. Some DMN (e.g., that of the temporoparietal junction) and some MD regions (e.g., inferior frontal sulcus, anterior insula) are likely to be sensitive to the task-ness of the period, regardless of its content and are not about issues like internal/external attention, mind-wandering/focussed thought. In contrast, other DMN (e.g., the posterior cingulate) and MD (e.g. intraparietal sulcus) regions do seem to be sensitive to these latter issues.

Disclosures: I. Giray: None. **A.A. Farooqui:** None.

Poster

PSTR422

Functional Networks in the Human Brain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.14/S2

Topic: H.08. Learning and Memory

Title: Dlpfc network during late rest periods correlates with offline learning gain.

Authors: *R. KAWASOE[1], H. SUGATA[2];
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Abstract: In daily lives, we often learn unconsciously through various experiences. Offline learning, a specific form of learning, refers to improvements in motor performance during rest periods. Recent research on offline learning has explored the relationship between different sets of rest intervals interspersed with practice and functional connectivity (FC). While individual differences in motor learning performance have long been recognized, research has emphasized averaged data to explain the physiological mechanisms of motor learning, thereby overlooking this critical aspect. Several studies have demonstrated a correlation between offline learning and FC in neurophysiological aspects. However, these studies have primarily focused on averaged FC across participants and resting-state duration, overlooking the dynamic changes in individual FC highlighted in recent research. Consequently, the temporal dynamics of FC related to offline learning remain unclear. Therefore, we aimed to investigate the relationship between skill learning and the temporal characteristics of resting-state FC.Thirty-four healthy right-handed participants performed a force-controlled motor task for 5 minutes before and after a 15-minute wakeful rest period. During the task, participants were instructed to pinch a pressure sensor with their right thumb and index finger, manipulating a yellow cursor on a computer screen to align with a white baseline. Throughout the rest period, EEG signals were continuously recorded using a 64-channel EEG system. For the FC analysis, the 15-minute rest period was segmented into three 5-minute intervals to investigate the temporal dynamics of resting-state FC associated with offline learning.The results demonstrated a significant improvement in task performance after rest period, indicating offline learning during the rest period. Additionally, whole-brain correlation analysis between FC and offline learning revealed significant positive correlations between the contralateral dorsolateral prefrontal cortex (cDLPFC) and contralateral primary motor cortex (cM1), as well as the ipsilateral primary somatosensory cortex (iS1) during the late rest period in the beta band. Our results may support previous studies showing the relationship between M1 and DLPFC during awake offline learning from the aspect of temporal characteristics of FC. Moreover, sensory feedback linked to force control may contribute to offline learning by facilitating interactions between the cDLPFC and iS1. Our study provides insights into the temporal characteristics of resting-state FC associated with individual differences in offline learning.

Disclosures: R. Kawasoe: None. **H. Sugata:** None.

Poster

PSTR422

Functional Networks in the Human Brain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.15/S3

Topic: H.06. Social Cognition

Support: NIH Grant R01 MH127006

Title: The asymmetric influence of paracingulate sulcus morphology on cingulum bundle connectivity

Authors: *I. A. DANSTROM[1], J. A. ADKINSON[2], M. E. ROBINSON[6], A. MAHESHWARI[1], G. BANKS[1], M. HASEN[1], B. SHOFTY[7], S. A. SHETH[2], E. BARTOLI[3], A. M. GOLDMAN[4], S. R. HEILBRONNER[2], K. R. BIJANKI[5];
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Abstract: Current efforts to characterize the role of the cingulum bundle (CB) in mood disorders and evaluate its viability as a target for stimulation-based therapeutics overlook the importance of inter-individual variability in local sulcal morphology. The paracingulate sulcus (PCS) is a tertiary sulcus coursing dorsal and parallel to the cingulate sulcus and is present in at least one cerebral hemisphere across 70% of the population. PCS presence has been linked to reorganization of cytoarchitecture and changes in functional connectivity of the anterior cingulate cortex (ACC), a brain region related to emotional processing and social behavior. However, the influence of PCS on the CB remains largely unknown. To better understand the impact of sulcal variability on electrographically defined CB connectivity, we examined evoked potential responses to single-pulse electrical stimulation (SPES) as a function of intra-hemispheric PCS presence or absence. Stimulation was directed to the right and/or left hemisphere CB in 19 patients undergoing intracranial monitoring for treatment refractory epilepsy with stereo-electroencephalography (sEEG). Evoked potential responses were extracted from brain areas and a connectivity robustness ratio was computed for each brain region. Network-level robustness ratios were compared at the group-level across right and left hemisphere CB and present or absent PCS pattern. Stronger right CB electrographic engagement was detected to ipsilateral and contralateral brain regions in the absence of right hemisphere PCS morphology compared to the present PCS morphology condition (U=114, p=0.0279). Alternatively, left hemisphere CB engagement was stronger in the presence of left hemisphere PCS compared with the absent PCS condition (U=364, p=0.0049). Interestingly, similar connectivity robustness was observed across the left and right hemisphere CB when PCS morphology was dissimilar. However, when PCS morphology was mirrored across hemispheres, unbalanced CB connectivity robustness was observed in the left and right CB. Taken together, these data support that CB engagement is modulated by PCS morphology, seemingly in an asymmetric manner. Paired with the finding of balanced bilateral CB connectivity with asymmetric PCS distribution, our results may point toward the PCS as an important lateralized brain feature. Future research should focus on grouping by inter-hemispheric distribution of PCS morphology. Implications of these findings are relevant for characterizing a hemispheric specialization of the CB and for considering optimal CB electrode placement in stimulation-based interventions in psychiatric disorders.

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Poster

PSTR422

Functional Networks in the Human Brain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.16/Web Only

Topic: H.06. Social Cognition

Support: HIV Clinical Trials Network (CTNPT 026; CTN 273)
CIHR Team Grant (TCO-125272)

Title: Structural and functional brain correlates of loneliness among older people with HIV

Authors: *V. HARI[1], M. BROUILLETTE[2], N. MAYO[2], L. K. FELLOWS[3];
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Abstract: Background: Loneliness significantly impacts the quality of life and brain health, defined as both mental health and cognitive ability, of older individuals, especially those living with HIV. Research indicates that loneliness correlates with diminished grey matter volume and altered functional connectivity in the default mode network among healthy older adults. Notably, individuals with HIV experience a six-fold higher prevalence of loneliness compared to their peers. Moreover, this population faces increased risks of cognitive and mood symptoms, potentially influenced by various factors, including loneliness. However, the impact of loneliness on neuroimaging measures has not been studied in HIV. Here, we estimate the extent to which loneliness is associated with grey matter volume in 5 apriori regions of interest and resting-state functional connectivity in the default model network among older people living with well-controlled HIV infection. Methods: Fifty-seven HIV-positive participants (54 men) from the Canadian Positive Brain Health Now cohort underwent multimodal MRI. Loneliness was assessed using a single self-reported item from the Older Americans Resources and Services questionnaire. Imaging outcomes were selected based on prior work in health adults: grey matter volume assessed using voxel-based morphometry in five regions of interest, and default mode network resting state functional connectivity. Results: Loneliness was associated with reduced grey matter volume in the left amygdala and left hippocampus with a small-medium effect size and with functional connectivity within the default mode network with a medium effect size. Exploratory analysis revealed reduced connectivity associated with loneliness between the left amygdala and a cluster of regions comprising the left hippocampal gyrus, temporal pole, and orbitofrontal cortex, with a small effect size. Conclusion: We provide evidence that self-reported loneliness is associated with brain structural and functional measures in older people living with

HIV. These findings emphasize that social as well as biological factors are important in understanding brain health in those with HIV. Loneliness is modifiable, and thus could be a novel target for intervention to improve brain health in people living with this serious chronic illness. Supported by the HIV Clinical Trials Network (CTNPT 026; CTN 273)and CIHR Team Grant (TCO-125272)

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Poster

PSTR422

Functional Networks in the Human Brain

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.17/S5

Topic: H.01. Attention

Support: Donation from Catharon and Brian Miller

Title: Changes to resting state brain network connectivity under gluten challenge in celiac patients

Authors: *M. S. COHEN[1], B. JABRI[2], S. S. KUPFER[2], J. DECETY[1];
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Abstract: “Brain fog” is a commonly reported symptom in celiac disease and various other medical and psychological disorders. Little is known about the specific neural correlates of this symptomology, particularly in celiac disease. Here, we used fMRI to examine whether and how brain function changes in human celiac disease patients after a gluten challenge. Patients had two study visits, receiving a gluten challenge in one session and a sham gluten challenge in the other, with assignment randomized and double-blinded. Resting state fMRI was measured as a means of examining the activity of intrinsic networks in the brain, based on spontaneous low-frequency fluctuations in the BOLD signal. A parcellation by Schaefer et al. (2018) was used to divide the brain into 300 functionally distinct parcels, with each parcel assigned to one of seven functional networks, for analysis of resting state activity. Among a pilot sample of 10 participants (7 female, 3 male, mean age = 48 years, age range = 25-78 years, SD = 15 years), within-network functional connectivity during resting state was lower after consuming gluten than in the sham condition in the salience/ventral attention and default networks, while within-network connectivity was greater in the limbic network under gluten challenge. Graph theory analysis showed that network segregation (measured via mean clustering coefficient) tended to be lower after a gluten challenge. These preliminary results are promising in suggesting possible approaches for identifying and quantifying neural markers of brain fog. Prior work has shown reductions in mean clustering coefficient in diffusion-weighted (DWI) MRI structural connectivity data in age-related mild cognitive impairment (MCI; Berlot et al., 2016), with the degree of decline correlating with category fluency, a measure of cognitive control. A similar

reduction in clustering coefficient has been observed in structural connectivity following COVID infection (Mishra et al., 2023), which is notable because long-term effects of COVID can include brain fog. These past results are consistent with the preliminary findings reported here using more transient resting state connectivity measures in celiac patients.

Disclosures: M.S. Cohen: None. **J. Decety:** None.

Poster

PSTR422

Functional Networks in the Human Brain

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Topic: H.01. Attention

Support: NIH Grant T32GM007281 (NB)
National Science Foundation BCS 2043740 (MDR(

Title: Longitudinal stability of resting state functional connectivity predicts attention-related symptomatology in youth.

Authors: *N. BERRIAN[1], L. SAMS[2], A. CHAO[2], A. STIER[3], O. KARDAN[4], M. G. BERMAN[2], M. D. ROSENBERG[2];
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Abstract: Functional connectivity patterns have been shown to be relatively stable and unique at an individual level. Recent work suggests that the stability of these patterns across short time scales may relate to cognitive and social function. For example, Corriveau and colleagues (2023) demonstrated that adults who are more accurate on sustained attention and working memory tasks have more stable functional connectivity patterns across minutes and days. Cross-sectional studies have shown that youth connectomes become increasingly stable and distinct with age and that youth with increased clinical symptoms showed a marked delay in connectome distinctiveness (Kaufmann et al. 2017). However, it is unclear whether within-subject stability across years is related to clinical symptoms or cognitive function across development. Here, we investigated whether functional connectome stability in youth (N = 1109) across years can serve as a developmental marker of inattention indexed by Child Behavior Checklist DSM5-oriented Attention-deficit/Hyperactivity symptom (CBCL-ADH) scores and 0-back task performance, using data from the Adolescent Brain Cognitive Development (ABCD) Study. Analyses indicate that youth with more stable patterns of functional brain organization at rest from age 9-10 to 11-12 show better sustained attention task performance ($\beta = 0.11$, $p < 0.05$) and lower attention-related clinical symptoms ($\beta = -0.12$, $p < 0.05$), on average, across two years. Network-level analysis demonstrated that higher within-network stability in the medial frontal, frontoparietal, motor, and visual association networks—but not the default mode, subcortical-cerebellum, visual

I, visual II networks—is significantly associated with lower mean ADHD symptoms in youth. Higher stability across years within the medial frontal, frontoparietal, and default mode network significantly predicted average 0-back performance. These findings align with the idea that features of the pediatric functional connectome predict clinically relevant attentional performance across time at the individual level.

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Poster

PSTR422

Functional Networks in the Human Brain

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Program #/Poster #: PSTR422.19/T1

Topic: H.01. Attention

Support: Medical Research Council grant (MC_UU_00030/7)

Title: Precise topology of diverse cognitive activations within the multiple-demand core of the human brain

Authors: *D. FABER[1], G. SHIELDS[2], J. DUNCAN[2], M. ASSEM[2];
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Abstract: The multiple-demand (MD) system is a brain network crucial for flexible cognitive functions. Using the high-resolution multimodal MRI approach of the Human Connectome Project (HCP), we recently characterized 9 domain-general MD territories distributed in frontal, parietal, and temporal cortices. MD territories co-activate during multiple cognitive demands including reasoning and executive tasks. Our findings suggest a novel framework for MD's role in flexible cognition. For example, we revealed that three distinct executive functions showed overlapping activations within MD regions, yet each showed unique functional preferences extending to nearby resting-state networks (RSNs). These activations peaked at MD and adjacent RSNs borders, suggesting a likely substrate for integration between networks. Thus, we hypothesized that partially-specialized networks recruit adjacent MD territories from different spatial locations on the cortical sheet to generate flexible cognitive functions. To test this hypothesis, we scanned 40 participants using HCP approaches. Each participant performed five diverse cognitive tasks, designed to target different RSNs surrounding MD regions. To test the generalizability of findings we varied the presentation modality, task design (block vs event-related), and response type/speed across tasks: (1) language (verb generation vs noun repetition) (2) mental rotation (360 rotated vs unrotated (non-)mirrored letters/numbers) (3) memory (recollection vs recognition) (4) theory of mind (TOM) (higher-order vs first-order stories) and (5) salience (unexpected go/no-go vs expected go/no-go). As expected, each task primarily

engaged an independently defined large-scale cortical network: The language task activated the language network, the mental rotation task activated the dorsal attention network, the salience task activated the cingulo-opercular network, the memory task activated the default mode network, and the TOM task activated a TOM network. Further, all tasks significantly activated the core MD network. However, each task fractionated MD regions in a manner consistent with their fine-grained intrinsic connectivity with the engaged RSNs. Surprisingly, we observed coarse similarities in how supposedly distinct cognitive domains (e.g. language and mental rotation) engage core MD regions. These results support our framework and suggest that, occupying a central position between multiple domain-specific networks, core MD regions are well placed to integrate the components of a cognitive operation to generate flexible cognition.

Disclosures: D. Faber: None. **G. Shields:** None. **J. Duncan:** None. **M. Assem:** None.

Poster

PSTR423

Inhibitory Control and Cognitive Flexibility

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Univ. of MD Visiting Graduate Fellows in Neuroscience Program (SEA)

Title: Action signals in the rat nucleus accumbens during STOP-change performance are modulated by sex & early life inflammation

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Abstract: Nucleus accumbens (NAc) is thought to contribute to motivated behavior by signaling the value of reward-predicting cues and the delivery of anticipated reward. There are numerous theories regarding the functions of NAc signals and cell types, but most are in the context of reward processing with fewer considering that NAc might serve functions related to action selection more generally. Here, we recorded from NAc single neurons as rats performed a STOP-change task that is commonly used to study motor control and impulsivity. In this task, rats respond quickly to a spatial cue on 80% of trials (GO trials) and must stop and redirect planned movement on 20% of trials (STOP trials). Both NAc dysfunction and impulsivity have been associated with neuropsychiatric developmental disorders (NDDs), and similarly recapitulated by

rodent NDD models like early life inflammation; with that framework, we recorded from adult male and female rats treated with the viral mimic polyinosinic:polycytidylic acid (poly(I:C), 5 mg/kg) on postnatal days 8 and 10, which roughly correlates to late third trimester pregnancy in humans. We first divided cells based on whether they increased (n = 351) or decreased (n = 578) firing during the reward epoch relative to the baseline epoch. In increasing-type cells recorded from males and vehicle-treated females, activity signaled accurate response direction on GO, but not STOP trials; cells recorded from poly(I:C)-treated females uniquely represented direction on both trial types. Interestingly, increasing cells exhibited higher pre-cue firing after correct trials—an effect that was stronger in males compared to females, and blunted in males that experienced early life inflammation. In contrast, decreasing-type cells recorded from all groups accurately reflected correct response direction on both trial types. In this cell population, proactive modulation following correct trials was present in males but not females, suggesting that this firing pattern is overall more prevalent in males across two cell populations. Sex and early life inflammation also shifted the relative abundance of cell types we recorded from, with a greater proportion of decreasing cells recorded from poly(I:C)-treated males compared to that from all other groups. As neural recordings were conducted following a battery of behavioral tasks across the lifespan, we also identified several novel relationships between neural activity patterns and behavior earlier in life. Collectively, these data suggest that NAc neuronal populations differentially contribute to action selection, which can further be modulated by both sex and early life inflammation in a rat model of NDDs.

Disclosures: S. Ashton: None. **N. Kang:** None. **A.T. Brockett:** None. **M.R. Roesch:** None. **M.M. McCarthy:** None.

Poster

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Program #/Poster #: PSTR423.02/T3

Topic: H.04. Executive Functions

Title: Motor inhibition and impulsivity in cocaine use disorder: comparison between urine-positive/negative consumption

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Abstract: As part of inhibitory control, motor inhibition is related to impulsivity traits and severity in cocaine use disorder (CUD). Acute consumption of cocaine/crack is supposed to facilitate different cognitive processes. In contrast, literature has shown both improvement and impairment in performance in CUD depending on consumption status. Thus, we compared motor inhibition between participants with positive (COC+) and negative (COC-) cocaine consumption. Also, we aimed to relate motor inhibition with impulsivity trait. Here, we show basal data of an ongoing clinical trial using neuromodulation in CUD. The study followed the Declaration of Helsinki and was approved by the local ethics committee. Participants gave a written informed consent. We used the computerized Stop Signal Task to measure the stop signal reaction time (SSRT), defined as the time that takes the inhibitory process. Impulsivity was measured by self-report Barrat Impulsiveness Scale (BIS-11). Cocaine/crack consumption was confirmed by qualitative multi-drug test. A Welsch-t test was used to compare SSRT between groups and a lineal regression was performed to explore the relationship between SSRT and BIS-11. Thirty-two participants (COC+ n=23, COC- n=9) were recruited to the study. Sociodemographic information was as follow: age (COC+ 34.82 ±7.49; COC- 28.77 ±8.4), years of consumption (COC+ 8.91 ± 6.02; COC- 6.44 ± 5.83). In COC+ group mean SSRT were of 330.46 ± 81.08 s. while in COC- was of 313.45 ± 116.4 s. Comparison by Welsch-t test showed no difference between COC+ and COC- in SSRT (t=-0.42, df=12.8, p=0.6). Lineal regression analysis was performed by total sample (n=32) and was statistically significant (R sq ad= 0.10, $F_{1,30}$=4.59, p=0.04). This analysis showed a -1.51 ms. reduction in SSRT for each point augmentation in BIS-11 (t= -2.143, p=0.04). Recent cocaine/crack consumption could facilitate different cognitive processes, as part of the intoxication period and to be extensive afterhours. However, our results suggest that motor inhibition, as elementary cognitive ability, could not be susceptible of facilitation of recent cocaine/crack consumption. Moreover, higher impulsivity might reflect elevation in other compensatory abilities that contribute to motor inhibition.

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Poster

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Topic: H.04. Executive Functions

Support: NIDA DA031695

Title: Unilateral excitotoxic lesions to the submedius thalamic nucleus disrupt action selection and goal-directed behaviors

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Abstract: The ability to flexibly adapt goals and actions in the face of novel sensory information is an essential component of almost all goal-directed behaviors and cognitive health more generally. This ability has traditionally been studied in the context of the frontal lobe, however, increasing evidence has demonstrated that individual thalamic nuclei dynamically regulate these processes as well. Here we investigated the contributions of the submedius thalamic nucleus (SUB) to both inhibitory control and goal-tracking behaviors, separately. We show that unilateral ibotenic acid lesions to the SUB disrupt action selection on the stop-change task, a variant of the canonical stop-signal task, commonly used to study motor control and response inhibition. In this task, rats respond quickly to a spatial cue on 80% of trials (GO trials) and must stop and redirect planned movement on 20% of trials (STOP trials). Lesioned rats performed worse on GO trials that required a response to be made in the direction ipsilateral to the lesioned hemisphere but showed no response inhibition impairments on STOP trials or deficits in motivation or attention. We attribute this deficit in action selection to a failure to attend to goal-relevant cues, as in a separate experiment, we show that unilateral lesions to the SUB also attenuated goal-tracking performance on a Pavlovian autoshaping task. Collectively, our findings support a role for the SUB in attending to goal-relevant sensory information that may then inform frontal areas about changes in environmental contingencies.

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Poster

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Title: Noradrenergic modulation of neuronal population dynamics in the prefrontal cortex during inhibitory control

Authors: *A. LIU, Y. NONG, Q. WANG;
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Abstract: Inhibitory control stands as a critical executive function enabling animals to suppress impulsive behavior for goal achievement or punishment avoidance. The prefrontal cortex (PFC) has long been implicated in this capacity. Recent work underscores that both norepinephrine (NE) and acetylcholine (ACh) neuromodulators play an important role in regulating inhibitory control. We previously discovered the relevance of prefrontal NE-ACh phase synchrony to inhibitory control by selectively inactivating basal forebrain (BF)-projecting locus coeruleus (LC) neurons in mice performing an inhibitory control task. However, the effect of inhibiting this particular noradrenergic pathway on PFC population activity mediating inhibitory control remains elusive. Using Neuropixels 1.0 probes, we measured population activity in the prefrontal cortex of mice performing inhibitory control tasks. In this behavior paradigm, water-deprived animals were trained to withhold their habitual licking of the water spout for a variable period cued by a tone. Premature licking elicited a brief punishing air puff to the face, while successful withholding led to a sweet water reward. At the single cell level, we categorized neurons as encoding inhibitory control or predicting 'action' based on their unique firing pattern prior to behavioral outcomes, with the former exhibiting a significant firing rate difference between successful and failed trials, while the latter showing a rapid increase in firing rate before animal licking in failed trials. Following LC inhibition, animals exhibited lower success rates in lick-withholding. Our data showed a significant decrease in both firing rate and the number of encoding neurons, which was not observed among action-predicting neurons. At the population level, inhibiting BF-projecting LC neurons disrupted the correlation structure among encoding neurons more prominently than among non-encoding neurons in the PFC. Interestingly, a demixed principal component analysis (dPCA) further revealed that LC inhibition impaired the low-dimensional population firing structure representing inhibitory control and its correlation with inhibitory control performance. Altogether, our findings provide insights into how the LC-NE system modulates PFC population dynamics during inhibitory control.

Disclosures: A. Liu: None. **Y. Nong:** None. **Q. Wang:** Other; Conflict of interest: Q.W. is the co-founder of Sharper Sense, a company developing methods of enhancing sensory processing with neural interfaces.

Poster

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Title: Action conflict neurons in the cingulate cortex of non-human primates

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Abstract: The midcingulate cortex (MCC) has been implicated in cognitive control of actions. A theory regarding the monitoring of conflict between different action plans predicted that cells responsive to this conflict would be in this region (Botvinick et al., 2001). Conflict associated activity has been reported in this region using fMRI (Kerns et al., 2004), EEG (Yeung & Nieuwenhuis, 2009) and single unit recordings in humans (Sheth et al., 2012, Fu et al. 2019). However, investigations in non-human primates (NHPs) reported the absence of these cells in this region (Ito et al., 2003, but see Ebitz & Platt 2015). This brought into question the use of monkeys as a model for cognitive control (Cole et al. 2010 but see Schall & Emeric 2010). Previous findings may have suffered from incomplete sampling; therefore, we are using 32-channel linear electrodes that offer more reliable sampling. We trained two male NHPs (*Macaca mulatta*) to do a saccade countermanding task. In this task fixating animals are presented with a target at the same time as the disappearance of the fixation point (FP), and they are expected to make a saccade to receive reward. On a minority of trials, the FP reappears (stop signal) and the animal must inhibit the saccade to receive reward. The stop signal delay (SSD) before FP reappears varies, which modulates success probability, and increased SSD should increase the conflict between actions. We sampled both dorsal (area 6/32, dMCC) and ventral (area 24c, vMCC) banks of the cingulate sulcus, separated based on current-source/power spectral density. Unlike previous studies that have used population statistics, we are going to look at each neuron individually. We recorded from 32 grid locations spanning +26-34mm AP and 94 penetrations, yielding 1566 units (949 dMCC, 617 vMCC). We aligned activity to the SSD and used a linear model with a 100ms sliding window to detect instances where activity during cancelled trials was higher than latency-matched no-stop trials for at least five 10ms steps, of which we found 466 units (317 dorsal 149 ventral). We also assessed for when cancelled activity was higher than SSD matched non-cancelled, of which there were 322 units (234 dMCC, 88 vMCC). For these units, we fit cancelled activity based on SSD. We considered the 41 units (37 dMCC, 9 vMCC) where activity significantly increased with SSD to be action conflict units. Only 1 unit passed this test when we analysed a 500ms fixation window. Future analyses will assess whether densities are different between dorso-medial frontal cortex (Sajad et al. 2022) and dMCC. However, it does not appear that vMCC has the capacity to contribute significantly to conflict monitoring.

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Poster

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Title: Neural mechanism of pause-then-cancel model of movement inhibition: evidence from caudate nucleus

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Abstract: The ability to inhibit impending response warranted by a sudden change in the environment is crucial for flexible goal-directed behavior. It is often studied using stop-signal task (SST) in which movement is elicited by a 'go' signal, but then in a minority of the trials; needs to be inhibited in response to a 'stop' signal. Unitary process account of inhibition in SST is challenged by pause-then-cancel dual-process that suggests that any infrequent salient signal triggers a global suppression that supports a subsequent 'cancel' process. The hyperdirect pathway of basal ganglia is proposed to implement the 'pause' whereas the corticostriatal pathway implements the 'cancel'. However, no study directly investigated the pause and cancel using additional salient signal while recording the activity in the striatum. In this study, two monkeys performed saccadic eye-movement version of SST with additional infrequent continue (or ignore) trials wherein monkeys were required to ignore the signal and continue generating the movement. The reaction time in continue trials was longer than in go trials due to a brief pause in the saccade after the continue signal. We recorded extracellular activity of neurons from the caudate nucleus (2426 neurons) to study the neural mechanisms underlying stopping and pausing in continue. Neurons demonstrate the hallmark of participating in the go and stop process as the activity of direction-selective saccade related neurons increased in go trials, whereas in correct stop trials activity initially increased but then decreased with short latency after the stop signal appeared. Activity in response to the stop and continue signal initially evolved similarly as it was briefly interrupted (or paused) after the signal appeared. After this initial common processing, activity further decreased for correct stop trials and increased for continue trials. Interestingly, this second phase of increase resulted in higher activity around the saccade onset than go, suggesting an increase in the threshold. In sum, pause in reaction time distribution and caudate neurons' activity provide evidence for pause in the ongoing movement plan by infrequent salient signal (continue). Subsequently, the threshold for movement generation is set higher which probably 'buys time' for the cancel process. These findings provide evidence supporting the implementation of the pause-then-cancel model in the corticostriatal pathways.

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Poster

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Topic: H.04. Executive Functions

Title: Dopamine signal imaging from distinct subregions of the striatum in macaque monkeys performing a saccadic stop-signal task

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Abstract: Animals have the ability to inhibit inappropriate actions, known as "response inhibition". This ability involves the cortico-basal ganglia loop circuitry and is impaired in patients with dysfunctions of the dopamine system that is a key component of this loop circuitry. However, the circuit-level mechanisms by which the dopamine system regulates response inhibition remain ambiguous. To address this issue, we attempted to identify dopamine signals transmitted to the striatum, a principal input nucleus of the basal ganglia and the recipient of extensive dopamine input, in macaque monkeys who were performing a saccadic stop-signal task. At the beginning of each trial, the monkey was required to gaze a fixation point. Subsequently, this point disappeared and a saccadic target was presented. In 70% of the trials, the monkey was required to make a saccade to the target (no-stop-signal trials). In the remaining 30%, the fixation point reappeared as a “stop signal” (stop-signal trials). The monkey was then required to cancel a planned saccade. Here we utilized the dopamine biosensor, dLight1.1, and measured dopamine transients in distinct subregions of the striatum (i.e., the caudate nucleus, putamen, and nucleus accumbens). We found that dopamine release within the putamen increased when the monkey successfully canceled a planned saccade, while decreased when the monkey failed to do so. These dopamine transients were similar to dopamine neuron responses to reward and no-reward, but preceded the onset of reward delivery. Notably, the magnitude of dopamine release increment in stop-signal trials was negatively correlated with the latency of saccade in the next no-stop-signal trial, indicating that the larger the dopamine release increment in stop-signal trials, the shorter the saccade latency in the next no-stop-signal trial. Thus, the enhancement in dopamine signal seemed to facilitate the saccade in the next trial, as if the dopamine signal made the animal impulsive. Our findings suggest that the pathway from midbrain dopamine neurons to the putamen conveys a critical signal that regulates impulsivity and is involved in response inhibition in a proactive manner.

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Poster

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Title: The role of the Nucleus Reuniens in inhibitory control: prefrontal-thalamic pathways underlying inhibitory control over the hippocampus

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Abstract: Humans have mechanisms enabling them tocontrol memory retrieval process. Such control becomes important when one isconfronted with intrusive memories. The precise fronto-hippocampal inhibitorycontrol pathway(s) through which memory suppression is accomplished in humansis unknown. Evidence in rodents and primates suggests that a small but criticalstructure in the ventral midline area of the thalamus, the nucleus reuniens(NRe) plays a key role in mediating top-down control. Further, it has been hypothesizedthat fear extinction, the basis of exposure therapy, employed with PTSDpatients could engage retrieval stopping. Individuals could employ retrievalstopping while trying to extinguish fear. The NRe has been implicated in fearextinction in rodents. However, there have been no attempts in the human brainto examine the function of this small but vital nucleus. In the current study,we conducted a meta-analysis of fMRI studies (n=330) using the Think-No-think(TNT) task. The TNT task is typically associated with behavioural measures suchas suppression-induced forgetting (SIF) and intrusions which has revealedfronto-hippocampal control mechanisms that support retrieval stopping. Wesought to understand whether the NRe contributes to this fronto-hippocampalmodulation. fMRI data were analysed using an ROI approach andcorrelations between BOLD response in the NRe and behavioural measures (SIF andintrusions). We hypothesized that the dorsolateral prefrontal cortex (DLPFC)downregulates the hippocampus (HpC) via the NRe. We also quantified the levelof activity during No-Think trials and correlated it with correspondingactivity in the DLPFC and HpC. Additionally, we correlated this activity indexwith behavioural indices. Our results show that the NRe is engaged duringretrieval stopping, consistent with a role in top-down control of the memoryretrieval process. Ongoing work is examining how the human prefrontal cortexachieves control over hippocampal activity by driving interactions (i.e.,effective connectivity) between the DLPFC, the NRe and the hippocampus.Further, an ongoing fMRI study is examining whether retrieval stopping studiedvia

the TNT task, and fear suppression studied via Pavlovian fear conditioning,share common neural mechanisms.

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Poster

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Title: The role of human subthalamic nucleus in inhibition of competing task representations

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Abstract: Inhibitory control is a critical component of executive function processes that allow for flexible, intelligent behavior. In the motor system, inhibitory control relies on a fronto-subthalamic system, with the subthalamic nucleus (STN) playing a crucial role in implementing inhibition. Recently, accumulating evidence has suggested that this system is involved in domain general inhibitory control of both motoric and cognitive processes. In this study, we attempt to test the domain-general role of STN, by examining its contribution towards inhibiting conflicting task representations. Effects of conflicting task representations are measurable via the N-2 Repetition Cost (N2RC). The N2RC denotes a reaction time (RT) increase when the same task context across trials 1 and 3 of a successive trial triplet (ABA triplets) requires different responses, compared to triplets with no contextual overlap (CBA triplets). One existing theory holds that the N2RC reflects the need to inhibit the task representation of trial 1 when reactivated by the overlapping task context on trial 3. We hypothesize that the STN, through domain general inhibitory control, plays a role in the inhibition of these conflicting task representations. One way to collect causal evidence of STN involvement is through deep brain stimulation (DBS) of the STN. STN-DBS is commonly used to treat hypokinetic symptoms in Parkinson's disease as it decreases inhibition of the motor system. In this study, 16 STN-DBS patients (Age mean(SD) = 64.1 (8.0), 2 left handed, 1 female) performed two sessions - one with DBS on and one with DBS off - completing an N2RC task while undergoing EEG recording. We predicted that if the STN inhibited inappropriately active task representations on ABA-trials, the N2RC cost would be greater ON DBS, where STN-generated inhibition is reduced. The behavioral results showed no difference in N2RC between on and off states, neither in RT, nor in accuracy. While preliminary, these results suggest that STN may not be involved in this type of task representation inhibition.

Further analysis of the EEG data will be informative in assessing whether task representations are modulated by STN-DBS.

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Poster

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Title: Neuro-navigated cTBS of pre-SMA reveals complementary subprocesses underlying action-stopping

Authors: *J. R. TATZ[1], D. A. DIESBURG[2,3], N. CREMERS[1], B. D. UITERMARKT[1], A. D. BOES[1], J. R. WESSEL[1];
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Abstract: Recent theories of inhibitory control propose that action-stopping involves two sequential phases: Pause and Cancel. These phases are proposed to be implemented via a fronto-basal ganglia network including right inferior frontal cortex (rIFC) and pre-supplementary motor area (pre-SMA). The Pause process is triggered by attentional detection of salient events and is characterized by early reductions in electromyography (EMG). The Pause process is thought to be implemented by rIFC, and patients with lesions to this area show markedly higher rates of trigger failures, indicating potential lapses in attentional detection of the stop signal. However, later occurring neurophysiological signatures of action stopping and the subthreshold EMG that remains after the Pause phase may necessitate a "Cancel" process. To investigate the role of pre-SMA in the Cancel process, we utilized neuro-navigated continuous theta burst stimulation (cTBS), EMG recordings, and hierarchical Bayesian modeling in a stop-ignore task. In this task, subjects had to abort responses to the Stop signal while continuing responses to an Ignore signal with matching attention-capturing properties. Whereas overall stop-signal reaction time did not differ across stimulation conditions (no cTBS, active cTBS, sham cTBS), hierarchical Bayesian modeling indicated reduced attentional trigger failures after active cTBS. Further, although subthreshold EMG on successful stop trials was observed in each stimulation condition (indicating an intact Pause process), EMG after active cTBS showed a different pattern after this initial peak. Namely, the initial reduction in EMG showed a more rapid decline and was followed by a second subthreshold peak. These results suggest that subjects may compensate for

an impaired Cancel process by increasing their ability to implement the Pause process. Overall, our findings suggest that the pre-SMA may implement a Cancel process, which operates in tandem with a Pause process to achieve action stopping.

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Program #/Poster #: PSTR423.11/U1

Topic: H.04. Executive Functions

Support: NIH Grant NS102201

Title: The human subthalamic nucleus transiently inhibits active attentional processes

Authors: *C. SOH[1,2,3], M. HERVAULT[4,3], N. CHALKLEY[4,3], A. ROHL[5], Q. ZHANG[6], E. UC[7], J. D. GREENLEE[8], J. R. WESSEL[4,3,9];
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Abstract: The subthalamic nucleus (STN) of the basal ganglia is key to the inhibitory control of movement. Accordingly, it is a primary target for the neurosurgical treatment of movement disorders like Parkinson's Disease, where modulating the STN via deep-brain stimulation (DBS) can release excess inhibition of thalamo-cortical motor circuits. However, the STN is also anatomically connected to other thalamo-cortical circuits, including those underlying cognitive processes like attention. This suggests that the STN may also contribute to the inhibition of those processes. We here tested this hypothesis in humans. We used a novel, wireless outpatient method to record intracranial local field potentials from STN DBS implants during a visual attention task. We also modulated STN via DBS. In both cases, we simultaneously recorded high-density EEG to extract the steady-state visual evoked potential (SSVEP), a neural measure of visual attentional engagement. Introducing unexpected, distracting sounds lead to a momentary reduction of this SSVEP. This suppression was preceded by sound-related γ-frequency (>60Hz) activity in STN, which scaled with each sound's surprisal value. The STN activity statistically mediated the suppressive effect of surprisal on the SSVEP. Finally, modulating STN activity via DBS reduced the sound-related SSVEP-suppression. Together, these findings provide the first causal evidence that the human STN contributes to the inhibition

of attention, a non-motor process. Beyond their support for a domain-general inhibitory role of the STN, these findings also suggest a mechanism underlying known cognitive side-effects of STN DBS.

Disclosures: C. Soh: None. **M. Hervault:** None. **N. Chalkley:** None. **A. Rohl:** None. **Q. Zhang:** None. **E. Uc:** None. **J.D. Greenlee:** None. **J.R. Wessel:** None.

Poster

PSTR423

Inhibitory Control and Cognitive Flexibility

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR423.12/U2

Topic: H.04. Executive Functions

Support: Egg Nutrition Center Grant
Univ. of Illinois Personalized Nutrition Initiative Grant
Univ. of Illinois Division of Nutritional Sciences 50th Award

Title: Brain volume and the neuroelectric indices of inhibitory control in school-aged children

Authors: *L. ROSOK[1], C. N. CANNAVALE[1], C. ROMÁN[1], J. KIM[1], M. PASCUAL-ABREU[1], C. KINDER[1], S. KEYE[1], H. SCHWARB[2], B. SUTTON[1], N. A. KHAN[3];
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Abstract: The P3 and N2 event-related potential (ERP) components are thought to reflect resource allocation and information processing speed to support attentional and inhibitory control, respectively. Previous studies have suggested that the P3 may be generated by the superior parietal lobe, posterior cingulate gyrus, and inferior temporal gyrus, while the N2 may be generated by the anterior cingulate gyrus. However, the volume of these regions in relation to the characteristics of these neuroelectric outcomes have not been adequately studied, especially among children. This study aimed to examine how P3 and N2 amplitudes and latencies may relate to superior parietal lobe, cingulate gyrus, and inferior temporal gyrus white matter volume in school-aged children. Cross-sectional data were used to conduct analyses among 33 school-aged children ages 7-13 years ($\bar{X}$ = 10.92 ± 2.25). ERP components were recorded during a modified Eriksen flanker task, with incongruent trials requiring greater attentional inhibition relative to congruent trials. The P3 was extracted from a six-electrode ROI and the N2 was extracted from the FPZ electrode. T1-weighted scans were acquired with a clinical whole-body Siemens 3T Prisma MRI scanner (Siemens Medical Solutions; Erlangen, Germany). Following bivariate correlations, linear regressions were conducted to assess brain volume in relation to ERP outcomes, adjusting for age, sex, household income, and intracranial brain volume. Right hemisphere white matter inferior temporal gyrus volume was negatively related to incongruent N2 mean amplitude (β = -0.83, p = 0.04) and congruent and incongruent N2 peak amplitude

(congruent: $\beta = -0.79$, $p = 0.04$; incongruent: $\beta = -0.96$, $p = 0.02$). Bivariate correlations revealed that P3 amplitude and latency were not significantly related to superior parietal lobe, cingulate gyrus, or inferior temporal gyrus white matter volume. These results indicate that larger inferior temporal gyrus volume may be related to larger N2 amplitude in school-aged children, suggesting greater inhibitory resource allocation. While inferior temporal regions, crucial for selective visual processing (e.g., pattern, face, and object recognition), are implicated in P3 generation, these results are not surprising, as N2 is also an attentional component triggered by visual stimuli. Future interventional trials are needed to better understand the relationship between the neuroelectric indices of inhibitory control and brain volume in school-aged children.

Disclosures: L. Rosok: 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.; Egg Nutrition Center Grant. **C.N. Cannavale:** 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.; Univ. of Illinois Urbana-Champaign Personalized Nutrition Initiative Grant. **C. Román:** None. **J. Kim:** None. **M. Pascual-Abreu:** None. **C. Kinder:** None. **S. Keye:** None. **H. Schwarb:** None. **B. Sutton:** None. **N.A. Khan:** 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.; Egg Nutrition Center Grant, Univ. of Illinois Urbana-Champaign Personalized Nutrition Initiative Grant.

Poster

PSTR423

Inhibitory Control and Cognitive Flexibility

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR423.13/U3

Topic: H.04. Executive Functions

Support: CIHR

Title: Does Early Bilingual Experience Confer Cognitive Benefits? Investigating Inhibitory and Attentional Control in Bilingual Preschoolers

Authors: *S. SADE[1], B. E. KOLB[2], C. L. GONZALEZ[1], R. L. GIBB[3];
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Abstract: One of the most profound, constant, and integrative human experiences modulating brain function is language use. For more than half of the world's population this extends to managing two languages. Bilingual individuals rarely report intrusion errors or confusion paralysis, instead, they are capable of inhibiting one language to successfully attend to another.

The adaptive control hypothesis assumes there is an adaptation in domain general processes of attention subsequently shaping performance on nonverbal tasks recruiting such cognitive (executive) control. The objective of this study was to investigate the effects of bilingualism on executive control in 3-5-year-old children. To do this, children were assessed using a comprehensive battery of tests of executive function (EF), selective attention, inhibitory function, emotional regulation, and language. Tasks included the animal Stroop and Dimensional Change Card Sort for EF evaluation, and the flanker task for selective attention and inhibitory function. Language proficiency was measured using the Peabody Picture Vocabulary Test and emotional regulation through dyadic social play with LEGO. Caregivers completed subjective surveys, including the Behaviour Rating Inventory of Executive Function in Preschoolers (BRIEF-P), Adverse Childhood Experiences Questionnaire and Language Experience and Proficiency Questionnaire. Results indicated favourable performance among bilingual children across tasks. Notable distinctions were also observed in emotional control, flexibility and inhibitory self-control as indicated by the BRIEF-P. These findings provide support for the adaptive control hypothesis, suggesting bilingualism may elicit changes in domain general processes of attention.

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Poster

PSTR423

Inhibitory Control and Cognitive Flexibility

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR423.14/U4

Topic: H.04. Executive Functions

Support: the Campus Research Board University of Illinois Urbana-Champaign Grant No. RB20025

Title: Interrupting sitting with moderate-intensity physical activity breaks improves inhibitory control in adults with overweight and obesity: findings from the sitless pilot randomized crossover trial

Authors: *D. M. PINDUS[1], J. KUANG[1], K. M. MCDONALD[2], T. S. LIGEZA[3], H. MARTIN[1], L. PICKERILL[1], S. LIANG[1], T. SYED[1], A. SHARMA[1], F. B. QUIROZ[1], Q. YU[4], C. N. CANNAVALE[1], L. ZOU[4], N. A. BURD[1], N. A. KHAN[5], A. F. KRAMER[6], C. HILLMAN[7]; [1]Hlth. and Kinesiology, Univ. of Illinois Urbana-Champaign, Urbana, IL; [2]Dept. of Psychology, Northeastern Univ., Watertown, MA; [3]Inst. of Psychology, Jagiellonian Univ., Krakow, Poland, Kraków, Poland; [4]Sch. of Psychology, Shenzhen Univ., Shenzhen, China; [5]Hlth. and Kinesiology, Univ. of Illinois At Urbana-Champaign, Urbana, IL; [6]Psychology, Northeastern Univ., Boston, MA; [7]Dept. of Psychology, Northeastern Univ., Boston, MA

Abstract: This study tested the acute effects of interrupting three-hour prolonged sitting every 30 minutes with 3.5-minute moderate-intensity physical activity bouts (MPA+SIT) on inhibitory control relative to a sedentary social interaction condition (SOC+SIT) in young and middle-aged adults with overweight or obesity (O/O). Data from 19 adults (63% females; 29.9±7.5 years) were analyzed from the SITLess pilot randomized crossover trial. Inhibitory control was expressed as accuracy and reaction time (RT) on incongruent trials of a flanker task. Choice RT was expressed as accuracy and RT on congruent trials. Attentional resource allocation and stimulus evaluation speed were measured using P3b amplitude and latency of event-related brain potentials, respectively. Intervention effects were tested using Generalized Linear Mixed Models with Time (pre, post) by Condition (MPA+SIT vs. SOC+SIT) interactions and simple effects within each time point. Participants were faster on incongruent trials after MPA+SIT than SOC+SIT ($F(18.0, 54) = 5.59$, $p = 0.02$; $\Delta M = 16.7$ ms, 95% CI: 1.64, 31.7). A similar trend ($F(18.0, 54) = 4.03$, $p = 0.05$) emerged for congruent trials ($\Delta M = 17.3$ ms, 95% CI: 5.66, 29.0). P3b amplitude and latency did not differ between conditions. Interrupting sitting with short MPA bouts is a viable strategy to improve cognitive performance in adults with O/O. A definitive trial should test its efficacy in enhancing cognitive and brain health in O/O.

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Poster

PSTR423

Inhibitory Control and Cognitive Flexibility

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR423.15/U5

Topic: E.04. Voluntary Movements

Support: Royal Society Te Aparangi Marsden Fast-Start Grant 19-UOA-220

Title: Tired and out of control? Effects of total and partial sleep deprivation on response inhibition under threat and no-threat conditions

Authors: *A. NIEUWENHUYS[1], C. G. WADSLEY[2], J. CIRILLO[3], R. SULLIVAN[1], W. D. BYBLOW[1];
[1]The Univ. of Auckland, Auckland, New Zealand; [2]Dept. of Human Physiol., Univ. of Oregon, Eugene, OR; [3]Discipline of Physiol., The Univ. of Adelaide, Adelaide, Australia

Abstract: Study objectives: Neurophysiological evidence suggests that sleep deprivation may impair top-down inhibitory control over emotional responses. The current study examined behavioral consequences of this phenomenon and manipulated the magnitude of individuals' sleep deficit to determine effect thresholds. **Methods:** Twenty-four healthy human participants

were provided with 0hr, 2hr, 4hr and 8hr of sleep opportunity and, subsequently, performed a bimanual anticipatory response inhibition task under threat and no-threat conditions. Behavioral measures of going and stopping were taken and surface electromyographical (EMG) recordings were collected from task effectors to examine underlying neurophysiological activity. **Results:** Bayesian analyses of variance revealed that compared to 8hr sleep, go trial success was reduced with 0hr sleep. Stopping speed was reduced with 0hr and 2hr of sleep, as evidenced by longer stop-signal delays, but only in a selective stopping context. None of the outcome measures was impacted with 4hr sleep. Under threat, go trial success was maintained, whilst response times were slightly delayed and characterized by more attenuated response-related EMG-bursts. Stopping speed was increased under threat across both stop-all and selective stopping contexts. No evidence was observed for interactions between sleep and threat. **Conclusions:** Sleep deprivation negatively affects response inhibition in a selective stopping context, with stopping speed reduced following a single night of ≤ 2hr sleep. Performance-contingent threat improved response inhibition, possibly due to a prioritizing of stopping. No evidence was observed for increased threat-related responding after sleep deprivation, suggesting that sleep deprivation and threat may impact top-down inhibitory control via different mechanisms.

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Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR424.01/U6

Topic: H.07. Long-Term Memory

Support: FWO Funding G0B4520N
NIH Funding 1R01MH123508-01
CSC202006380043

Title: An investigation of the effect of external trigeminal nerve stimulation on the hippocampus: insight from rat experiment and human study

Authors: *L. CHEN[1], X. GUO[2], Q. SUN[1], N. SEMINCK[1], H. WU[2], M. MC LAUGHLIN[1]; [1]Neurosci., KU LEUVEN, LEUVEN, Belgium; [2]Dept. of Neurosurg., Second Affiliated Hosp., Zhejiang Univ. Sch. of Med., Hangzhou, China

Abstract: Recent studies have implicated a non-invasive neuromodulation method, external trigeminal nerve stimulation (eTNS), in modulating broader neural circuits, including those involved in cognitive functions, such as memory and attention. Within this complex neural network, one particularly intriguing aspect is its interaction with the hippocampus—a pivotal structure renowned for its role in learning and memory consolidation. Previous studies in animal

have revealed the possibility of hippocampal neuromodulation through trigeminal stimulation, potentially via indirect pathways involving activation of noradrenergic nuclei in the brainstem. However, the exact mechanisms underlying these effects remain poorly understood, particularly when considering neurotransmitter dynamics and electrophysiological correlates.
In 6 male Sprague Dawley rats, we implanted Nafion-coated carbon fiber electrodes (sensitivity: 37.1nA/uM) in the hippocampus CA1 to detect dopamine/norepinephrine (DA/NE) using fast scan cyclic voltammetry. We applied 5 minutes of high-frequency intermittent stimulation (200 Hz burst for 1 second, repeated every 30 seconds) on the dermatomal distribution of the mandibular branch of the trigeminal nerve in rats, followed by post-recording for 50 minutes after eTNS. In a clinical study, stereo electroencephalography (sEEG) electrodes were implanted in 8 patients with refractory epilepsy for the measurement of hippocampal evoked potentials (HEP) and local field potentials (LFP). We applied 20 minutes of eTNS in each patient, followed by post-recording for 1 hour. We analyzed the evoked potentials elicited after the onset of each burst stimulation and compared changes in LFP power spectra before, during, and after stimulation. The effect of TN-HFS on the amplitude of HEP and LFP spectra was analyzed using a linear mixed-effects model.
In 6 rats, we observed that during eTNS, DA/NE increased by 0.20±0.06μM, gradually reaching a peak increment of 1.725±0.74μM around 40 minutes, where it remained stable. Data from 8 patients showed that eTNS caused a distinct evoked potential with a negative peak (-74.60±20.44mv) at 401.5ms in the hippocampus, which was not present in other brain structures. Analysis of the LFP showed that eTNS caused an increase in high gamma activity, which remained elevated 60mins after eTNS was stopped.
Our findings demonstrate the efficacy of eTNS in modulating hippocampal activity and neurotransmitter dynamics. These results underscore the potential therapeutic application of eTNS for cognitive enhancement and neurological disorders, while highlighting the need for further behavior investigations.

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Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR424.02/U7

Topic: H.07. Long-Term Memory

Support: NSF 2123474
Texas Woman's University Woodcock Institute Research Grant

Title: Early Neural Predictors of Threat-Related Long-Term Memory Reinstatement in Hippocampus

Authors: *B. TANRIVERDI[1,2], L. SKALABAN[3], D. F. GREGORY[3], I. R. OLSON[3], J. M. CHEIN[3], V. P. MURTY[3];
[1]Temple Univ., Philadelphia, PA; [2]Psychology and Neuroscience, Temple University, Philadelphia, PA; [3]Psychology and Neurosci., Temple Univ., Philadelphia, PA

Abstract: While the hippocampus is influenced by threat in complex ways, and thus implicated in psychiatric conditions like PTSD, our understanding of how threat-related representations are reinstated in hippocampus over time and how they relate to subjective experience of remembering is limited. Here we test how reinstatement of threat memories are related to subsequent memory and assessments of threat representations 1-week after encoding (N = 39). On Day1, participants encoded six (3 aversive, 3 neutral) short movie clips in the scanner, each followed by a rest scan. Twenty-four hours later on Day2, participants completed a temporal order memory test. A week later on Day8, they watched a disrupted version of each clip in the scanner, during which the screen alternated between the original video (On period: 10 second) and a black screen (Off period: 20 second), with continuous audio cues. Importantly, on both Day1 and Day8, they provided valence ratings following each clip. Using representational similarity analysis, we found that higher Day1-Day8 neural similarity in right posterior hippocampus was associated with changes in valence across days, wherein clips with higher neural reinstatement (On (p<.01) and Off (p=0.08) periods) were rated more positively on the second watch (Day8). Further, we found that higher temporal memory accuracy on Day2 was associated with lower right posterior hippocampal reinstatement on Day8 (Off (p<0.05)), suggesting a consolidation effect wherein stronger representations are no longer reinstated in hippocampus. Future analyses will test how neural reactivation of threat-related information during post-encoding rest relate to the long-term reinstatement patterns observed.

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Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR424.03/U8

Topic: H.07. Long-Term Memory

Support: NIH Grant P50MH109429

Title: Human hippocampal and cortical ripple oscillations as a coordinating mechanism in the processing and memory of continuous, naturalistic stimuli

Authors: *A. MISHRA[1], G. TOSTAEVA[1], M. NENTWICH[1], E. ESPINAL[2], N. MARKOWITZ[1], S. GHERMAN[1], A. D. MEHTA[1], S. BICKEL[1];
[1]Zucker Sch. of Med. at Hofstra/Northwell, Manhasset, NY; [2]Drexel Univ., Philadelphia, PA

Abstract: The real world is a continuous, multimodal experience that humans store as rich episodic memories. Event boundaries are specific timepoints in these experiences that represent key intervals where the brain may segment narratives into smaller event chunks and consolidate event memory. Coincident ripple oscillations are a powerful mechanism that may synchronize neural activity during these processes. Here, we use intracranial recordings from 32 human participants who viewed a continuous 10-minute stimulus from the film *Despicable Me*. This stimulus contains 9 event boundaries (defined as timepoints that exhibited high consistency in a group of 200 online participants who were instructed to identify event boundaries), hence segmenting the clip into 10 discrete events. Thirteen participants also performed a free recall after stimulus viewing to describe specific aspects of the clip that they remembered. We quantified recall performance as whether a viewed event was subsequently recalled or not. We find that hippocampal ripples transiently increase in rate between 1-2 seconds following event boundaries ($p=0.026$). This effect is primarily driven by electrodes in the right anterior hippocampus ($p=0.006$). In this post-event boundary window, coincident hippocampal-cortical ripples are more present in select cortical regions that have previously shown activation around event boundaries in functional magnetic resonance imaging studies (inferior parietal, precuneus, lateral occipital, inferior frontal; $p<0.01$). Further, increases in co-ripple rate following event boundaries in these regions correlates with subsequent scene recall ($p<0.001$). Finally, coincident ripples are assembled in specific spatial and temporal patterns that occur during viewing of an event. These patterns of coincident ripples re-occur immediately following the event boundary ($p<0.001$) and again during free recall ($p=0.023$). The magnitude of ripple pattern re-occurrence relates to recall performance ($p=0.046$). Taken together, ripple oscillations serve as a powerful mechanism that coordinate activity in widespread event segmentation regions. They also are optimally positioned to function in the encoding of naturalistic memory and binding of memory representations to assemble a coherent, continuous experience.

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Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR424.04/U9

Topic: H.07. Long-Term Memory

Title: Memory strength dynamics in the primate brain

Authors: *A. PICCATO[1], G. M. STINE[2], M. JAZAYERI[3];
[1]Brain and Cognitive Sci., [2]Neurosci., MIT, Cambridge, MA; [3]Brain and Cognitive Sci., Massachusetts Inst. of Technol. Dept. of Brain and Cognitive Sci., Cambridge, MA

Abstract: A signature feature of long-term memory is its time- and experience-dependent strength. Memory strength directly impacts behavior: we recall recent and repeated experiences faster and more accurately than infrequent and remote ones. However, the neural and computational principles that link memory strength to accuracy and reaction time are not known. We designed a novel visuo-spatial association task that offers a window onto memory strength dynamics via parametric control of recency and repetition of prior experiences. Subjects acquire and retrieve visuo-spatial associations over tens of trials. Behavioral data from human and non-human primate subjects performing the task revealed that recency and repetition independently influence behavior, causing more accurate choices and shorter reaction times. The observed relationship between accuracy and reaction time led us to hypothesize that memory-based decision-making is governed by a mnemonic evidence accumulation process parametrized by memory strength. We constructed a multi-alternative bounded drift-diffusion model in which the choice and reaction time are determined by the diffusion process that first reaches the bound. Critically, the drift rate for each alternative is controlled by the corresponding memory strength, a value that is incremented after each exposure and decays exponentially with time. We found that human behavioral data can be parsimoniously explained with memory-strength dynamics that are invariant to set size, suggesting that these dynamics may be fundamental to intrinsic memory processes. Accuracy and reaction time patterns of a trained non-human primate are similar to those of human subjects, indicating that the mechanisms underlying dynamics of mnemonic strength and decision-making are shared across species. Previous work has identified the hippocampus (HC) as a critical region for the formation of long-term associative memories. We have begun recording the activity of neurons in the HC of a trained macaque performing the task with the aim of identifying a neural correlate of behavioral memory strength. Together, our results suggest that memory retrieval is a dynamic process and establishes a behavioral platform for studying the neural basis of these dynamics in the primate brain.

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Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

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Program #/Poster #: PSTR424.05/U10

Topic: H.07. Long-Term Memory

Support: NINDS Grant 1RF1NS127128-01
NEI (P30 EY008126)
Whitehall Foundation Grant 100001391

Title: Selective activation of cell assemblies during sequenced associative learning and their reactivation in subsequent sleep in freely-behaving macaques

Authors: *S. ABBASPOOR[1], K. L. HOFFMAN[2];
[1]Vanderbilt Univ., Nashville, TN; [2]Psychological Sci., Vanderbilt Univ., Nashville, TN

Abstract: The ability to flexibly learn from specific past experiences is crucial for animals to adapt to ever-changing environments. Hippocampal CA1 plays a key role in this process across species, with evidence from rodent models that such memory formation requires reliable, cooperative firing among subsets of neurons, termed "cell assemblies." Sequential activation of these assemblies during experiences supports learning and memory retrieval, and their reactivation during post-learning sleep supports memory consolidation. The existence of cell assemblies in the macaque hippocampus and their relationship to learning and memory remain largely unknown. To investigate this in freely-moving macaques, we developed a naturalistic learning paradigm. In this paradigm, monkeys learned multiple item-context sequences presented in alternating blocks between opposite corners of the environment, thus requiring flexible learning in 3D space. Within each session, one corner was allocated for learning a new set of items that the animal had not seen before, while the other corner was assigned for testing remote memories that the animal had learned 2 to 5 weeks earlier. Two female macaques successfully learned multiple sets (N=14 and 22 sets). Performance was significantly greater for remotely learned sets (2-5 weeks prior to testing) compared to novel sets, indicating long-term memory savings. During training and post-learning sleep, we used high-density laminar probes to wirelessly record ensemble spiking activity (40-300 isolated units per session) in the hippocampus and extrahippocampal areas. We applied component analysis methods to detect cell assembly patterns for each session (Total: 1197). During the task, most detected cell assemblies were biased to activate in either recently- or remotely learned sequences, but not both. During post-learning sleep, neural patterns that appeared during the task reactivated, sometimes concurrently with hippocampal sharp-wave ripples. Segregating CA1 pyramidal cells into superficial and deep layers revealed strata-specific spike-timing interactions with inhibitory cell groups, suggestive of segregated functional circuits. Correspondingly, these distinct subpopulations formed cell assemblies that were activated according to their substrata. Together, these findings highlight the functional organization of cell assemblies in the macaques, which includes their context-dependent expression and reactivation during sleep, as a potential mechanism for structuring and stabilizing memory.

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Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: MCP Hall A

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Topic: H.07. Long-Term Memory

Support: NINDS Grant 1RF1NS127128-01
NEI (P30 EY008126)
Whitehall Foundation Grant 100001391

Title: Coupling of hippocampal sharp-wave ripples to spindles signals stronger cell assembly reactivation in sleeping macaques

Authors: *A. ALJISHI, S. ABBASPOOR, K. L. HOFFMAN;
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Abstract: Non-rapid eye-movement (NREM) sleep is important for the formation of durable associative memories. One theorized role relies on the synchronization between two sleep oscillations: hippocampal sharp-wave ripples (SWRs) and sleep spindles. In rodents, these oscillations reflect conditions that are conducive to reactivation of task-related neural ensemble "cell assemblies". Both sleep reactivation of cell assemblies and the coupling of SWRs to spindles have been independently linked to memory in rodents. Consequently, sleep oscillations are posited to reflect the underlying neural circuit dynamics that organize and modify cell assemblies across regions. Whereas human studies showed that SWRs co-occur with spindles in the hippocampus and neocortex during NREM, it is unclear whether the coordination of these rhythms modulates cell assembly reactivation in primates. To address this, we implanted two female macaques with high-density laminar probes in the hippocampus and extrahippocampal areas. We wirelessly recorded local field potentials (LFPs) and ensemble spiking activity (40-300 isolated units per session) during sequenced-associative learning (N=14 and 22 item-context sets) and subsequent overnight sleep. For each sleep session (N=35), we detected hippocampal CA1 SWRs (100-180 Hz) and spindles (10-16 Hz) from the LFP, and applied component analysis methods to identify cell assemblies and detect their reactivations during sleep (N = 1197 assemblies). In line with previous reports from animal and human recordings, we observed that the highest incidence of SWRs during spindles occurred just before the spindle peak. On average, across sessions, 13% of SWRs occurred during spindle events and 11% of spindles contained SWR events. As expected from the rodent literature, cell assemblies were preferentially reactivated during SWRs. Furthermore, we found that reactivation rates in both animals during spindle-associated SWRs exceeded those occurring during isolated SWRs (average of 18% increase in peak reactivation rate, $p < 0.05$, permutation test). This uptick in reactivation during key hippocampo-cortical network events may indicate a privileged influence of hippocampal ensembles on the location and organization of memory traces across the expansive cortical mantle of primates

Disclosures: A. Aljishi: None. **S. Abbaspoor:** None. **K.L. Hoffman:** None.

Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR424.07/U12

Topic: H.07. Long-Term Memory

Support: FAPERGS-Pq2021
CNPq

Title: Different memory engrams regulate fear and extinction processes

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Abstract: Memories are encoded within engram cells, essential for memory retrieval, which can undergo reconsolidation or extinction upon recall. During reconsolidation, the original memory engram is reactivated, allowing for updates. Conversely, extinction forms a new memory trace that suppresses the original engram. Yet, it remains unclear whether extinction creates a new engram or modifies the original. Utilizing the Daun02 procedure in c-Fos-lacZ rats, we induced apoptosis in strongly activated neurons to investigate the emergence of new memory traces following short or long reactivation. We focused on the basolateral amygdala (BLA) and infralimbic (IL) cortex. Eliminating neurons activated during consolidation and reactivation significantly impacted fear memory, emphasizing the BLA's importance. Disrupting the IL extinction engram reactivated suppressed aversive memories, indicating its critical role in extinction networks. Our findings support the formation of new memory traces during extinction, contrasting with reconsolidation's reactivation of the original memory trace.

Disclosures: L. Alvares: None. **J. Griebler Luft:** None. **F.C. Cruz:** None.

Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR424.08/U13

Topic: H.07. Long-Term Memory

Support: JSPS Grant-in-Aid for Scientific Research (A) (18H04081)
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Title: Facilitating Memory Consolidation through Light Exercise: The Role of the Coeruleo-Hippocampal Dopaminergic Pathway

Authors: *T. HIRAGA[1], T. HATA[1,2], S. SOYA[3], J. P. JOHANSEN[4], T. TAKEUCHI[5,6,7], M. OKAMOTO[8,2], H. SOYA[8,2];
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Abstract: Recent research increasingly acknowledges the benefits of physical exercise in enhancing learning and memory, particularly through the dorsal hippocampus (dHPC). Our long-standing translational research with animals and humans has focused on light exercise below the lactate threshold, such as yoga and slow running. This type of exercise offers clinical benefits that almost anyone can safely perform with minimal stress and has been found to effectively increase hippocampal activity and enhance memory performance. However, prior studies, including our findings, have not fully addressed the circuit mechanism underlying this benefit. Our recent findings demonstrate that light exercise can stimulate tyrosine hydroxylase-positive (TH^+) neurons in the locus coeruleus (LC) and the ventral tegmental area (VTA), and increase the release of noradrenaline (NA) and dopamine (DA) in the dHPC. This raises the hypothesis that dopaminergic and/or noradrenergic projections to the dHPC contribute to the beneficial effect of light exercise on memory enhancement. Here, we examined this hypothesis using a rat treadmill exercise model based on the lactate threshold, combined with a dHPC-dependent object location task, pharmacological and chemogenetic manipulation, neuroanatomical tracing, and in vivo microdialysis. Our results confirmed that post-encoding-light-exercise enhanced memory retention over 24 hours in the object location task. Notably, this was inhibited by blocking the DA D1/D5 receptor, but not the β-adrenergic receptor, in the dHPC. Further investigation through anterograde viral tracing showed dense projections from LC-TH^+ to the dHPC, in contrast to the sparse projections from VTA-TH^+ neurons. Besides, retrograde tracing combined with c-Fos-based neuronal activation mapping indicated that light exercise activated TH^+ neurons projecting to the dHPC at the LC, but not the VTA. Subsequent experiments using excitatory DREADD hM3Dq to specifically activate LC-TH^+ neurons increased DA release in the dHPC and improved memory retention in a manner of hippocampal DA D1/D5 receptor-dependent. Conversely, chemogenetic inhibition of LC-TH^+ neurons using inhibitory DREADD KORD diminished memory enhancement induced by light exercise. Overall, our findings unveil a pivotal role for the coeruleo-hippocampal dopaminergic pathway in light-exercise-induced memory consolidation. This research not only illuminates the circuit mechanisms of exercise-enhanced memory but also opens avenues for accessible memory-enhancement strategies based on light exercise regimens.

Disclosures: T. Hiraga: None. **T. Hata:** None. **S. Soya:** None. **J.P. Johansen:** None. **T. Takeuchi:** None. **M. Okamoto:** None. **H. Soya:** None.

Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR424.09/U14

Topic: H.07. Long-Term Memory

Support: CNRS
Université de Strasbourg

Title: A role for the Claustrum in sleep dependent memory consolidation

Authors: *C. PORTET[1], F. THELLIER[2], K. HERBEAUX[2], D. BATTAGLIA[2], J. BAHUGUNA[3], J. C. JACKSON[4], R. GOUTAGNY[2];
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Abstract: For memories to last, they should be transformed from an initially labile state to a more permanent one (Buzsaki,1989; Frankland, 2005; Diekelmann, 2010). This memory "stabilization", also called memory consolidation, involves a dialog, mainly during sleep, between the hippocampus, where traces are initially formed, and the neocortex, where they are stored for long-term retention (Buzsaki, 1989; Euston, 2007; Diekelmann, 2010). The slow waves arising during NREM sleep (non-rapid eye movement sleep) have received particularly prominent attention as increasing slow oscillations enhances memory consolidation in both Human and animal models (Binder et al., 2014, Marshall et al., 2006). We have recently shown that the Claustrum (CLA), a small sub-cortical structure at the boundary between the striatum and the insular cortex plays a facilitating role in memory consolidation, likely through the modulation of cortical slow-waves dynamics. As the Claustrum is thought to 'integrate' several pieces of information coming from different cortical areas, we hypothesize that the claustrum might biased slow wave dynamics in multiple cortical territories, enhancing long range cortical communication and memory consolidation during sleep. Yet, the dynamics of cortical interactions during NREM following CLA activation and its possible implication in memory remain to be tested. To this aim, we performed simultaneous local field potential recordings in different cortical regions (prefrontal, retrosplenial and entorhinal cortices as well as the dorsal subiculum) while optogenetically stimulating the CLA specifically during NREM sleep following a weak learning (i.e. during the consolidation period). In accordance with our previous results, we showed that such stimulation allows memory retrieval 24 hours after the sampling, permitting the consolidation of a memory otherwise intended to be forgotten. Confirming our and other previous results, we showed that CLA stimulation tend to increase slow-waves power in cortical regions but not in the subiculum. Slow wave is however a generic term encompassing different phenomena. One of them is related to cortical up and down states, and termed delta waves. We therefore counted the delta during sleep period and showed that CLA activation increases delta occurrence and amplitude, mainly in the prefrontal cortex. We are currently

analyzing cross cortical functional connectivity to investigate Claustrum implication in long range communication through delta wave. Altogether, our results highlight a new role for the Claustrum in memory consolidation likely through the modulation of cortical delta waves.

Disclosures: C. Portet: None. **F. Thellier:** None. **K. Herbeaux:** None. **D. Battaglia:** None. **J. Bahuguna:** None. **J.C. Jackson:** None. **R. goutagny:** None.

Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR424.10/U15

Topic: H.07. Long-Term Memory

Support: NSF 1703340

Title: Hippocampal Replay and Sleep's Hidden Language: Methods for Detecting Functional Connectivity from Spike Trains

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Abstract: Hippocampal replay is characterized by the reactivation of population-wide neural sequences during non-exploratory states, such as sleep and rest. Replay is often correlated with firing sequences observed during preceding spatial navigation tasks. The generation of replay is crucial for memory consolidation and retention and is key to retrieving previous memories. Replay episodes contain information about the causality structure within a network of neurons, which helps to experimentally track how the memories are formed during learning. We investigate the relationship between replay and functional connectivity by simulating the activity of a network of CA3 place cells as seen in rats during spatial navigation. We use the NEURON simulation environment to implement a single compartment multi-current pyramidal cell network connected with realistic AMPA and NMDA synapses and fitted with realistic in vivo-like excitatory and inhibitory uncorrelated background noise. The conductances of the synaptic currents are scaled by a connectivity matrix that we manipulate to introduce controlled causality structures in subgroups of neurons. We compare the results from three approaches designed to identify those subgroups of neurons and their firing order, if any. The first approach uses spike counts over some fixed synchrony window, the second approach leverages spectral analysis with Fourier transform, and the third approach extracts a directed acyclic graph that best describes the causality relationship between neurons by capturing the underlying generative model using the mathematical framework of Markov Decision Process. All three methods are tested against the ground-truth connectivity matrix to evaluate the robustness and degradation profiles of the algorithms. We discuss how extracting the neurons involved in replay, and the order in which the

episodes propagate, highlights the role of place cells in spatial navigation, providing insights into learning and decision-making in complex environments. In future work, these algorithms will be used to assess hippocampal replay in datasets collected as the rats solve complex navigation problems in megaspace.

Disclosures: M. Yousuf: None. **M. Chertkov:** None. **J. Fellous:** None.

Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: MCP Hall A

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Topic: H.07. Long-Term Memory

Support: NIH Grant RF1NS132913
NIH Grant R01MH125557
NSF Grant 2223839

Title: Selective strengthening or weakening of memory traces during sleep depends on the synchrony of local slow waves

Authors: R. GOLDEN[1], A. MIZRAHI-KLIGER[2], G. P. KRISHNAN[3], K. GANGULY[4], ***M. BAZHENOV**[5];
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Abstract: Studies suggest the critical importance of sleep to the process of memory consolidation. However, most of this work has focused on how sleep helps to strengthen memory traces. In this new study, we focus on how, under the appropriate circumstances, slow-wave sleep (SWS) helps to forget in a selective way in order to allow for greater behavioral flexibility. We make use of a biophysical thalamocortical model capable of learning neural sequence memories during an awake state and producing spontaneous memory replay during autonomously generated Up states of SWS. The model implemented spike timing dependent plasticity (STDP), as well as a heterosynaptic plasticity (HSP) which keeps the total incoming synaptic strength to a given neuron approximately constant. Two cortical regions, representing prefrontal cortex (PFC) and motor cortex (MC), with local, recurrent connectivity within each region and feedforward connectivity from PFC to MC were simulated. Before training, the model generated local (asynchronous) Up states. During an awake phase, we first encoded a nonlinear sequence memory in MC to represent an animal learning to perform a reach movement. Subsequently, we trained a static pattern memory in PFC to represent the animal learning the task-relevance of a go-cue. During a subsequent SWS stage, we implemented a model of hippocampal indexing in order to associate the two representations. We found that

hippocampal indexing caused the Up states to synchronize, allowing for synaptic strength increases from PFC to MC. Following sleep, activation of the static pattern in PFC was sufficient to induce pattern completion of the nonlinear sequence in MC. Next, we modeled the effect of changing the motor reach target by encoding a new nonlinear sequence memory in MC, and changing the indexing protocol to associate the go-cue with the new memory. In this case, synchronized reactivation of the go-cue and the new sequence memory facilitated STDP-induced increases in synaptic strength between these representations, while the unsynchronized reactivation of the go-cue and the old sequence memory caused HSP-induced decreases of synaptic strength between these representations. Our model predicts that systems consolidation processes can promote both selective strengthening or weakening of memory representations depending on the synchrony of reactivation during local Up states. Crucially, the selective weakening relies on HSP type mechanisms being actively involved in synaptic plasticity during SWS.

Disclosures: R. Golden: None. **A. Mizrahi-Kliger:** None. **G.P. Krishnan:** None. **K. Ganguly:** None. **M. Bazhenov:** None.

Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: MCP Hall A

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Topic: H.07. Long-Term Memory

Support: NSF (2223839)
NIH (R01MH125557; RF1NS132913)

Title: Sharp-wave ripples induce interleaved memory replay within individual cortical Up states

Authors: *R. GOLDEN[1], R. SAXENA[2], O. C. GONZALEZ[3], E. DELANOIS[4], S. KILIANSKI[6], B. L. MCNAUGHTON[7], M. V. BAZHENOV[5];
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Abstract: Sleep plays a crucial role in the consolidation of new memories, and recent work has shown that slow-wave sleep (SWS) helps to facilitate this process while minimizing interference to existing memories. Although the exact dynamics behind these processes remain poorly understood, much research has implicated a causal role for the reciprocal interactions between the cortex and hippocampus during SWS in general, and the slow oscillations (SOs) and sharp wave-ripples (SWRs) produced by these regions, in particular. To elucidate the synaptic mechanisms that facilitate consolidation without interference, we used a biophysical

thalamocortical model capable of learning neural sequence memories in an awake state and producing spontaneous memory replay during Up states of the SO. We implemented hippocampal indexing to the cortex via simulated SWR input that was applied at variable delays from the detected Down-to-Up state transition (DUt) of each SO. We found that indexing induced 1) robust novel memory consolidation when it was applied just after the DUt, 2) moderate novel memory consolidation when applied near the end of the Up state just before the Up-to-Down state transition (UDt), 3) no novel memory consolidation when applied in the middle of an Up state. Importantly, outside of the period the index was applied, the cortex reliably replayed the familiar memory, suggesting that interleaved memory replay within individual Up states can drive novel memory consolidation without interference to competing familiar memories. To test model predictions, we analyzed single unit activity from the mouse retrosplenial cortex while they completed a virtual maze task; first in a highly familiar environment, then in a novel environment. Reactivation projectors for both tasks were obtained via PCA and used to score familiar and novel memory reactivation during a subsequent urethane-induced sleep period. We found three different patterns of memory replay: 1) novel memory replay near the DUt with familiar memory replay during the rest of the Up state (~ 10% of Up states); 2) novel memory replay near the DUt and UDt with familiar memory replay in the middle (~ 20-30% of Up states); 3) novel memory replay at the UDt with familiar replay during the early phase of the Up state (~ 40% of Up states). Together, our results suggest that replay of familiar and novel memories are tightly coordinated within Up states of sleep SO. SWRs arriving near the DUt are most efficient to entrain cortical replay, while the middle phase of Up state tends to replay old memories. The study presents a novel theory of how replay of the familiar and novel memory traces is organized during SWS to enable continual learning.

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Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR424.13/Web Only

Topic: H.07. Long-Term Memory

Title: It isn't the same. Evoked activity in lateral entorhinal cortex in known and new spaces.

Authors: E. N. SHEFFIELD, A. L. KELLEY, ***M. C. ZRULL**;
Psychology, Appalachian State Univ., Boone, NC

Abstract: The lateral entorhinal cortex (LEC) leads and ends a processing loop through the hippocampal formation (HF) that contributes to neural representations of objects and their locations in an animal’s environment. LEC is believed to provide the HF with information about

non-spatial content of a space, as well as object location in a space, that is updated as an animal interacts within the space. We used c-FOS to examine neural activation in superficial (sLEC), which receives input from various regions and relays it to the HF, and deep LEC (dLEC), which receives HF output and is reciprocally connected with sLEC, evoked by exposure to a new or known environment with or without various moveable objects. After weaning, 40 rats were group housed in standard cages and spent 1.5 h, 5 days/week in an enclosure with ramps and platforms with (EEob) or without (EEno) objects or were only handled (No groups). At 49 days old, rats from each test group spent 1.5 h in the enclosure with (NoEEob, EEobEEob, EEnoEEob groups) or without (NoEEno, EEobEEno, EEnoEEno) or in a standard cage in a quiet and dark room (NoNo, EEobNo, EEnoNo controls). Brains were processed to visualize c-FOS+ neurons, and cell counts were made using digital microscopy and stereological technique with counts standardized to the NoNo group. For sLEC, exposure to the environment with (+569%) or without (+420%) objects increased neural activity over no exposure (any No controls) (both $p<.001$) suggesting activity in sLEC is dependent upon spatial and non-spatial features and changes that occur within an environment without regard to prior experience in a similar or different setting. For dLEC, neural activity increased in rats with no prior experience, whether objects were present or not (>+1000% for both NoEEob and NoEEno over No-No, both $p<.001$) suggesting novelty affects dLEC neural activity. For rats with a history in a space, increased neural activity was greatest when the current did not match historical context. Active neurons for EEobEEno were +138% compared to EEobEEob ($p<.041$) and were +54% for EEnoEEob compared to EEnoEEno ($p<.037$) suggesting recall of past context, including contents, and updating to accommodate changes. Overall, data support the idea that LEC may provide a representation of a space through its non-spatial content, rather than just mapping object placement within the environment.

Disclosures: E.N. Sheffield: None. **A.L. Kelley:** None. **M.C. Zrull:** None.

Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

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Topic: H.07. Long-Term Memory

Support: European Research Council 715968
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Title: Role of clustered protocadherins in engram formation and memory function

Authors: *C. ORTEGA-DE SAN LUIS[1,2], O. CLEMENT[3], E. URRIETA[1], C. HERRING[3], L. MARKS[1], R. LISTER[3], T. J. RYAN[1,2,4,5];

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Abstract: The brain encodes learned information as specific changes to engram cells, populations of neurons that act as building-blocks of memory. These cells, whose activity is sufficient and necessary for the recall of the memory, modify their connections across brain regions in a way that sustains the physical storage of information. We investigate how connections between engram cells are regulated by transsynaptic adhesion molecules. In particular, we assess the role of clustered protocadherins (cPcdhs), a superfamily of cell surface proteins that act as a barcode system for cell to cell recognition and synapse specification. Using engram labelling technology, in situ hybridization, spatial transcriptomic and genomic approaches, we examine the impact of experience-encoding in cPcdhs expression in the hippocampus. We further investigate the role of cPchds in engram formation and function. Our findings suggest that cPcdhs play a significant role in shaping memory formation and function, influencing the dynamics of engram cells.

Disclosures: C. Ortega-De San Luis: None. **O. Clement:** None. **E. Urrieta:** None. **C. Herring:** None. **L. Marks:** None. **R. Lister:** None. **T.J. Ryan:** None.

Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

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Program #/Poster #: PSTR424.15/U19

Topic: H.07. Long-Term Memory

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Title: Representational drift as a correlate of memory consolidation

Authors: *D. ALEVI[1,2], F. LUNDT[1], K. HEINEY[1], H. SPREKELER[1,2,3];
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Abstract: Neural representations often change over time, in spite of stable behavior. This phenomenon, termed representational drift, challenges the established concept of stable memory engrams. Existing theories focus either on potential mechanistic causes of drift (e.g. Qin et al., 2023; Ratson et al., 2024) or on ways to compensate for drift and ensure stable behavior (e.g.

Kalle Kosio et al., 2021; Rule & O'Leary, 2022). But is representational drift merely a bug or does it serve a computational function? Memory consolidation is an obvious candidate function, but it is unclear how the area-centered perspective of classical systems consolidation relates to the population view of representational drift. Here, we present a computational model for the population dynamics of memory engrams under memory consolidation and explore the phenomenology of the resulting representational drift. The model assumes that changes in memory engrams are driven by reactivations. It is structurally similar to a recurrent neural network (RNN) evolving on long time scales of memory consolidation. This allows us to reinterpret common dynamical phenomena in RNNs in light of memory and relate them to experimentally observed representational drift. The model adopts a population view, where memory engrams can be transferred between brain regions or redistributed across or within regions. Memory recall remains stable by confining engram dynamics to the null space of a memory readout. We show that low-rank dynamics induce selective forgetting: some memories remain stable during consolidation, while others transform or are forgotten. Moreover, the model can display power-law forgetting of distributed memory engrams without the need for a wide diversity of forgetting times (Roxin & Fusi 2013). The model also reproduces hallmarks of representational drift: Neuronal tuning curves show a diversity of changes, including stability, gradual drifts in preferred stimulus, abrupt remapping, and loss or gain of tuning. Decoders trained on neural activities from a single day show limited generalization over time, while multi-day decoders reveal invariant subspaces in population activity. However, the performance of multi-day decoders trained on a subset of neurons degrades over time, suggesting that apparently random aspects of representational drift (Rule et al., 2020) may in part result from subsampling. Our model bridges the gap between the traditional area-centered perspective of systems consolidation and the population-level perspective of representational drift and offers a functional interpretation of drift as a means to redistribute engrams for improved memory retention.

Disclosures: D. Alevi: None. **F. Lundt:** None. **K. Heiney:** None. **H. Sprekeler:** None.

Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

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Topic: H.07. Long-Term Memory

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Title: The causal role of prefrontal and motor cortex coupling during sleep-dependent systems consolidation

Authors: *J. KIM[1], L. HE[2,3], K. GANGULY[2,3];
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Abstract: Systems consolidation is a process by which new experiences are initially encoded in the hippocampus and subsequently 'transferred' to the cortex, facilitating integration into long-term memory networks. Our recent work has found that the prefrontal cortex (PFC) may play an essential role during intermediate phases of consolidation. Here, we demonstrate the causal role of the sleep-dependent cross-area coordination between PFC and primary motor cortex (M1) for long-term motor memory consolidation. Using a reach-to-grasp task in rats, we monitored precise coupling in PFC-M1 sleep slow oscillation (SO) during NREM sleep following motor skill learning. Our study shows that the temporal dynamics of PFC-M1 SO coupling are precisely associated with the changes in motor task performance, such as success rate and reaching trajectory stability. To test the causal relationship, optogenetic interventions were applied to manipulate PFC-M1 SO coupling. Interestingly, disturbances in PFC activity during PFC-M1 coupled SO in sleep led to delayed increases in PFC-M1 coordination and slower stabilization of motor task performance - indicating a causal role for changes in PFC-M1 coupling in systems consolidation. Notably, the slower increases in PFC-M1 SO coupling were associated with disruptions and delayed enhancements of cross-area coupling during spindle trains, i.e., spindles that occur in temporal clusters. Our results provide evidence for the causal role of PFC-M1 dialogue during intermediate phases of systems consolidation.

Disclosures: J. Kim: None. **L. He:** None. **K. Ganguly:** None.

Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

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Program #/Poster #: PSTR424.17/U21

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH R01 MH123466
NSF GRFP 2236868

Title: The Relationship Between Hippocampal Sharp Wave Ripples and Ventral Striatal Dopamine

Authors: *M. JANSSEN, M. A. VAN DER MEER;
Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Animals learn both through direct experience and through remembering that experience offline later. The hippocampus of rodents and humans replays past and possible

experiences offline, and this replay is causally important for subsequent memory. Computational theories of the function of replay, rooted in reinforcement learning, posit that such 'experience replay' speeds learning through coupling to a fictive teaching signal (e.g. Mattar & Daw 2018; Sagiv et al. 2023). A major candidate for how such offline teaching signal may be implemented in the brain is ventral striatal (VS) dopamine (DA) which is known to signal reward prediction errors (RPE) during experience. However, it is currently unknown whether there is any relationship between hippocampal replay and ventral striatal DA in the brain, and if so, whether that relationship is modified by experience. As an initial step towards testing if replay and VS DA are related, we used fiber photometry to measure extracellular DA concentration ([DA]) by expressing the fluorescent dopamine sensor $GRAB_{DA}$ in mice (n = 5). We simultaneously recorded local field potentials in the dorsal CA1 area of the hippocampus to detect putative replay events (sharp wave ripples, SWRs). We first verified that we were measuring true VS [DA] by confirming we could detect a clear RPE signal by observing the expected bidirectional [DA] response across unexpected reward omission and larger-than-expected reward delivery. Further validating the [DA] signal, we also observed a clear increase following amphetamine administration (2.5 mg/kg, i.p.). Having established we could measure RPEs in VS dopamine, we next examined the relationship between putative replay events (hippocampal SWRs) and VS [DA] during rest periods before and after the reward prediction error task. We computed peri-event histograms of [DA] triggered on SWR occurrence, and assessed the statistical significance of any deviations against a null distribution of circularly shifted events. Averaged across sessions and subjects, there was a clear increase in VS [DA] following SWRs, peaking about 0.5-0.75s after SWR time. However, substantial variability in the magnitude of SWR-triggered [DA] remained, even across sessions recorded from the same subject. We have demonstrated a link between hippocampal SWRs and ventral striatal dopamine, which on average showed a transient increase following SWRs. This temporal asymmetry is consistent with SWRs causing increased DA release, although this would require experimental intervention to test more directly. The main contributors to the substantial session-by-session variability remains unclear.

Disclosures: M. Janssen: None. **M.A. van der Meer:** None.

Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR424.18/U22

Topic: G.02. Reward and Appetitive Learning and Memory

Support: CIHR Project Grant #367017
CIHR Project Grant #377074
CIHR Project Grant #463403
NSERC Discovery Grant #74105
Canada Research Chairs Program

Title: Parvalbumin interneurons regulate recall of associations and novelty coding in ventral hippocampus

Authors: *E. J. MAES[1], M. P. BRANDON[2];
[2]Psychiatry, [1]McGill Univ., Montreal, QC, Canada

Abstract: The hippocampus (HPC) is thought to support memory retrieval through reactivation of specific neural ensembles previously active during encoding. It has also been shown that environmental novelty provokes increases in the firing rates of hippocampal neurons. However, a common neural basis supporting these two processes remains unknown. We addressed this question using chemogenetic inhibition of vHPC parvalbumin-positive (PV+) interneurons and Nucleus Accumbens GRAB-DA2m fiber photometry in freely-moving mice. We found that mice learned to discriminate different cue-outcome associations across valence. Inhibition of PV+ interneurons led to impaired recall of specific CS-US associations while leaving performance intact. Furthermore, PV+ interneuron inhibition prevented familiarization and habituation. These data suggest that at the population level, reduced global inhibition supports novelty, while at the individual cell level, lateral inhibition supports independent recruitment of partly overlapping ensembles during recall. Revealing how the hippocampus orchestrates prediction and learning is fundamental to understanding the neural basis of behavior, as well as disorders with salience and associative deficits such as schizophrenia.

Disclosures: E.J. Maes: None. **M.P. Brandon:** None.

Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR424.19/U23

Topic: H.07. Long-Term Memory

Support: LSU College of Sciences and Department of Biological Sciences Startup funds (J.H.)
Board of Regents Research Competitiveness Support Program, LEQSF(2022-25)-RD-A-08 (J.H.)

Title: Effect of membrane hyperpolarization and depolarization on entorhinal cortical delta oscillations and memory consolidation

Authors: *S. GUNIN, J. VU, J. HAAM;
Biol. Sci., Louisiana State Univ., Baton Rouge, LA

Abstract: Delta oscillations in the temporoammonic (TA) pathway of the entorhinal cortex via hyperpolarization-activated cyclic-nucleotide-gated (HCN) channels are known to mediate

memory consolidation during sleep. While we have shown that the blockade of these oscillations via an HCN channel antagonist impairs long-term memory formation, the impact of membrane hyperpolarization and depolarization on delta oscillations and memory consolidation remains unclear. We hypothesized that chemogenetic modulation of the TA pathway membrane potential would influence memory consolidation via changes in delta oscillations during sleep. To record the activities of the TA pathway neurons in vivo, a virus cocktail containing the calcium indicator GCaMP6f and TdTomato genes under the glutamatergic CaMKII promoter was injected in the entorhinal cortex layer III (ECIII) of adult male C57BL/6J mice. Optical probes were implanted in the hippocampal area CA1 targeting GCaMP6f-expressing axon terminals for photometry recordings in freely moving animals. Membrane hyperpolarization and depolarization of the TA pathway neurons were achieved by the viral expression of the chemogenetic receptors hM4Di and hM3dq respectively, which can be activated by the synthetic ligand deschloroclozapine (DCZ). In vivo recordings were performed for six consecutive days with three days of habituation sessions, one day with saline injection, one day with DCZ injection, and one day of wash-out. Memory consolidation performance was examined using the Y-maze delayed non-match to sample (DNMS) and the Morris water maze (MWM) tasks. DCZ was delivered immediately after the training sessions. Membrane hyperpolarization following DCZ injection caused a significant increase in the power of delta oscillations, whereas membrane depolarization caused a significant decrease in the power of delta oscillations. Mice with chemogenetic hyperpolarization of the ECIII exhibited enhanced performance in DNMS tasks, indicating enhanced consolidation of spatial memory. Chemogenetic hyperpolarization of the ECIII also caused an enhanced performance during the MWM probe trials without causing a significant change in the performance during the training sessions. On the other hand, membrane depolarization caused a decrease in performance in DNMS and MWM tasks. Our findings suggest that chemogenetic manipulation of the membrane potential effectively alters the power of delta oscillations of the TA pathway. Further, chemogenetic hyperpolarization of ECIII significantly enhances the consolidation of spatial memory whereas chemogenetic depolarization has the opposite effect.

Disclosures: S. Gunin: None. **J. Vu:** None. **J. Haam:** None.

Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR424.20/U24

Topic: H.07. Long-Term Memory

Support: LSU College of Sciences and Department of Biological Sciences Startup funds
Board of Regents Research Competitiveness Support Program, LEQSF(2022-25)-RD-A-08

Title: Effect of chronic stress and sleep deprivation on long -term memory

Authors: *A. DINGANKAR, J. CARIGNAN, J. HAAM;
Biol. Sci., Louisiana State Univ., Baton Rouge, LA

Abstract: Chronic stress and sleep deprivation (SD) are recognized contributors to memory impairments. However, the specific mechanisms underlying these memory impairments remain unclear. Previous work in our laboratory uncovered that the temporoammonic (TA) pathway neurons in the entorhinal cortex exhibit delta oscillations during sleep, that are driven by hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channels and are crucial for memory consolidation. While the entorhinal cortex is known to be susceptible to chronic stress, the specific effects of chronic stress and SD on entorhinal cortical delta oscillations and their consequences for memory consolidation remain unexplored. The purpose of this study is to examine the effect of chronic stress and SD on memory consolidation mediated by the TA pathway**.** Wild-type C57BL/6 mice were subjected daily to the chronic variable stress (CVS) paradigm, in which a combination of two to three out of seven stressors are presented unpredictably, followed by SD for 30 days. Subsequently, a battery of behavioral tasks assessed their memory performance. Corticosterone levels, pre- and post-stressor exposure, were systematically monitored via fecal samples over the stress exposure period, and anxiety levels were evaluated with the open field task. Following the behavioral battery, mouse brains were collected for immunohistochemical and western blot analyses to examine the impact of CVS and SD on HCN channels. Additionally, mice, injected with AAVs carrying genes for GCaMP6f and TdTomato (AAV9-CaMIIα-GCaMP6f and AAV9-CaMIIα-tdTomato-WPRE) and implanted with an optical probe targeting the TA pathway terminals onto hippocampal CA1 were subjected to CVS and SD to investigate its effect on entorhinal cortical delta oscillations using fiber photometry. Mice that received CVS and SD displayed impaired long-term spatial memory, evident from the equal time spent in the target quadrant compared to non-target quadrants in the Morris water maze task. However, these mice exhibited intact spatial working memory in the spontaneous alternation task. Mice subjected to prolonged periods of CVS followed by SD exhibit compromised long-term spatial memory while retaining intact working memory. This indicates a reparative role of sleep in ameliorating the detrimental impacts of chronic stress on long-term memory storage. The interference with this compensatory mechanism, particularly over protracted durations, leads to discernible long-term memory impairment.

Disclosures: A. Dingankar: None. **J. Carignan:** None. **J. Haam:** None.

Poster

PSTR424

Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR424.21/U25

Topic: H.07. Long-Term Memory

Support: NSF 2209874
NSF 2223839

Title: Continual Learning with Limited Data: Biological Principles and Application to Artificial Neural Networks

Authors: A. BAZHENOV[1], P. DEWASURENDRA[1], G. P. KRISHNAN[2], *E. DELANOIS[3]; [1]Del Norte High Sch., San Diego, CA; [2]Georgia Inst. of Technol., Atlanta, SOCORRO, NM; [3]UCSD, San Diego, CA

Abstract: Artificial neural networks (ANNs) show limited performance with scarce or imbalanced training data and face challenges with continuous learning, such as forgetting previously learned data after new tasks training. In contrast, the human brain can learn continuously and from just a few examples. Sleep has been hypothesized to play an important role in memory consolidation and generalization of knowledge in biological systems. During sleep, neurons are spontaneously active without external input and generate complex patterns of synchronized activity across brain regions. Two critical components believed to underlie memory consolidation during sleep are spontaneous replay of memory traces and local unsupervised synaptic plasticity.
Our new study explores the impact of sleep - implemented as unsupervised phase incorporating stochastic network activation with local Hebbian learning rules - on ANNs trained incrementally with limited and imbalanced datasets, specifically MNIST and Fashion MNIST. We previously reported that applying the sleep replay consolidation (SRC) algorithm significantly enhances accuracy in ANNs trained with limited data. In this new study, we applied SRC to scenarios when the model was trained sequentially on several tasks with limited data. We found that sleep-like replay can both improve the performance of models trained with limited data as well as rescue previously trained task performance that was damaged by new training. Interestingly we observed that applying SRC to the network also aided preserving old task performance during subsequent new task training, suggesting SRC encoded information in the weight matrix in a robust manner that was difficult to overwrite. Analysis of synaptic weight dynamics revealed that while SRC increased the strength for a subset of presumably critical synapses, many others were weakened. This suggests that the overall increase in accuracy after SRC was a result of sparser responses. In multiple task scenarios, SRC reduced weights biased towards the most recent tasks, thus allowing older tasks to be correctly classified as long as information about these tasks was still present in the synaptic weights matrix.
Our study sheds light on a potential synaptic weight dynamics strategy employed by the brain during sleep to enhance memory performance for continual learning when training data are limited or imbalanced. Applied to ANNs, sleep-like replay improves performance in a completely unsupervised manner, requiring no additional data, and can be applied to pre-trained models.

Disclosures: A. Bazhenov: None. **P. Dewasurendra:** None. **G.P. Krishnan:** None. **E. Delanois:** None.

Poster

PSTR425

Hippocampal Circuits II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR425.01/U26

Topic: H.08. Learning and Memory

Title: Encoding of predictable and unpredictable odor sequences in the mouse hippocampus and ventral striatum

Authors: *M. MOHAPATRA[1], K. LEAMAN[2], M. A. VAN DER MEER[3];
[1]Dartmouth Col. Psychological & Brain Sci., Hanover, NH; [2]Dartmouth Col., Haover, NH;
[3]Psychological & Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Associative learning, and its various generalizations to more complex knowledge structures such as maps and schemas, are fundamental to adaptive behavior. Yet, even basic questions of how associations between stimuli are encoded in ongoing neural activity remain poorly understood. For instance, under circumstances where A predicts B, presentation of A must evoke some kind of neural representation of B, as demonstrated by behavioral phenomena such as second-order conditioning and sensory preconditioning. This suggests an "additive" view on associative encoding, where neural activity signaling presentation of A is supplemented with activity signaling expectation of B. Yet, expectation of B can suppress stimulus-evoked encoding of B when it actually occurs, as formalized in predictive coding and predictive processing accounts. This highlights a "subtractive" side to associative encoding where associative activity patterns do not supplant, but rather cancel out, evoked activity. How are these operations realized in neural circuits, and what determines whether two activity patterns add or subtract?
In this study, we explored these questions by presenting sequences of discrete odors to head-fixed, awake mice who could lick for sucrose reward. Each day, the mice were exposed to two separate blocks of odor sequences: one block consisted of predictable sequences (odors D, E, F in that order), and the other comprised random sequences (A, B, C in random order). 10% of programmed stimulus presentations were catch trials where the stimulus was omitted. A first set of analyses focused on the encoding of the odors themselves between the predictable and unpredictable blocks, as quantified by decoding accuracy of odor identity and changes in representational similarity. Complementary analyses investigated the encoding of predictions and prediction errors during the catch trials. Using simultaneous Neuropixels recordings in dorsal CA1 of the hippocampus and in the ventral striatum, differences in odor and odor prediction encoding were compared.

Disclosures: M. Mohapatra: None. **K. Leaman:** None. **M.A. van der Meer:** None.

Poster

PSTR425

Hippocampal Circuits II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR425.02/U27

Topic: H.08. Learning and Memory

Support: Dartmouth Fellowship
NIH R01 MH123466

Title: Reactivation of discrete odor stimuli in the mouse hippocampus

Authors: K. LEAMAN, M. MOHAPATRA, *M. A. VAN DER MEER;
Dartmouth Col., Hanover, NH

Abstract: Internally generated activity in the hippocampus (HC), in the form of sharp wave-ripples (SWRs) contributes both to off-line systems consolidation of past experience into cortical knowledge structures, and to on-line memory-guided decision making. Despite decades of experimental work in rodents, and more recently in humans, we still do not understand the internal logic of what exactly determines the content of such "replay" and how it relates to subsequent memory.

One persistent challenge has been that reactivation studies in humans generally (although not exclusively) involve learning about highly controlled discrete stimuli and the associations between them, whereas reactivation studies in rodents overwhelmingly (but not exclusively) are done in freely moving animals learning about spatial trajectories, making it hard to connect findings across these different settings. Moreover, many open questions about reactivation in rodents would benefit from the increased experimental control afforded by discrete stimuli, e.g. the ability to parametrically vary stimulus intensity, timing, and predictability.

To address these issues, we presented sequences of discrete odor stimuli to head-fixed mice. Previous work has demonstrated clear odor-selectivity in hippocampal neurons (e.g. from Eichenbaum, Manns, Fortin and colleagues) but it is currently unknown whether odor signals in HC are reactivated during SWRs, and if so, what the properties of that reactivation are. Using Neuropixels recordings in dorsal CA1, we addressed two foundational questions about odor reactivation. First, do cells selective for a particular odor participate in SWR events? Second, is this reactivation modifiable by experience?

In data from 5 mice, we found that odor-selective cells reliably participated in SWR events, opening the door to further analysis to expose the factors that govern this participation. In preliminary analysis, we found that 67% of odor-selective cells increased their SWR participation after, compared to before, novel presentation of their preferred odor ($p < 0.05$, chi-squared test). These results suggest that experience modifies the reactivation of odor-encoding neurons in the hippocampus, and opens the door to further analyses to probe the internal logic of hippocampal replay with discrete stimuli.

Disclosures: K. Leaman: None. **M. Mohapatra:** None. **M.A. van der Meer:** None.

Poster

PSTR425

Hippocampal Circuits II

Location: MCP Hall A

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Topic: H.08. Learning and Memory

Support: NIMHR01MH124047 (AL)
NIMHR01MH124867 (AL)
NINDSR01NS121106 (AL)
NINDSU01NS115530 (AL)
NINDSR01NS133381 (AL)
NINDSR01NS131728 (AL)
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ISF personal grant 2354/19 (IS)
BSF US-Israel binational grant 2021281 (IS)
Burroughs Wellcome Fund (SAH)
Israel Ministry of Absorption Fellowship (DK)
Horowitz Foundation Fellowship (DK)

Title: 2p-nuctag: on-demand phototagging for molecular analysis of functionally identified cortical neurons

Authors: *J. SHI[1], B. NUTKOVICH[2], D. KUSHINSKY[2], B. Y. RAO[1], S. A. HERRLINGER[1], T. S. MIHAILA[1], K. COHEN-KASHI MALINA[2], C. K. O'TOOLE[1], M.-E. CONDE PAREDES[1,3], H. YONG[1], E. VAROL[4], A. LOSONCZY[1], I. SPIEGEL[2];
[1]Columbia Univ., New York, NY; [2]Weizmann Inst. of Sci., Rehovot, Israel; [3]New York University, New York, NY; [4]New York Univ., New York, NY

Abstract: Neural circuits are characterized by genetically and functionally diverse cell types. A mechanistic understanding of circuit function is predicated on linking the genetic and physiological properties of individual neurons. However, it remains highly challenging to map the functional properties of transcriptionally heterogeneous neuronal subtypes in mammalian cortical circuits in vivo. Here, we introduce a high-throughput two-photon nuclear phototagging (2P-NucTag) approach optimized for on-demand and indelible labeling of single neurons via a photoactivatable red fluorescent protein following in vivo functional characterization in behaving mice. We demonstrate the utility of this function-forward pipeline by selectively labeling and transcriptionally profiling previously inaccessible ‘place’ and ‘silent’ cells in the mouse hippocampus. Our results reveal unexpected differences in gene expression between these hippocampal pyramidal neurons with distinct spatial coding properties. Thus, 2P-NucTag opens a new way to uncover the molecular principles that govern the functional organization of neural circuits.

Disclosures: J. Shi: None. **B. Nutkovich:** None. **D. Kushinsky:** None. **B.Y. Rao:** None. **S.A. Herrlinger:** None. **T.S. Mihaila:** None. **K. Cohen-Kashi Malina:** None. **C.K. O'Toole:** None. **M. Conde Paredes:** None. **H. Yong:** None. **E. Varol:** None. **A. Losonczy:** None. **I. Spiegel:** None.

Poster

PSTR425

Hippocampal Circuits II

Location: MCP Hall A

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Program #/Poster #: PSTR425.04/U29

Topic: H.08. Learning and Memory

Support: R00MH128772
NIH 1R01MH124867
NIH 5U19NS104590
NIH 1R01MH124047

Title: Inferring Neuronal Identity from Functional Correlation Patterns in Mouse Hippocampal Circuits

Authors: *M.-E. CONDE PAREDES[1,2], S. A. HERRLINGER[3], B. Y. RAO[4], S. CHEN[1], T. GEILLER[5], A. LOSONCZY[6], E. VAROL[7];
[1]Columbia Univ., New York, NY; [2]Tandon School of Engineering, New York University, New York, NY; [3]Zuckerman Inst., Columbia Univ., New York, NY; [4]Neurobio. and Behavior, Columbia Univ., New York, NY; [5]Zuckerman Inst., Columbia Univ., New Haven, CT; [6]Neurosci., Columbia Univ., Westport, CT; [7]Tandon Sch. of Engin., New York Univ., New York, NY

Abstract: Neuronal cell-type is hypothesized as a key determinant of functional roles within neural circuits, yet linking these roles to specific molecular signatures remains challenging. This study addresses this gap by exploring whether neuronal identity can be accurately inferred from patterns of interactions among interneuron subtypes in local circuits. To investigate this possibility, we used a probabilistic graphical model that quantifies cell-specific functional correlations as variables within a hierarchical framework, encapsulating both individual cell-to-cell-type and broader cell-type-to-cell-type interactions. We applied this analytical framework to in vivo two-photon (2P) imaging data collected from inhibitory neurons located across CA1 and CA3 layers of the hippocampus. Following imaging, we conducted post hoc immunostaining using specific molecular markers to identify and categorize six distinct interneuron subtypes. This approach allows for a detailed examination of how individual and network-level functional interactions correlate with distinct molecular identities.Our probabilistic model successfully predicted the identity of six out of six interneuron subtypes with significant accuracy above random chance, demonstrating distinct interaction patterns among cell types. Furthermore, the discernible clusters in the low dimensional projection of interaction parameters suggest that our model captures salient features of cell interactions which facilitate cell type classification. These findings pave the way for future investigations to expand on the structural correlates of functional connectivity patterns, potentially leading to a more comprehensive map of neural circuitry. The implications of this work extend to the predictive modeling of neuron types based

on their functional interactions, offering a novel approach to understand the complex interplay between the brain's molecular structure and its dynamic functional patterns.

Disclosures: M. Conde Paredes: None. **S.A. Herrlinger:** None. **B.Y. Rao:** None. **S. Chen:** None. **T. Geiller:** None. **A. Losonczy:** None. **E. Varol:** None.

Poster

PSTR425

Hippocampal Circuits II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: H.08. Learning and Memory

Support: NIMHR01MH124047
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NINDSR01NS121106
NINDSU01NS115530
NINDSR01NS133381
NINDSR01NS131728
NIARF1AG080818

Title: Recurrent connectivity captures distinct spatial memory features along the proximodistal axis of hippocampal CA3

Authors: *E. KONG, T. S. MIHAILA, E. ZABEH, Z. LIAO, D. S. PETERKA, A. LOSONCZY, T. GEILLER;
Zuckerman Inst., Columbia Univ., New York, NY

Abstract: Recurrent connectivity in the brain is a major anatomical feature underlying higher cortical functions, from sensory perception to learning and memory. This study focuses on the CA3 subregion of the hippocampus, which is instrumental in the rapid encoding of contextual representations critical for memory-guided behaviors. The CA3 subregion is known for its anatomical heterogeneity, organized along a proximodistal gradient, which suggests functional diversity among its pyramidal neurons. However, detailed functional characterization of these neurons has been limited, particularly in deeper segments (CA3c) near the dentate gyrus, due to imaging constraints.
To address this gap, we utilized *in vivo* two- and three-photon microscopy combined with a virtual reality (VR) behavioral setup to assess the functional heterogeneity of the CA3 subregions at a cellular level. Head-fixed mice were trained to navigate a virtual track with hidden rewards, allowing us to capture the dynamics of spatial representations over multiple days. We also investigated context-dependent network remapping followed by context-switching or VR-off tasks. Our analysis revealed pronounced disparities in the functional connectivity and stability of place cells between proximal (CA3c) and distal (CA3a-b) regions. Furthermore, to

test if recurrent anatomical differences could account for these functional discrepancies, we employed recent advances in neural network modeling where recurrent neural networks (RNNs) with forced connectivity properties mimicking those of the proximal and distal circuits were constructed and trained on the same VR tasks. Reverse engineering of distal and proximal RNNs demonstrated artificial cells with spatial tuning aligned with each subregion's characteristics. Our combined experimental and computational approach not only confirms the functional heterogeneity within hippocampal CA3 subregions but also illustrates how subtle differences in local connectivity can lead to significant variability in the functionality and stability of neuronal circuits in awake, behaving animals. These findings provide deeper insights into the mechanisms by which hippocampal subregions contribute to spatial memory and navigation.

Disclosures: E. Kong: None. **T.S. Mihaila:** None. **E. Zabeh:** None. **Z. Liao:** None. **D.S. Peterka:** None. **A. Losonczy:** None. **T. Geiller:** None.

Poster

PSTR425

Hippocampal Circuits II

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Program #/Poster #: PSTR425.06/U31

Topic: H.08. Learning and Memory

Support: NIMHR01MH124047
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NINDSR01NS121106
NINDSU01NS115530
NINDSR01NS133381
NINDSR01NS131728
NIARF1AG080818

Title: In vivo dendritic voltage mapping of CA3 autoassociative circuits during hippocampal sharp-wave ripples

Authors: *A. NOGUCHI, G. ZAKKA, A. LOSONCZY, S. TERADA;
Columbia Univ., New York, NY

Abstract: The hippocampal CA3 region is essential for rapid memory encoding and generating sharp-wave ripples, attributed to the unique circuit architecture characterized by recurrent connections, mossy-fiber, and direct entorhinal cortical inputs. Despite decades of work, the underlying in vivo synaptic and computational mechanisms of CA3 network are poorly understood. Using 3-dimensional acousto-optic deflection microscopy, we performed ultrafast two-photon imaging of dendritic and somatic membrane potential dynamics from single CA3 pyramidal cells in awake behaving mice. We observed sub-millisecond voltage dynamics throughout the dendritic tree of basal, apical, and tuft domains coupled with somatic

depolarization. The dendritic depolarization latency is correlated to the path distance from the soma. We also found dendritic depolarization locally evoked in the absence of somatic depolarization, and these local events are prominently observed in tuft dendrites. Combining voltage imaging of CA3 pyramidal cells with simultaneous CA1 local field potential recordings, we observed soma depolarized before CA1 sharp-wave ripples and subsequently hyperpolarized. This preceding depolarization followed by hyperpolarization is broadly distributed across the apical dendrites, while tuft dendrites tend to depolarize during CA1 sharp-wave ripples. Our results provide a deeper, mechanistic understanding of how electrical compartmentalization in distinct dendritic domains is organized in CA3 autoassociative network.

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Poster

PSTR425

Hippocampal Circuits II

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Support: Medical Research Council UK (award MC_ST_BNDU_2019)
Medical Research Council UK (programme MC_UU_00003/4 and award MR/W004860/1)

Title: Linking Hippocampal Population Firing Dynamics to Ripples Laminar Profiles

Authors: *M. CASTELLI[1], V. LOPES DOS SANTOS[2], D. DUPRET[2];
[1]MRC Brain Network Dynamics Unit, Univ. of Oxford, Oxford, United Kingdom; [2]MRC Brain Network Dynamics, Univ. of Oxford, Oxford, United Kingdom

Abstract: The collective spiking activity of hippocampal principal cells supports information processing and integration for memory-guided behaviour. This population spiking is temporally structured and reflect behavioural states. That is, hippocampal principal cell spiking is sparsely paced by theta oscillations during active exploration but tightly synchronised by sharp-wave/ripples (SWRs) during sleep/rest. Notably, previous studies have related SWRs to a current sink in the *stratum radiatum*, reporting CA3 inputs that serve memory consolidation. Here, we explore whether individual SWRs deviate from this general pattern of activities. We start by using Principal Components Analysis to analyse and characterise the currents along the somato-dendritic axis of CA1 principal cells during SWRs. We observe a substantial diversity in single SWR events, involving two distinct current profiles: Rad^{sink} ripples with a stronger current sink in the *stratum radiatum* and LM^{sink} ripples with a stronger current sink in the *stratum lacunosum-moleculare*. We relate these distinct current profiles to different ripple waveforms and ripple frequencies. We show that single-ripple variability unveils a functional link between individual ripple current profiles and transient modulation of principal cell joint firing. These preliminary

observations suggest a role for hippocampal laminar somato-dendritic currents in shaping the activity features and mnemonic functions of SWRs.

Disclosures: M. Castelli: None. **V. Lopes Dos Santos:** None. **D. Dupret:** None.

Poster

PSTR425

Hippocampal Circuits II

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Topic: H.08. Learning and Memory

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ANR-10-IAIHU-06
ICM-OCIRP
ANR-21-CE17-0029

Title: Comparative analysis of oscillatory dynamics in the human and rodent brains

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Abstract: Brain oscillatory dynamics are ubiquitous signatures of operating neural circuits. Multiple oscillations have been described in many neural structures of the rodent brain, being commonly associated with various behaviours, computations, and codes, in the sensory, cognitive, and motor domains. The extent to which such oscillatory organisation of neural activity features the human brain remains elusive. To contribute to such an investigation, here we present ongoing cross-species work where we apply the same intra-cranial multi-channel recording technique and analytical framework to cortical and subcortical regions from the human and rodent brains. We monitored neuronal ensemble spiking and network oscillations in drug-refractory epileptic patients with hybrid electrodes that contain tetrodes, as in behaving rodents. Having extracted oscillatory patterns, we present our observations about the frequency content of

the local field potentials, describing distinct rhythms ranging millisecond to second timescales and relating these to neuronal spiking across wake, rest, and sleep epochs.

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Poster

PSTR425

Hippocampal Circuits II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR425.09/U34

Topic: H.08. Learning and Memory

Support: BB/S007741/1
BB/N002547/1
MC_UU_00003/4
MR/W004860/1

Title: Organising the coactivity structure of the hippocampus from robust to flexible memory

Authors: *G. GAVA[1], D. DUPRET[1], L. LEFÈVRE[1], T. BROADBELT[1], S. B. MCHUGH[2], P. V. PERESTENKO[1], V. LOPES-DOS-SANTOS[1];
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Abstract: New memories are integrated into prior knowledge of the world. But what if consecutive memories exert opposing demands on the host brain network? We report that acquiring a robust (food-context) memory constrains the hippocampus within a population activity space of highly correlated spike trains that prevents subsequent computation of a flexible (object- location) memory. This densely correlated firing structure developed over repeated mnemonic experience, gradually coupling neurons of the superficial CA1 pyramidale sublayer to whole population activity. Applying hippocampal theta-driven closed-loop optogenetic suppression to mitigate this neuronal recruitment during (food-context) memory formation relaxed the topological constraint on hippocampal coactivity and restored subsequent flexible (object- location) memory. These findings uncover an organizational principle for the peer-to-peer coactivity structure of the hippocampal cell population to successfully meet memory demands.

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Poster

PSTR425

Hippocampal Circuits II

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: H.08. Learning and Memory

Support: HHMI Hanna Grey Fellowship

Title: A mechanism for internal sequence generation in the mammalian brain

Authors: *C. MALLORY[1], J. WIDLOSKI[2], D. J. FOSTER[3];
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Abstract: Temporally extended neural activity sequences are generated by the brain in the absence of external input. How these sequences are produced is unknown. The hippocampus offers a unique lens to uncover the mechanisms of offline internal sequence generation. During movement, the hippocampus conveys information about an animal's current location, while during sleep or awake immobility it depicts spatial trajectories, called replay, that relate to the animal's past or predicted future. By simultaneously monitoring the spiking activity of hundreds of individual hippocampal neurons in combination with optogenetic manipulation of cortical input in freely behaving rats, we reveal an unexpected temporal organization to hippocampal replay with profound implications in its generation. Immediately upon cessation of movement, replay of the future predominates while replay of the past behavior, which involves the most recently active neurons, is strongly avoided; seconds into stopping, this pattern reverses and replay preferentially depicts past behavior. This temporal organization contrasts with early reports and theoretical predictions but is well-recapitulated by a symmetry-breaking attractor model of sequence generation utilizing cellular adaptation. The model further predicts replays to avoid recently reactivated paths over a shorter timescale, which we observe experimentally. While cellular adaptation alone is sufficient to produce the early past-avoidance, the model requires a facilitating input to produce the later past-preference. Optogenetic perturbations demonstrate that this input is provided by medial entorhinal cortex, revealing a novel role for the region in maintaining a memory of past experience that biases hippocampal replay. These data provide the first evidence that a particular class of model underlies the generation of intrinsic neural sequences in the mammalian brain.

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Poster

PSTR425

Hippocampal Circuits II

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Program #/Poster #: PSTR425.11/V1

Topic: H.08. Learning and Memory

Support: NS113557
MH103325

Title: Spontaneous emergence of alternating hippocampal sequences in 2D in a simple adaptation model.

Authors: *J. WIDLOSKI[1], D. J. FOSTER[2];
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Abstract: Animals often exhibit alternating left-right search patterns to track and localize targets. These alternating behaviors are mirrored at the neural level, particularly in mammals during locomotion, where spatial sequences encoded by cells in the hippocampal region have been observed to activate in sweeps called "theta sequences" that alternate across the animal's midline during navigation in the open field. However, the brain mechanisms generating such alternating neural activity are unknown. Here, we propose a model integrating continuous attractor dynamics with short-term adaptation to explain alternating theta sequences in a 2D environment. We demonstrate that this model exhibits robust alternating theta sweeps that arise rapidly at the onset of movement and exhibit a precise angular offset relative to heading direction. Crucially, we confirm all model predictions using a novel hippocampal data set consisting of hundreds of place cells recorded simultaneously from animals navigating in an open field. Our results suggest that hippocampal alternating theta sequences in 2D may be generated by spontaneous self-organization of neural activity.

Disclosures: J. Widloski: None. **D.J. Foster:** None.

Poster

PSTR425

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Program #/Poster #: PSTR425.12/V2

Topic: H.08. Learning and Memory

Support: ERC-ST2019 850769
PCEGP3_194220

Title: Divergent recruitment of birthdated hippocampal neuronal ensembles supports memory dynamics

Authors: *V. KVEIM, L. SALM, T. ULMER, M. LAHR, S. KANDLER, F. DONATO; Univ. of Basel, Basel, Switzerland

Abstract: Memories are dynamic constructs whose properties change with time and experience. The biological mechanisms underpinning these dynamics remain elusive, particularly concerning how changes in the composition of memory-encoding neuronal ensembles influence the properties of a memory over time. Here we employed a developmental approach to target distinct subpopulations of principal neurons ("birthdated neurons"), and used calcium imaging, optogenetics, chemogenetics, in combination with multiple hippocampus-dependent learning tasks to investigate their contribution to memory engrams over time. Our results show that (1) different subpopulations of developmentally-defined neurons in the hippocampus have distinct recruitment dynamics to the engram during memory consolidation, recent, and remote recall, while similar dynamics are not observed in the amygdala and subiculum; (2) birthdated neurons in CA3 segregate into separate subnetworks with distinct firing properties, functional connectivity, and capacity for plasticity, and (3) exhibit divergent activity dynamics upon the processing of a fear memory; (4) the recruitment of CA3 birthdated neurons at specific delays after acquisition is necessary for successful memory retrieval, but manipulation of the same populations in CA1 did not exert any behavioral effect; and (5) the recruitment of a transient memory trace in CA3, but not CA1, supports plasticity of recently-acquired memories. Together, our results indicate that the divergent recruitment of developmentally-defined and functionally segregated populations in hippocampal CA3 can support various memory functions over time, and reveal possible mechanisms by which information across multiple temporally adjacent learning episodes can be integrated into cohesive memories.

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Poster

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR425.13/V3

Topic: H.08. Learning and Memory

Support: ERC-ST2019 850769

Title: Adaptive brain-machine-interface learning uses similar neuronal strategies in motor and hippocampal networks

Authors: *C. MITELUT[1], A. DE VICENTE DONDERIS[3], R. MENDES[4], L. MARIANELLI[2], M. COLOMER-ROSELL[2], F. DONATO[5];
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Abstract: Brain-machine interfaces (BMIs) are a vital technology for reinstating mobility in individuals with paralysis and offer a promising avenue for exploring the neural substrates of learning. While BMIs have been used in rodent models to investigate decoding and adaptive processes in motor cortex during voluntary activation of individual neurons, the broader network restructuring remains poorly understood.Here we employed 2-photon (2P) imaging over an eight-day period as mice learned to volitionally activate pairs of neurons within the motor cortex (MC) or hippocampal CA3 regions to obtain water rewards. We developed an open-source pipeline that integrates longitudinal tracking of individual neurons with real-time neural activity analysis.We found that mice can learn to volitionally activate single neurons within both the MC and CA3 regions. Interestingly, in the majority of successful trials, only one of the two targeted ensemble neurons was responsible for eliciting the reward. However, within both regions, the neuron predominantly driving reward acquisition could switch identity over the course of training. At the network level, we unveiled several adaptation strategies, including positive ensemble cells increasing their correlation with the broader network, negative ensemble cells diminishing their network correlation, and overall reductions in non-ensemble cell correlations. Our findings underscore systematic changes in both single-neuron and network-level activity across two brain regions engaged in the same BMI task. Furthermore, they shed light on diverse strategies supporting BMI performance and offer insights that may inform the development of personalized BMIs in future research and clinical applications.

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Poster

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Program #/Poster #: PSTR425.14/V4

Topic: H.08. Learning and Memory

Support: ERC-ST2019 850769
PCEGP3_194220

Title: Dissecting the impact of memory processes on the generation of cognitive maps

Authors: *N. IMMERZEEL, C. MITELUT, L. SALM, S. MAUL, F. DONATO;
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Abstract: As we go through life, we encounter many unique experiences that shape us into who we are and change the way in which we interact with the world. The hippocampus plays an important role in encoding and storing these experiences as memories, while also performing computations that are essential for our ability to navigate the world. These computations rely on neurons whose activity is modulated by our movement through the environment, known as place cells, and underpin the generation of cognitive maps that are used to navigate and adapt our behavior in response to external and internal contingencies. Whether the underlying computational principles that support the generation of cognitive maps are preserved independently from the recent experiences one has is still an open question. Here, we use calcium imaging in freely behaving mice to dissect how the recent encoding of a contextual fear memory impacts the hippocampus' ability to create and exploit cognitive maps of unrelated experiences. Our focus lies on the CA3 region of the hippocampus due to its unique recurrent circuitry, which enables the efficient generation of distinct maps for distinct experiences. Our results show that introducing mice to a new environment when the CA3 network is involved in the processing of a fear memory leads to the generation of a cognitive map that recruits a larger fraction of place cells, making it resemble the map of an environment that the animal is highly familiar with. Moreover, initial evidence suggests that this is accompanied by alterations in fundamental properties of the map, from place field characteristics to the accuracy by which we can decode the animal's location from it. Thus, our preliminary results suggest that the recent encoding of a fear memory could enable the fast formation of a mature, stable map upon minimal exposure to an environment. Future work will focus on exploring how changes to network structure and dynamics could underly these effects, and the impact they have on behavior. Overall, our ambition is to uncover whether the computations carried out by CA3, and how it encodes, stores, and revises information from the world, is impacted by the individual experiences one has, and to reveal mechanisms that might be key in keeping track of an animal's recent history and adapting its behavior to external contingencies.

Disclosures: N. Immerzeel: None. **C. Mitelut:** None. **L. Salm:** None. **S. Maul:** None. **F. Donato:** None.

Poster

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Topic: H.08. Learning and Memory

Support: ERC-ST2019 850769
PCEGP3_194220

Title: Developmental dynamics shape the ontogeny of hippocampus-dependent memories

Authors: *T. ULMER, V. A. KVEIM, F. IMHOF, M. LAHR, F. DONATO;
Univ. of Basel, Basel, Switzerland

Abstract: The ability to encode and store memories from personal experiences is crucial for an animal's survival and develops over a prolonged period of postnatal time in mammals. In rodents, memories formed during the first three weeks of life are quickly forgotten, a phenomenon resembling "infantile amnesia". This forgetting process has been linked to the gradual development of the entorhinal-hippocampal circuit, which, in adults, is responsible for encoding, consolidating, and storing these memories long-term. The development of such networks continues well into the first month of a mouse's life, when neurons responsive to spatial cues tune and refine their responses, while new cohorts of neurons are integrated into the hippocampal network according to their birth date. How such neurogenesis-defined developmental trajectory influences the emergence of spatial representations and memory processes remains unknown. Hippocampal neurons with distinct birthdates (isochronic neurons) segregate into subpopulations with distinct functional tuning, gene expression, plasticity, and connectivity profiles. Most interestingly, they might have distinct contributions to memory processes in adult animals. It has recently been shown that while late-born neurons are recruited into memory engrams at recent recall and exhibit more plastic activity dynamics upon learning, early-born neurons support memory retrieval at remote times, and exhibit rigid co-activity dynamics during learning. Thus, a fundamental open question is understanding how developmental dynamics influence hippocampus-dependent memory ontogeny. Here, we used targeted labeling and manipulation of specific isochronic subpopulations, with histological and functional identification of engram neurons, to dissect their contribution to the encoding and retrieval of hippocampus-dependent memory in infant mice. Our results suggest that the sequence of maturation exhibited by isochronic cohorts is recapitulated in the recruitment of principal neurons to the engram, with infant memories being supported by the activation of a rigid, early-born subnetwork over a memory’s lifetime. Moreover, through c-Fos based brain-wide network reconstructions, we show that in adults, activating distinct subpopulations recruits distinct brain-wide networks in naïve and trained animals, which in infants might be responsible for the types of memory correlates exhibited by early-life memories. Thus, we speculate that infant memory traces are quantitatively different than those encoded during adulthood and plan on charting brain-wide mechanisms that might underlie their markedly different memory attributes.

Disclosures: T. Ulmer: None. **V.A. Kveim:** None. **F. Imhof:** None. **M. Lahr:** None. **F. Donato:** None.

Poster

PSTR425

Hippocampal Circuits II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: H.09. Spatial Navigation

Support: Fundación La Caixa (LCF/PR/HR21/52410030; DeepCode)
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EPSRC (EP/T020970/1)
European Research Council (ERC-2020-StG- 949660)

Title: Cell-type-specific geometric transformations in the hippocampal spatial code

Authors: *J. ESPARZA IBANEZ[1], J. QUINTANILLA[1], E. CID[1], J. GALLEGO[2], L. M. DE LA PRIDA[1];
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Abstract: Survival hinges on the ability to effectively navigate the environment, which requires building cognitive maps that represent the world in different reference frames, e.g., local and global. However, how these two reference frameworks are realized into the hippocampal cognitive map remains elusive. Here, we address this question in mice by considering the role of two genetically-defined circuits of the hippocampal output region, CA1.
We focused on deep and superficial CA1 pyramidal cells and asked whether they hold different representations of space in a linear track when local and global cues were set in conflict. After surveying gene expression atlases and mouse repositories, we restricted expression of calcium indicators to either deep or superficial CA1 cells using viral and transgenic strategies. Single- and dual-color miniscope imaging permitted recording the activity from hundreds of cells as mice performed a rewarded alternation task. Using topological methods, we found that the population activity of each CA1 sublayer was spanned by a tridimensional ring manifold, each of which continuously maps position and running direction. Despite sharing this similar topology, sublayer-specific representations displayed geometric differences. By manipulating local and global cues, and leveraging cell-type-specific chemogenetic manipulations coupled to calcium imaging, we identified specific rotational and translational transformations of spatial representations between sublayers.
Our results show how local and global frameworks are integrated into the hippocampal spatial representation, and highlight the importance of genetically dissecting the vast neural space to unveil hidden differences between neural populations from different circuits.

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Poster

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Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR425.17/V7

Topic: H.09. Spatial Navigation

Title: The role of mossy cells in dentate gyrus on detection of changes in environment

Authors: *A. SARIEV[1,2], D. JUNG[3,2], S. ROYER[4];
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Abstract: The dentate gyrus (DG) is thought to be crucial for detecting change in the environment based on both spatial and non-spatial information from the entorhinal cortex. However, the specific mechanisms underlying this process, particularly the involvement of granule cells (GC) and mossy cells (MC) - the primary neurons in the DG - remain largely unknown. In our study, we investigated DG neural responses to alterations in object layouts while mice ran on a treadmill belt. We conducted two experiments: in the first, we shifted the position of a detachable landmark along the belt; in the second, we replaced the landmark with another one without altering its position. Under normal conditions (vehicle condition), manipulating the landmark's position led to a corresponding shift in DG neuron place fields associated with the landmark. However, when the landmark was replaced, various modifications in place field activity occurred throughout the belt. Interestingly, chemogenetic silencing of MCs reduced the rate and extent of remapping triggered by alterations in the belt layout compared to the vehicle condition. Based on these findings, we developed a simulation model. In this model, mossy cells detect changes between the current and previous representations of the layout received from GCs and CA3 pyramidal cells, respectively. The information is then spread throughout the DG via specific connectivity. The model successfully reproduced distinct responses of DG neurons to spatial and non-spatial alterations by normalizing MCs activity. Overall, our results suggest that mossy cells play a critical role in detecting, amplifying, and disseminating information about change in the environment.

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Poster

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Topic: H.09. Spatial Navigation

Support: NIH R01NS119503
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Title: Ramping activity of hippocampal neurons supports path integration

Authors: *R. HELDMAN, D. PANG, Y. WANG;
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Abstract: Animals navigating through their environment often estimate their position by measuring distance traveled from a starting point. This measure is formed by a process known as path integration, where self-motion cues are accumulated over time into distance traveled. In the hippocampus, a brain region crucial for spatial navigation, individual neurons develop firing fields that correspond to specific distances traveled along a path. Firing fields are thought to be the mechanism through which the hippocampus encodes distance. Here, we use head-fixed mice performing a path integration task in virtual reality to uncover a novel coding scheme, in which position along a path is encoded by gradual ramping activity of individual neurons. This ramping activity took one of two forms, with one population of neurons increasing their activity at the beginning of integration before gradually ramping down, and another with the opposite response, shutting off at the beginning of integration before ramping as the animal approached the reward. Using closed-loop optogenetic stimulation, we demonstrate that silencing SST interneurons disrupts the activity of the first population, resulting in an impairment of path integration accuracy. On the other hand, silencing PV interneurons disrupts the activity of the second population, leading to disruptions in path integration specifically at the earlier points. Overall, these studies highlight the local circuit mechanisms behind a novel coding scheme in the hippocampus used to support path integration.

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Poster

PSTR425

Hippocampal Circuits II

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Topic: H.09. Spatial Navigation

Support: EPSRC/Wellcome grant EP/W024020/1
EPSRC grant EP/Y020316/1

Title: How does learning a new memory reorganize the functional network topology of an old memory in hippocampal area CA1?

Authors: *S. CAI[1], M. SAEEDIAN[2], M. GO[1], M. BARAHONA[2], S. R. SCHULTZ[1];
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Abstract: The brain has a finite memory capacity. This means that at capacity, for new memories to be acquired, either old memories must be degraded, or their representation must be continually adapted to take advantage of redundancies with new items (or both). To investigate the latter hypothesis, we used two-photon calcium imaging to record the activity of hippocampal (HPC) neurons in head-fixed mice (C57BL/6J, 2-3 mo, n=11 fields from 6 animals.) running around a circularair-suspended track (Neurotar). Mice were trained over one week in a familiar environment (fam) marked by distinct visuotactile cues. On imaging days, they were introduced to a new environment (nov). To study changes in the representation of an old memory after a new one is learned, we imaged hippocampal neural activity (n=1761 cells from 11 fields) in mice as they navigated fam, were moved to nov and then returned to fam (fam → nov →fam*). Individual sessions (fam, nov, or fam*) typically lasted 20 mins. Mice exhibited hesitant exploratory behavior (assessed using a chi-square test) in specific environmental locations more of the time in nov (31%±4.43%, mean±s.e.m.) than fam (12±2.58%, p=0.004, paired t-test, n=11). In fam*, 74±3.06% of CA1 neurons showed stronger firing (compared to fam)in locations corresponding to the exploratory behavior in nov. We then analyzed directed functional networks of HPC neurons in different environments, as derived from induced spike similarity (Billeh et al., J Neurosci Meth 92:106, 2014). At the network level, we found a higher average clustering coefficient (ACC) and lower geodesic path length (GPL) in the first 4 mins of exploring the novel environment (ACC fam: 0.551±0.009, nov: 0.609±0.012,p=0.018. GPL fam: 1.635±0.058, nov: 1.437±0.039, p=0.011), but in fam* these returned to the level of fam (ACC: fam 0.551±0.009, fam* 0.539±0.012, p=0.549; GPL: fam 1.635±0.017, fam* GPL=1.617±0.007, p=0.601). We detected communities in the directed networks using multiscale Markov Stability analysis (Schaub et al., PRE 99:6, 2019), finding at the subgraph level, asymmetry of connections between subgraphs was reduced in fam* compared to fam(fam: 0.77±0.06; famr2: 0.61±0.08; p=0.048). Overall, we find that reorganization of HPC functional networks during memory encoding is driven by exploratory behavior, and see evidence for changes in the structural properties of old spatial memories at subgraph level due to an intervening spatial learning episode.

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Poster

PSTR425

Hippocampal Circuits II

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Topic: H.09. Spatial Navigation

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Synapsis Foundation/Dementia Foundation Switzerland
DFG TRR 274 - Checkpoints of Central Nervous System Recovery

Title: Brain-wide microstrokes affect the stability of memory circuits in the hippocampus

Authors: *H. HEISER[1], F. KIESSLER[2], A. ROGGENBACH[1], M. J. WIECKHORST[1], J. GJORGJIEVA[2], F. HELMCHEN[1], A.-S. WAHL[3,1,4];
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Abstract: Cognitive decline after multiple brain-wide microstrokes is reported regularly in patients diagnosed with vascular dementia. However, the neuropathological mechanisms linking microstrokes to cognitive decline remain unclear, with no specific treatment options available. We present a mouse model to study hippocampal memory circuits in the healthy condition and after induction of cerebral microstrokes via microsphere injections. Mice are trained to navigate in a virtual corridor while recording the same hippocampal neurons via chronic two-photon calcium imaging before and after stroke for up to six weeks. Our approach allows tracking individual neurons in health and disease in relation to the cognitive performance in the spatial navigation task. We identify different classes of neurons based on their stability to encode for spatial information including stable and unstable place cells. While neurons in healthy networks largely maintain their function across days, this functional imprinting is disrupted in rewiring networks after stroke. Animals with full cognitive recovery exhibited a higher number of stable place cells than mice with a persistent cognitive deficit, where networks show a reduction of the quality and stability of neuronal representation of spatial information across session in the VR corridor. Furthermore, we identified synchronous activity of surviving neurons significantly enhanced in recovered mice, suggesting functional stability and neuronal synchronicity as protective mechanisms preventing cognitive decline.
Our results provide insights into fundamental principles of network rewiring and reorganization as intrinsic repair mechanisms of the brain after multiple microstrokes, laying the basis for the development of novel therapeutic approaches or optimized cognitive rehabilitation strategies in vascular dementia.

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Poster

PSTR425

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Topic: H.03. Decision Making

Support: Simons Foundation; SCGB

Title: Representational changes in the hippocampus are related to goal-directed learning

Authors: *P. D. RICH[1], S. THIBERGE[1], N. D. DAW[2], D. W. TANK[3];
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Abstract: Neural representations in the hippocampus change their responses over days and hours. How such a dynamic representation may support ongoing decision-making is poorly understood, as observations are usually performed during simple behavioral tasks that do not allow identification of the cognitive strategy an animal is using to solve the task.
We trained rats to perform a dynamic navigation task that required the continual learning of new routes to various goals. Animals were presented with an odor cue which indicated the availability of reward at a certain location in the maze. In blocks of trials, the configuration of the maze was changed so that a new route would need to be taken to get to some goals. Animals showed fast switching for different goals, and incremental learning of the new routes after a block change. Goal-directed learning is a behavioral strategy that can be detected by its reliance on the use of indirect experience to guide decisions. In some block transitions, only a single goal has a changed route, so animals could learn the new route from direct experience with a single odor only. In other block transitions, more than one goal has a changed route, and animals could learn from indirect experience with different odors. We found that animals learned faster in block transitions with relevant indirect experience, indicating a reliance on a goal-directed strategy in this task. We used a reinforcement learning model of behavior that explained the dynamics of learning with a combination of an incrementally updated estimate of maze configuration, and a fast planning step for goal switching.
Neural activity in dorsal CA1 during the odor cue presentation period showed a strong odor encoding, but was invariant to the immediate left right-choice and the current configuration of the maze. Moreover, the neural representation changed throughout the 4-hour sessions, despite revising the same experimental conditions. We found that odor responses showed increased changes across block transitions when more than one goal has a changed route (indirect learning) compared to block transitions when only one goal has a changed route (direct learning only). This provides evidence that the representational changes in the hippocampus are directly related to goal-directed learning.

Disclosures: P.D. Rich: None. **S. Thiberge:** None. **N.D. Daw:** None. **D.W. Tank:** None.

Poster

PSTR425

Hippocampal Circuits II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR425.22/V12

Topic: H.03. Decision Making

Support: NIH Grant 5U19NS132720

Title: What is memory made of? An all-optical cellular-resolution study of engram reactivation during decision-making.

Authors: *M. IOFFE[1], J. B. JULIAN[2], A. DRINNENBERG[4], J. KAMINSKY[3], T. L. DAIGLE[6], L. SIVERTS[8], B. TASIC[7], H. ZENG[9], C. RAMAKRISHNAN[5], K. DEISSEROTH[10], C. D. BRODY[11], D. W. TANK[1];
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Abstract: Partial retrieval cues can invoke recall of past experiences. Such partial-cued recall is hypothesized to occur through pattern completion within hippocampal `memory encoding' engram neurons. To test this hypothesis, we trained mice to perform a virtual-reality decision-making task. During learning of a particular stimulus-choice association, we labeled the population of neurons expressing the immediate early gene Arc with the calcium indicator GCaMP6M/S and channelrhodopsin ChrMINE (or ChrimsonR). Using an all-optical cellular-resolution stimulation and imaging approach, we subsequently optogenetically reactivated small groups (N=5-100 targets) of individual hippocampal (CA1) engram neurons (a random “part” of a memory) during the task. Reactivation of as few as 5 individual engram neurons was sufficient to bias choice behavior on a trial-by-trial basis, weeks after the encoding event used to tag the neurons. The magnitude of the evoked behavioral bias was largely invariant to the number of directly reactivated engram neurons, and was similar to the bias observed following bulk reactivation of large populations of CA1 engram neurons using implanted optic fibers. We are investigating whether and how memory recall depends on perturbation timing, as well as whether recall is preferentially evoked when reactivation drives pattern completion within engram vs. untagged neurons.

Disclosures: M. Ioffe: None. **J.B. Julian:** None. **A. Drinnenberg:** None. **J. Kaminsky:** None. **T.L. Daigle:** None. **L. Siverts:** None. **C. Ramakrishnan:** None. **K. Deisseroth:** None. **C.D. Brody:** None. **D.W. Tank:** None.

Poster

PSTR425

Hippocampal Circuits II

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR425.23/V13

Topic: H.03. Decision Making

Support: NIH Grant U19NS104648

Title: Hippocampal engrams are causal to flexible context-dependent decision-making

Authors: *J. JULIAN[1], J. KAMINSKY[1], D. W. TANK[2], C. D. BRODY[3];
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Abstract: The context in which we find ourselves strongly impacts the choices we make. The prefrontal cortex (PFC) has figured prominently in most accounts of context-dependent decision-making (CDM), but the neural source of contextual signals that shape PFC choice dynamics for flexible behavior is unknown. Recent studies of hippocampal 'engram' cells suggest that these populations act as a neuronal substrate for contextual memory; however, CDM often involves working memory, executive function, and other cognitive processes that are distinctly separate from the classical conditioning assays that have dominated the engram literature. We thus tested whether and how hippocampal engram activity guides flexible CDM behavior. We developed a virtual-reality CDM task for mice: across different contexts, the same sensory information was associated with different choices, and the same choice was associated with different sensory information. To test whether hippocampal engrams are causal to decisions during this task, we labeled the population of neurons activated in one context with channelrhodopsin-2 and later optically reactivated these same neurons in either the same or different context after mice have learned context-specific decision rules. Optogenetic reactivation of context-specific hippocampal engrams biased mice to retrieve context-specific decision rules on a trial-by-trial basis. Ongoing work is exploring whether hippocampal engrams guide flexible decision-making by gating choice-related signals in the medial PFC. Together, our observations suggest that hippocampal engrams serve as a neural source of contextual information during flexible decision-making.

Disclosures: J. Julian: None. **J. Kaminsky:** None. **D.W. Tank:** None. **C.D. Brody:** None.

Poster

PSTR425

Hippocampal Circuits II

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR425.24/V14

Topic: H.03. Decision Making

Support: NIH Grant U19NS132720
C.V. Starr Fellowship
Burroughs Wellcome Fund

Title: Low-dimensional manifolds in frontal cortex and hippocampus during spatial cognition

Authors: *M. SCHOTTDORF[1], C. D. BRODY[2], D. W. TANK[1];
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Abstract: Frontal cortex and hippocampus (HPC) play key roles in decision-making. Here, we explore neural representations in these brain-areas in a complex virtual reality task in which mice accumulate transiently visible and stochastic left/right cues in a T-maze to infer the correct reward location. Neural data was recorded simultaneously with up to 10 Neuropixel shanks in bilateral HPC and medial prefrontal cortex (mPFC). To compare population activity patterns, we analyze these data with state space models, in which each neuron is represented by an activity axis, and population activity constitutes a trajectory in this high-dimensional space. We demonstrate that mPFC neural activity lies on a structured, low-dimensional and non-linear subspace. mPFC population activity can therefore be parameterized by a relatively small number of latent variables that span this neural activity manifold. We find that behavioral and cognitive variables are represented as smooth gradients and individual neurons are highly tuned to inferred latent variables. Similar to our previous findings with Ca^{2+}-imaging (Nieh*, Schottdorf* et al. 2021), we also found a low-dimensional neural manifold in HPC. While the mPFC manifold was significantly higher-dimensional than HPC, they share surprising similarities: a decoder trained on the HPC manifold can decode behavioral variables from mPFC and vice versa. The results reported here, together with a growing literature of related findings across tasks, provide evidence that hippocampal representations might be used for scaffolding cortical computations, such as working memory.

Disclosures: M. Schottdorf: None. **C.D. Brody:** None. **D.W. Tank:** None.

Poster

PSTR426

Human Navigation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR426.01/V15

Topic: H.09. Spatial Navigation

Support: TBD

Title: Conjunctive Encoding in Human Place and Time Cells and Their Relation to Spatial Memory

Authors: *S. MAESTA-PEREIRA[1], T. DONOGHUE[1], S. E. QASIM[2], H. AZAB[3], E. H. SMITH[6], R. MATHURA[4], J. MYERS[5], A. ANAND[5], J. ADKINSON[5], T. DAVIS[7], Z. KURTH-NELSON[8], H. G. REY[9], J. D. ROLSTON[10], T. E. BEHRENS[11], M. M. BOTVINICK[12], S. A. SHETH[5], J. JACOBS[1];
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Houston, TX; [6]Neurosurg., [7]Univ. of Utah, Salt Lake City, UT; [8]Max Planck UCL Ctr. for Computat. Psychiatry, Univ. Col. London, London, United Kingdom; [9]Dept. of Neurosurg., Med. Col. of Wisconsin, Milwaukee, WI; [10]Neurosurg., Brigham and Women's Hosp., Boston, MA; [11]FMRIB Centre, Univ. Oxford, Oxford, United Kingdom; [12]Princeton Univ., Princeton, NJ

Abstract: To navigate a dynamic world, the brain must be able to keep track of the 'when' and 'where' of actions in the world, as well as be able to integrate these information streams together to plan future actions. Work in animal models has demonstrated that single-neurons, especially in the medial temporal lobe (MTL), can encode place and time, as well as conjunctive encoding of such information - providing a potential mechanism for encoding this information as required for navigating complex environments. In this work, we extend this work into the human brain, examining single-unit responses collected from the MTL and surrounding regions of neurosurgical patients with implanted microwires. Patients (n=23) completed a computer-based virtual navigation task, including navigation decisions (alternating directions across subsequent trials at a choice point), as well as a spatial memory component in which subjects learn and then recall specified locations. In this data, we first replicate that we find single neurons in the human brain that encode place or time, with firing rates relating to subjects' virtual spatial position or elapsed time during stationary periods. We then looked for conjunctive encodings (or 'splitter cells'), finding novel results whereby single neurons in the human brain encode a conjunctive representation of multiple features, including for current location + future turn direction and current time + future turn direction. Finally, we analyzed the relationship between conjunctive encodings, neural responses to the chest-encounter events, and behavioral performance on the memory component of the task (computed as the distance error between response and true locations), finding that single-neuron activity related to behavioral performance on the task. Overall, our results serve to further establish conjunctive encoding in the human brain altogether contributing to the scientific understanding of how we navigate through space and time.

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Poster

PSTR426

Human Navigation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR426.02/V16

Topic: H.09. Spatial Navigation

Support: NIH Grant MH104606

Title: Human single-neuron correlates of spatial navigation and memory performance across encoding and recall

Authors: *C. HAN[1], T. DONOGHUE[1], M. TSITSIKLIS[2], J. JACOBS[1];
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Abstract: A multitude of investigations have explored the neural basis of spatial navigation and memory-related processes represented in hippocampus and surrounding structures in the medial temporal lobe (MTL). A large body of the existing literature demonstrates that the activity of specific cell types can show selective spatial modulation (e.g. place cells) using naturalistic tasks that involve complex human behaviors. While previous research has focused on analyzing single-neuron activity mostly during encoding periods of a task, what has been less examined is the precise relationships between such neural activity and behavioral performance by analyzing activity during recall periods. To address these questions, we recorded neural data from the hippocampus and surrounding areas in the MTL of human neurosurgical patients with implanted microwires while they performed a spatial episodic memory task named Treasure Hunt. In each trial, participants navigated an open arena, learning and later recalling the location of presented items. Examining the encoding and recall phase of the task, we first analyzed single-neuron activity for general stimulus responses as well as specific spatial modulations such as spatial target cells whose responses reflect the position of presented items. We found a significant number of cells that not only showed stimulus-related responses during either encoding and recall periods separately but also were highly active in both task phases. In addition, we observed an emerging pattern of cells that are spatially tuned to target location during both phases of the task. Next, we examined the relationship of firing patterns between encoding and recall periods and found that single-neuron activity during recall is positively correlated with activity during encoding and such association is strongly modulated by memory performance. Altogether, these results demonstrate the benefits of directly comparing the neural activity during encoding and retrieval to specify the relationship between neural activity during encoding and behavioral performance during recall.

Disclosures: C. Han: None. **T. Donoghue:** None. **M. Tsitsiklis:** None. **J. Jacobs:** None.

Poster

PSTR426

Human Navigation

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR426.03/V17

Topic: H.09. Spatial Navigation

Support: R01-MH104606
R01-NS125250
R01-NS107357

Title: Cholinergic modulation of human hippocampal oscillations during memory retrieval

Authors: *T. GEDANKIEN[1], J. L. KRIEGEL[2], B. C. LEGA[2], J. JACOBS[1];
[1]Biomed. Engin., Columbia Univ., New York, NY; [2]Neurosurg., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Cholinergic dysfunction is a hallmark of Alzheimer's disease and other types of memory disorders. Yet, we don't fully understand the neural mechanisms implicating the cholinergic system in memory. Previously, we showed that administering scopolamine, a cholinergic blocker, to humans during a free recall task impaired memory encoding and simultaneously disrupted the amplitude and timing of theta oscillations in the hippocampus (Gedankien et al., 2023, Nature Communications). Here, we expand this approach to an associative recognition task that allows us to compare the effects of cholinergic blockade between encoding and retrieval. We show that scopolamine significantly impairs memory when delivered before encoding, but spares memory if delivered prior to retrieval only. Thus, our results indicate that the disruptive behavioral effects of cholinergic blockade are specific to memory encoding, rather than retrieval. Examining hippocampal brain recordings following scopolamine administration, we describe how cholinergic blockade impacts theta amplitude and phase alignment during retrieval, as well as how it modulates the reinstatement of spectral patterns during retrieval. Together, our results suggest that cholinergic blockade impairs memory by disrupting oscillatory dynamics in the theta and gamma bands which normally facilitate synaptic plasticity within the hippocampus. These findings provide insights into the neural basis underlying memory encoding and retrieval in humans, which may aid in the development of new treatments for memory loss.

Disclosures: T. Gedankien: None. **J.L. Kriegel:** None. **B.C. Lega:** None. **J. Jacobs:** None.

Poster

PSTR426

Human Navigation

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Program #/Poster #: PSTR426.04/V18

Topic: H.09. Spatial Navigation

Support: NIH R01MH104606
NIH K00EY031607

Title: Coordinated traveling waves between the MTL and neocortex support memory encoding

Authors: *S. E. FAVILA[1], U. R. MOHAN[2], J. JACOBS[3];
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Abstract: Coordinated activity between the medial temporal lobe (MTL) and surrounding brain regions is thought to support successful memory encoding. Traveling waves, or neural oscillations that propagate across the brain, are one potential mechanism for the flexible orchestration of neural activity. Low frequency traveling waves have been observed in the human hippocampus and, more recently, across wide swaths of the human cortex, where they correlate with memory behavior. Those separate findings raise the possibility that previously identified traveling waves in the hippocampus and neocortex are coordinated with each other to support memory. Here, we explored this question in a large dataset of intracranial recordings acquired while human neurosurgical patients performed the free recall episodic memory task (N = 160 patients). From this dataset, we identified low frequency oscillatory activity in approximately 1,500 electrodes within the MTL (hippocampus, entorhinal cortex, perirhinal cortex, parahippocampal cortex, and amygdala) and in approximately 15,000 electrodes simultaneously implanted in the rest of the neocortex. We found evidence for significant frequency and phase coupling between approximately 20% of all MTL and cortical electrode pairs during word encoding. The percentage of electrode pairs with reliable phase coupling was highest for electrode pairs co-oscillating around 8 Hz. In this subset of electrodes, MTL activity was phase coordinated with the temporal lobe and the insula in approximately 50% of electrode pairs, and with frontal and parietal cortex in approximately 40% of electrode pairs. We next considered whether such MTL-neocortical phase coordination occurred in electrodes that have been previously identified to exhibit cortical traveling waves (Mohan et al., 2024). We find that approximately 50% of electrodes that participated in cortical traveling waves were also phase coupled to one or more MTL electrodes, suggesting that cortical traveling waves are coordinated with the MTL during memory encoding. Together, these data suggest that traveling waves may be a framework for coordinated hippocampal-cortical communication that supports memory encoding.

Disclosures: S.E. Favila: None. **U.R. Mohan:** None. **J. Jacobs:** None.

Poster

PSTR426

Human Navigation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR426.05/V19

Topic: H.09. Spatial Navigation

Support: NSF CAREER award to J.J.

Title: Planar, spiral, and concentric traveling waves distinguish cognitive states in human memory

Authors: *A. DAS[1], E. ZABEH[2], B. ERMENTROUT[3], J. JACOBS[4];
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Abstract: Neurons in the brain have tight anatomical connections through dendritic arborizations that integrate diverse inputs and axons that project outputs to multiple, distributed areas. How do individual neurons or regions reorganize their activity so that they selectively process particular inputs and direct their outputs to match the timescale of behaviors? In this work, we show that large-scale cortical "traveling waves" underlie this rapid and selective reorganization, by propagating in specific directions, thus flexibly and efficiently communicating between the given brain regions. We designed a novel, flexible analytical framework for measuring general patterns of traveling wave propagation during human cognition, and applied this procedure to direct brain recordings from neurosurgical patients performing multiple memory experiments. In previous work, traveling waves were shown to play a critical role for certain behaviors such as visual processing and spatial navigation in rodents (Lubenov & Siapas, 2009, *Nature*) and non-human primates (Davis et al., 2020, *Nature*), and recently in human cognition (Zhang et al., 2018, *Neuron*; Mohan et al., 2024, *Nature Human Behavior*), however, these waves were planar waves, severely limiting their ability to rapidly and dynamically reorganize to adapt to complex behaviors in humans. Our results showed an array of complex traveling wave propagation patterns including sources, sinks, spirals, and heterogeneous directional propagation patterns that extended beyond those seen previously in humans. Moreover, waves with specific complex shapes correlated with particular cognitive processes. Our findings of complex patterns of traveling waves converge well on recent theoretical predictions from biologically plausible neural models (Bhattacharya et al., 2021, Scientific Reports; Sato, 2022, Scientific Reports), which posit that waves can be generated locally based on the initial spatial activation of neurons, where each neuron is connected to a few of its neighbors. These locally generated waves can propagate across widespread regions between locally connected neurons and interact with other locally generated waves, to generate complex patterns of propagating oscillations. Crucially, we were able to robustly predict a subject's behavioral state at the single trial level based on the direction and strength of the observed patterns of complex traveling waves which could be the potential basis for a novel brain computer interface. Our findings provide a fundamental new advance in explaining how the brain dynamically reorganizes large-scale neuronal processes that underlie complex human behaviors.

Disclosures: A. Das: None. **E. Zabeh:** None. **B. Ermentrout:** None. **J. Jacobs:** None.

Poster

PSTR426

Human Navigation

Location: MCP Hall A

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Program #/Poster #: PSTR426.06/V20

Topic: H.09. Spatial Navigation

Title: Distinct trajectories of traveling waves co-exist in the human prefrontal cortex.

Authors: *E. ZABEH[1], **S. KANG**[2], M. TSITSIKLIS[3], E. H. SMITH[5], G. M. MCKHANN, II[6], C. SCHEVON[7], S. A. SHETH[8], J. JACOBS[4];
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Abstract: Oscillations are present in the brain during a wide range of behaviors including memory and navigation; however, their functional role remains hard to characterize. Here, to better understand the role of neural oscillations in human cognition, we conducted high-resolution measurements of oscillations in the prefrontal cortex (PFC) of a patient undergoing intracranial monitoring for epilepsy engaging in a memory-navigation task, using multi-electrode "Utah" arrays. We used a distinctive analysis framework to measure the spatial characteristics of these waves, probing how they are organized across electrodes. As the subject performed a memory task, electrodes showed traveling waves across various frequency bands, each exhibiting distinct spatial characteristics. In the alpha and theta bands, oscillations behaved as traveling waves that often propagated in a posterior-superior direction. There were also beta oscillations that behaved as traveling waves, but they propagated differently, in anterior or posterior directions. Furthermore these waves were linked to behavior, with theta waves exclusively indicating relevance with patient memory performance: successful memory encoding led to anterior inferior propagation, while unsuccessful attempts propagated anteriorly. Importantly, we found that these directional characteristics of the traveling waves were robustly maintained across testing sessions conducted on different days, even as the specific frequencies of the oscillations on each electrode shifted. Together these findings show the importance of measuring the spatial characteristics of oscillations, as the robustness of wave directions across sessions suggests a role for oscillations in consistently linking spatially organized neural populations. These findings hold practical significance, suggesting that the propagation direction of traveling waves could offer a more stable signal for brain-computer interfacing than traditional measures based on local oscillation properties.

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Poster

PSTR426

Human Navigation

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Program #/Poster #: PSTR426.07/V21

Topic: H.09. Spatial Navigation

Support: NIH Grant U01NS121472
NIH Grant U01NS117838

Title: Theta representations of sequential task structure in the human medial temporal lobe during free walking

Authors: *U. TOPALOVIC[1], H. AZAB[6], M. STANGL[1], M. SEEBER[2], M. VALLEJO[3], D. BATISTA[1], M. JENKENS-DRAKE[1], S. HILLER[1], M. EL-GABY[11], S. SHAH[7], R. MATHURA[8], E. BARTOLI[9], A. WATROUS[10], A. ANAND[10], J. ADKINSON[10], T. DONOGHUE[12], S. MAESTA PEREIRA[12], J. J. SAKON[4], Z. KURTH-NELSON[14], E. H. SMITH[15], C. S. INMAN[16], I. FRIED[17], J. JACOBS[13], M. M. BOTVINICK[18], T. E. BEHRENS[19], S. A. SHETH[10], N. A. SUTHANA[5];
[2]Semel Inst. for Neurosci. and Human Behavior, [3]neurology, [4]Neurosurg., [5]Neurosurg. / Psychiatry / Bioengineering, [1]UCLA, Los Angeles, CA; [6]Baylor Col. of Med., Evington, VA; [7]Neurosci., [9]Dept. of Neurosurg., [10]Neurosurg., [8]Baylor Col. of Med., Houston, TX; [11]MRC Brain Network Dynamics Unit, Univ. of Oxford, Oxford, United Kingdom; [12]Biomed. Engin., [13]Dept. of Biomed. Engin., Columbia Univ., New York, NY; [14]Max Planck UCL Ctr. for Computat. Psychiatry, Univ. Col. London, London, United Kingdom; [15]Neurosurg., [16]Psychology, Univ. of Utah, Salt Lake City, UT; [17]DEPT NEUROSURGERY, UCLA Sch. Med., Los Angeles, CA; [18]Princeton Univ., Princeton, NJ; [19]FMRIB Centre, Univ. Oxford, Oxford, United Kingdom

Abstract: The study investigated how abstract and physical positions are represented in the human brain using virtual reality (VR) combined with intracranial electroencephalography (iEEG) recordings from the medial temporal lobe (MTL). The underlying hypothesis was that the MTL can encode navigational tasks abstractly, independent of the spatial characteristics traditionally associated with navigation. Participants in the study were five patients who had undergone implantation of the responsive neurostimulation (RNS) system to manage their medication-refractory epilepsy. We utilized our custom hardware system to wirelessly record and synchronize the RNS system's iEEG data with precise location and eye-tracking measurements. The VR experiment took place within a simulated 10 x 10 m^2 room featuring a grid of nine target locations (nodes) arranged in a 3 x 3 pattern. Each target node potentially concealed a hidden reward. During the learning phase, participants were tasked with learning the spatial locations of four sequentially ordered hidden rewards. In the subsequent retrieval phase, participants revisited these locations eight times. Interactions with nodes involved participants clicking a handheld joystick button, followed by visual feedback indicating whether the node contained a reward (positive indicator) or not (negative indicator). After repeated retrieval of one reward combination, participants proceeded to the next sequence, with six unique reward sequences shuffled and repeated three times each. Analysis revealed a significant increase in hippocampal and entorhinal iEEG power (3-12 Hz) during correct responses (±2 s) compared to incorrect ones. This differentiation showed significantly elevated pre-interaction and declined post-interaction neural activity for correct responses, with the opposite pattern observed for incorrect interactions (permutation test, $p = 1.7x10^{-4}$). Further, neural power analysis at each of the nine nodes confirmed that these patterns were consistent regardless of node location, indicating that task-related neural activations were driven by the abstract task sequence rather than specific spatial locations (permutation test, $p = 1.2x10^{-3}$). Tests across participants revealed statistically

significant distinctions in brain activity patterns between correct and incorrect interactions, supporting the hypothesized abstract encoding of navigational tasks. These findings suggest that the MTL's processing of navigation tasks may be abstracted from physical space, corroborating single-neuron findings from freely moving mice (Behrens lab) and stationary human experiments (Sheth lab).

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Poster

PSTR426

Human Navigation

Location: MCP Hall A

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Topic: H.09. Spatial Navigation

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NIH Grant K99NS126715
Keck Junior Faculty Award
McKnight Foundation, Technological Innovations Award in Neuroscience

Title: Human MTL-PFC interactions during real-world and imagined navigation

Authors: *M. SEEBER[1], M. STANGL[1], M. VALLEJO[2], U. TOPALOVIC[1], S. HILLER[1], C. H. HALPERN[5], J.-P. LANGEVIN[6], V. R. RAO[7], I. FRIED[8], D. ELIASHIV[3], N. A. SUTHANA[4]; [2]neurology, [3]Dept. of Neurol., [4]Neurosurg. / Psychiatry / Bioengineering, [1]UCLA, Los Angeles, CA; [5]Stanford Univ., Stanford, CA; [6]Dept. of Neurosurg., Univ. of California Los Angeles, Los Angeles, CA; [7]Neurol., Univ. of California, San Francisco, San Francisco, CA; [8]DEPT NEUROSURGERY, UCLA Sch. Med., Los Angeles, CA

Abstract: The medial temporal lobe (MTL) is a key structure for spatial navigation and episodic memory, and a large body of rodent studies proposed close parallels of its functions supporting both tasks. During spatial navigation, sensory inputs, such as physical constraints, are present but might be absent when recollecting episodic memories. However, how internal neural organization mechanisms needed to recollect episodic memories are realized in the human MTL and how they interact with cortical areas remains unclear. This study investigates how internally generated neural dynamics in the human MTL are coupled to cortical structures during real-world and imagined navigation. We analyzed intracranial electroencephalography (iEEG) in

freely moving humans recorded from a chronically implanted device in five individuals receiving responsive neurostimulation therapy. Simultaneously, we recorded scalp EEG (64 channels) co-registered to individual magnetic resonance images to enhance its spatial interpretability with EEG source imaging. Participants were asked to walk two trajectories in an indoor room (14.6 × 13.5 m^2) and refined their walking trajectories based on motion capture feedback displaying actual and instructed routes. After each actual walk (30-35, left/right walks each), participants walked on a treadmill while imagining these learned walking trajectories in their minds. In agreement with previous reports, theta oscillations in the 3-10 Hz frequency range were evident at intracranial MTL electrodes. After aligning motion trajectories to each other, we accordingly analyzed theta modulations dependent on their position. These theta dynamics effectively encoded participants' positions within the linear segments of their walking trajectories. Given the imagined navigation trials' start, duration, and end times, we found theta modulations significantly correlated (r=0.30, p=0.003) between real-world and imagined navigation. We subsequently used these theta dynamics as priors to decompose scalp EEG and suppress unrelated activity, such as motion artifacts. We identified a subspace of components that significantly co-modulated with the intracranial data. Using EEG source imaging, we found that the generators of at least one component in every participant overlap with the actual location of the intracranial dynamics given by the implantation sites. In addition, we found other components that were functionally coupled to MTL dynamics, but their sources were localized to the prefrontal cortex (PFC). These findings open up novel possibilities for studying human MTL interactions with cortical areas in freely behaving and imagining humans.

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Poster

PSTR426

Human Navigation

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR426.09/V23

Topic: H.09. Spatial Navigation

Support: R01MH119086
R01HD097619

Title: Investigating the role of cognitive maps in representing sensorimotor programs

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Abstract: Humans have an exceptional ability to recall and organize information from diverse modalities. For example, basketball players dynamically adjust the force and timing of arm movements when taking a shot depending on their court location. However, it remains unclear how the brain organizes different sensorimotor features to recall and execute complex motor tasks. The cognitive map hypothesis posits that the brain constructs mental representations of spatial and abstract environments to guide memory formation and future actions. Cognitive maps are neurally instantiated by grid cells in the entorhinal cortex (ERC), which have receptive fields arranged in a hexagonal pattern. Here, we investigated whether cognitive maps are used to represent different features of sensorimotor information. We hypothesized that in humans, the ERC utilizes a hexagonal grid-like code to construct a cognitive map that represents sensorimotor information. Using functional magnetic resonance imaging (fMRI), we investigated ERC activity as healthy human participants performed effortful isometric hand-grip contractions. These contractions varied in force and duration and were organized in a 2D force-duration space. We found that participants' neural modulation in ERC was explained by six-fold periodicity representing a hexagonal structure. This result indicates that the ERC uses a hexagonal grid-like code to represent sensorimotor information using a cognitive map. Importantly, the ERC has shown to utilize hexagonal grid-like codes when navigating spatial and abstract environments. Our finding suggests that the ERC can also utilize a cognitive map to navigate different sensorimotor features while recalling and executing movements. Our subsequent fMRI analyses will investigate how sensorimotor regions such as the primary motor cortex and the supplementary motor area represent the sensorimotor features of the contractions. In addition, we will investigate how sensorimotor information is relayed from sensorimotor regions to form grid-like representations in the ERC.

Disclosures: J. Lee: None. **Y. Wang:** None. **A. Casamento Moran:** None. **D. McNamee:** None. **V.S. Chib:** None.

Poster

PSTR426

Human Navigation

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Topic: H.09. Spatial Navigation

Support: NIH/NEI Grant, 2R01 EY022350
NRSA Fellowship 1F32EY036266-01

Title: Cognitive maps acquired from afar: Allocentric representations of scene-space in the human brain

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Abstract: When we look out at a visual scene, we perceive it as a space, we can individuate different locations within that space, and we can understand the intrinsic (allocentric) spatial relationship between those locations. That is, we can create a cognitive map of the space "out there", akin to the cognitive map that humans and animals more typically create through physical exploration. What are the neural mechanisms that allow us to do this? To address this question, we scanned participants with fMRI while they attended to locations within a virtual courtyard, viewed from outside the courtyard. Stimuli were courtyard images taken from 4 possible viewpoints outside and slightly above the courtyard, spaced 90 degrees apart. Each image contained an indicator object (a car), in one of six possible allocentric locations. On each trial, participants reported whether the indicator object was facing the same or different allocentric direction as in the previous trial. This 1-back task was designed to direct attention to the location of the indicator object within the allocentric framework of the courtyard without requiring explicit reporting of that location. The fact that each location was viewed from all possible viewpoints allowed us to identify allocentric representations of the object's location. Two preliminary behavioral studies (N=30 each) found that reaction times were faster and accuracy greater when the same courtyard location was repeated in sequence, thus providing evidence for an allocentric representation of attended locations. Preliminary fMRI data (N=15 of 30) show that multivoxel patterns in the scene-selective retrosplenial complex (RSC) distinguished between these allocentric locations, whereas the scene-selective parahippocampal place area (PPA) and occipital place area (OPA) distinguished between courtyard views. This pattern of results underscores the RSC's purported role in transformations between egocentric and allocentric coordinate frames, as well as implicating it in the formation of allocentric representations from afar. This motivates further analyses and studies investigating whether anatomically distinct mechanisms could be responsible for allocentric representations of spaces viewed from afar vs. spaces that have been traversed from the ground level.

Disclosures: A. Shafer-Skelton: None. **T. Japaridze:** None. **R.A. Epstein:** None.

Poster

PSTR426

Human Navigation

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Topic: H.09. Spatial Navigation

Support: Marga und Walter Boll Stiftung grant 210-05.01-21

Title: Human cognitive representations of horizontal, vertical and three-dimensional space.

Authors: *O. BOCK;
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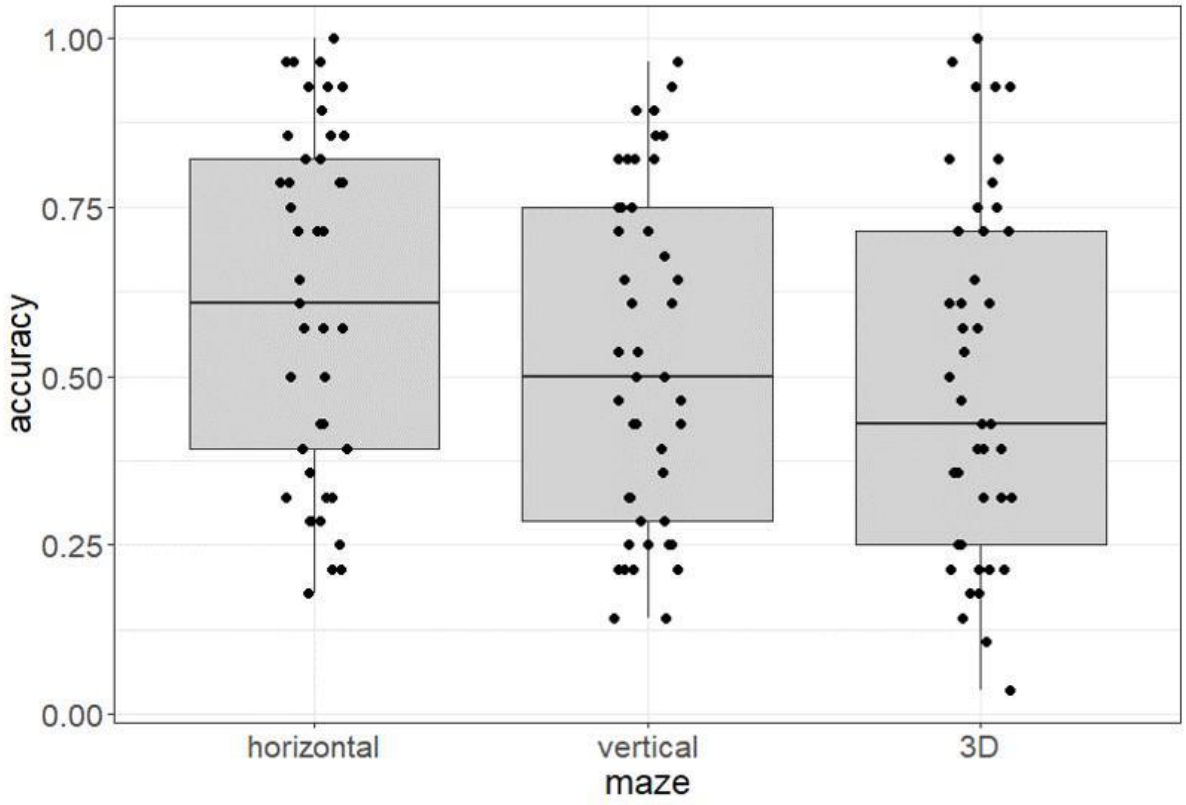
Abstract: Spatial orientation and wayfinding is often based on cognitive representations of the environment ("mental maps"). It is known that these representations can be two- or three-dimensional, depending on task demand. This study evaluates whether two-dimensional representations are easier to form compared to three-dimensional ones. Participants were guided through a virtual maze where a unique object appeared at each of 12 neighboring intersections, and then had to revisit those objects in a different order. The objects were arranged in a vertical or a horizontal plane, or in 3D; the order of these three conditions was balanced across participants. Accuracy (= proportion of correct decisions at intersections) was moderately but significantly higher in the horizontal plane compared to both the vertical plane and 3D, with no significant difference between vertical and 3D, as shown in the figure. Decision time (= time from next-object presentation to participants' decision) was comparable in all three conditions. In conclusion, forming a cognitive map with 12 objects was more efficient in the horizontal plane compared to the vertical plane and 3D, but all three spatial representations were read out at a comparable speed. The accuracy advantage of horizontal representations has potential implications for domains such as VR gaming, neurorehabilitation and urban design.



Disclosures: O. Bock: None.

Poster

PSTR426

Human Navigation

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Program #/Poster #: PSTR426.12/V26

Topic: H.09. Spatial Navigation

Support: Collaborative Research in Computational Neuroscience Grant (01GQ2106) of the German Ministry of Education and Research (BMBF)

Title: How altered coding of environmental boundaries affects spatial memory in human aging: An fMRI Study

Authors: *V. SEGEN[1], M. STANGL[2,3], T. WOLBERS[4];
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Abstract: Environmental boundaries provide an important spatial cue for navigation and are coded in the entorhinal cortex (EC) and subiculum. In humans, boundary-related behavior has been shown to be sensitive to aging. Specifically, older adults are less sensitive to boundary manipulations while older adults at increased risk of developing Alzheimer's disease show a preference towards navigating closer to environmental boundaries. At present, however, the mechanisms giving rise to altered boundary coding in older adults and their impact on spatial memory precision is not well understood. To address this important question, twenty young and twenty healthy older adults underwent functional magnetic resonance imaging (fMRI) while performing an object location memory task in a virtual environment, with objects placed at varying proximities to boundaries. We found that older adults have less precise memory for object locations, but only when these locations are further from boundaries. In addition, older adults exhibited a response bias, often re-placing objects too close towards boundaries. Parametric fMRI analysis revealed increased BOLD signal modulation in older adults relative to the proximity to boundaries in both the EC and the subiculum. Notably, greater sensitivity to boundary distance was correlated with poorer performance, particularly among older adults. This increased signal modulation in response to boundary proximity in EC was also linked to greater response bias towards boundaries observed in older adults. Moreover, group-level analysis revealed higher precuneus activity in young adults correlating with distance to boundaries at trial onset. Collectively, our findings indicate that older adults with poorer performance demonstrate a response bias towards boundaries and exhibit a reduction in BOLD signal with increased distance from boundaries in both the EC and subiculum. Although we have no direct evidence for the involvement of border/boundary vector cells in our paradigm, our results would be consistent with altered boundary coding in healthy aging. Specifically, while older adults are as precise as young adults when objects are near boundaries, their spatial coding deteriorates for objects placed further away, potentially due to an over-reliance on border cells, which are densely activated near boundaries but sparsely elsewhere. In contrast, young adults displayed less variation in BOLD signal based on object distance from boundaries, suggesting a reliance on more comprehensive spatial coding mechanisms that could rely on boundary vector cells that are not biased towards proximity and provide more accurate location coding.

Disclosures: V. Segen: None. **M. Stangl:** None. **T. Wolbers:** None.

Poster

PSTR426

Human Navigation

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Program #/Poster #: PSTR426.13/V27

Topic: H.09. Spatial Navigation

Support: Project-ID: 425899996 – SFB 1436, Deutsche Forschungsgemeinschaft (DFG)

Title: Object Vector Coding in the Human Brain

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Abstract: A key property of mammalian navigation is that locations of interest are often stored with respect to local or global landmarks. In rodents, this coding scheme has been related to vectorial information derived from object vector cells in the medial temporal lobe (MTL, Wang et al., 2018), which encodes both the distance and direction towards local objects. Furthermore, the caudate nucleus has also been shown to provide vectorial information but in an egocentric reference frame (Hinman et al., 2019). In humans, so-called egocentric bearing cells have been observed in the MTL, while the role of the caudate is often reduced to simple stimulus-response associations in service of route knowledge. However, the exact spatial properties being processed in this area still remain unclear.
To elucidate mechanisms of allocentric object vector coding in the human brain, we tested healthy young adults with functional Magnetic Resonance Imaging (fMRI) and a virtual reality based object location memory task. Prior to scanning, participants learned four target locations in relation to a fixed landmark, with the landmark providing both directional and distance information. In the subsequent fMRI session, participants were able to retrieve object locations within a predefined performance criterion, indicating effective learning of each location. Critically, even though we ensured that visual input was identical across the four test locations, we were able to decode the target positions from multivariate BOLD response patterns in the caudate nucleus, lateral occipital cortex and precuneus. Furthermore, model-based representational similarity analysis revealed that the lateral occipital cortex and the precuneus were sensitive to the allocentric direction towards the landmark, while the caudate nucleus might encode the distance component. Together, these results point towards a distributed code of allocentric object vectors in the human brain, which complements the boundary related processing typically observed in the entorhinal cortex and the subiculum.

Disclosures: J. Chen: None. **T. Wolbers:** None.

Poster

PSTR426

Human Navigation

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Topic: H.09. Spatial Navigation

Support: AMED Grant No. JP21wm0425016

Title: Impaired path integration ability correlates with reduced activation of the retrosplenial cortex during virtual navigation task

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Abstract: Studies have shown that navigation involves the entorhinal cortex (EC) for egocentric strategies and the retrosplenial cortex (RSC) for both allocentric and egocentric strategies. Both regions are also involved in the early stages of Alzheimer's disease (AD). Therefore, there is a growing interest in using navigation dysfunction as an early biomarker for AD. In this study, we used functional MRI (fMRI) to investigate the neural correlates associated with impaired navigation ability. Participants were classified as good (n = 18) or poor (n = 13) navigators based on their path integration ability, represented by an error distance metric measured using a 3D virtual reality navigation task performed outside the scanner. The task fMRI used a block design consisting of a video instruction block, followed by a self-navigation block, and a rest block, repeated 5 times. In the video instruction blocks, participants were presented with a first-person perspective video showing a pre-specified route in a virtual town that they had to navigate during the self-navigation blocks. We also used resting-state fMRI to examine connectivity changes in poor navigators using a network metric called functional connectivity overlap ratio (FCOR), which quantifies voxel-to-network connections. Compared to good navigators, poor navigators had lower activation in the brainstem, left RSC, bilateral precuneus, bilateral angular gyrus, and right cerebellum. The mean RSC activation also showed significant negative correlation ($r = -0.72$, $p = 4.6 \times 10^{-6}$) with the mean error distance. FCOR analysis revealed that the RSC is a hub region connected with the primary processing networks (sensorimotor and visual) as well as the default mode network (DMN). Additionally, the left hippocampus and the right middle frontal gyrus (MFG) exhibited widespread connections with the DMN and the left executive control network (LECN), respectively, in the poor navigator group. Taken together, our results suggest that poor navigation performance is associated with lower activation in cortical regions involved in visual and visuospatial processing during navigation. Specifically, the reduced activation of the RSC, a connector hub region, may have contributed to the reduction in navigation ability, which requires the integration of spatial information from multiple sensory modalities. However, the widespread connectivity of the hippocampus and MFG to the DMN and LECN, respectively, may also suggest compensatory actions of the core neurocognitive networks to mitigate

navigation dysfunction. The involvement of DMN and its associated regions also suggests a potential link with AD.

Disclosures: E. Bagarinao: None. **R. Ohdake:** None. **H. Isoda:** None. **A. Takashima:** None. **H. Watanabe:** None.

Poster

PSTR426

Human Navigation

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Topic: H.09. Spatial Navigation

Support: CIHR Grant 163014-2019
CIHR Doctoral Research Award: Canada Graduate Scholarships

Title: Neural patterns during cognitive map formation and virtual navigation in blindness

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Abstract: Blindness significantly impacts the ability to acquire spatial information during navigation and to build cognitive maps for orientation in the environment. Possible consequences include reduced autonomy, increased anxiety, social isolation, and overall decreased quality of life. Therefore, it is essential to investigate non-visual cognitive map formation and its underlying neural mechanisms, which are still poorly understood. To this goal, we used *f*MRI to study the neural correlates of cognitive map formation through touch and audition in individuals with congenital and acquired blindness and sighted individuals as controls. We used a three-phased game-like paradigm designed to differentiate between 1) the formation of a cognitive map, 2) its retrieval, and 3) its use during navigation. In *phase 1 (maze exploration)*, participants used their fingers to learn the layout of a tactile maze containing multiple destinations. In *phase 2 (drop-off)*, participants were randomly placed inside a virtual rendering of the same maze and were given a goal destination. In *phase 3 (navigation)*, participants navigated this virtual space with auditory feedback to reach the destination. Results reveal distinct neural patterns during navigation in each group: sighted controls showed a reliance on frontal, insular, and parietal cortices, and blind subjects significantly activated visual occipital areas and cerebellum, combined with a generalized deactivation in the rest of the brain. However, when participants form, retrieve, or use their cognitive map, different activation patterns emerge across the navigation network (hippocampus, parahippocampal place area, retrosplenial complex, occipital place area), providing insights into their role and adaptation in blindness.

Disclosures: M. Bleau: None. **L. Dricot:** None. **M. Ptito:** None.

Poster

PSTR426

Human Navigation

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Program #/Poster #: PSTR426.16/V30

Topic: H.09. Spatial Navigation

Title: Theta rhythm predicts and prepares for itinerary replanning during an active spatial navigation task

Authors: *V. VITKOVA[1], S. SIEKIERSKI[1], A. CASTILLA[2], M. ZAOUI[3], M. PETIEAU[1], A. BERTHOZ[4], G. CHERON[1], A. CEBOLLA ALVAREZ[1];
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Abstract: The ”Virtual House Locomotor Maze” (VHLM) (Castilla et al., 2021) is a novel paradigm based on the “Virtual Carpet” (Berthoz & Zaoui, 2015) technology, assessing place and direction errors, as well as navigation strategy kinematics, during active navigation in a realistic behavioral situation (Del Lucchese et al., 2021; Kronovsek et al., 2021). Using VHLM, surface electroencephalography (EEG) and inverse modelling (swLORETA, Cebolla et al., 2011; Palmero-Soler et al., 2007), we studied the brain dynamics and signal sources characterizing cognitive flexibility during itinerary inhibition and replanning.A virtual city with 6 houses was projected on the floor. Participants navigated to the same target house 5 times along the same route (learning). On the 6th attempt, the overlearned path was obstructed, requiring participants to find a new route to the target (inhibition and replanning). The brain activity of 19 participants was recorded during the 5th (NAV5) and 6th (NAV6) navigation. Target onset (t0) occurs when the target house is visually cued. Epochs include a 2 sec baseline, followed by a 4 sec period after t0.A grand average topographical analysis of the event-related spectral perturbation (ERSP) in the theta rhythm revealed significant differences between NAV5 and NAV6 from -1350 ms to 1800 ms relative to t0. Here we focus on the time interval before t0. From -1350 to -1050 ms, NAV6 elicits an overall increase in theta power, compared to NAV5, at right parietal-occipital and bilateral frontal and central locations. From -600 to -500 ms we observe a decrease in theta power in NAV6, compared to NAV5, at left parietal-occipital locations. From -100 to 0 ms we observe a decrease in theta power in NAV6, relative to NAV5, at right frontal locations. Source localization analysis revealed that in the -1150 to -1050 ms interval, the left cingulate gyrus (BA24) contributes significantly more to the replanning task (NAV6), compared to the learned navigation task (NAV5).Results suggest that the brain: 1) anticipates obstructed trials pre-target onset; 2) shows reduced theta during well-known itineraries (NAV5); 3) resumes theta synchronization when predicting obstruction and replanning (NAV6). The anterior cingulate gyrus (BA24) has been linked to error awareness, monitoring and dynamic regulation of

performance (Magno et al., 2006; Orr & Hester, 2012). Our results suggest that this role might relate to tracking and trying to predict the deviant events, once a routine is installed. In addition to revealing the normal brain activity, this paradigm may be useful for detecting executive function, inhibition and visuo-spatial deficits in patients or during rehabilitation.

Disclosures: V. Vitkova: None. **S. Siekierski:** None. **A. Castilla:** None. **M. Zaoui:** None. **M. Petieau:** None. **A. Berthoz:** None. **G. Cheron:** None. **A. Cebolla Alvarez:** None.

Poster

PSTR426

Human Navigation

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Topic: H.09. Spatial Navigation

Support: JST CREST/JPMJCR225P5

Title: The relationship between eye position bias and walking deviation under occluded vision: Evidence that spatial orientation estimation is manifested in eye movements

Authors: T. HIRATA[1], S. TADOKORO[2,3,4], T. YAMANAKA[2], Y. SHINJI[5], N. KAWAI[1,6], *Y. HIRATA[7,6,8,9];
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Abstract: Humans continuously estimate self-postural and motion states in relation to the surrounding environment, a perception known as spatial orientation. This perception is unconsciously formed by integrating multimodal sensory information, primarily from the vestibular and visual systems, with contribution from the somatosensory system and other modalities. While the vestibular system senses dynamical components of self-motion, such as velocity and acceleration, the visual system also provides static positional information. Consequently, in conditions of poor visual information, such as darkness, we must rely on temporally integrated dynamic vestibular signals to estimate our positional state, potentially leading to biased estimations over time. This may explain the challenges in maintaining a straight walking or running path when vision is occluded.In the present study, we hypothesized that the spatial orientation formed under these conditions is reflected in the eyes. Five healthy participants provided written informed consent and were asked to walk straight after looking straight ahead for 10 seconds in a large room. They wore a VR head-mounted display (HMD. HTC Vive Pro). A uniform gray stationary image was displayed in the HMD to minimize visual

cues and white noise was delivered to the participants through earphones to prevent auditory directional cues. In this situation, participants predominantly rely on vestibular and somatosensory information to walk straight. Binocular eye positions were measured in relation to the head by the HMD. Additionally, the head position and its direction within the room were simultaneously recorded.Results showed that four out of 5 participants exhibited a slight but significant bias in their averaged eye positions, either to the left or right, while looking straight ahead before beginning to walk. Interestingly, these participants deviated from the straight path in the direction of their biased eye positions after starting to walk. Furthermore, the greater the initial deviation of eye positions from straight ahead, the more pronounced the deviation of walking direction from the straight path toward the direction of the eye position bias. These simple experimental findings suggest that a biased estimation of the self-position and direction in the situation where visual cues are unavailable are reflected in the bias of eye positions. This provides evidence that spatial orientation estimation is manifested, if not always, in eye movements.

Disclosures: T. Hirata: None. **S. Tadokoro:** None. **T. Yamanaka:** None. **Y. Shinji:** None. **N. Kawai:** None. **Y. Hirata:** None.

Poster

PSTR426

Human Navigation

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Program #/Poster #: PSTR426.18/V32

Topic: H.09. Spatial Navigation

Support: NIMH R01MH131532

Title: Exploring Depressive Symptoms Effect on Learning in a Rewarding Environment

Authors: *K. JORDAN[1], C. A. SHARP[2], C. MARINO[1], S. BAVDEKAR[1], T. GARG[1], J. TAYLOR[3], M. HALVORSEN[1], E. PINEDA[1], B. SUAREZ-JIMENEZ[2];
[1]Univ. of Rochester, Rochester, NY; [2]Neurosci., Univ. of Rochester, Rochester, NY; [3]City Col. of New York, New York, NY

Abstract: Depression symptoms affect how a person reacts to their environment (e.g., decreased interest in exploring new and potentially rewarding environments). Recent research has shown that depression can affect spatial memory in spatial navigation tasks. Monetary studies discuss how depressed individuals have diminished behavioral responsiveness in shifting rewarding contingencies. However, little is known about how depressive symptoms affect learning and memory in a rewarding environment. By using a VR conditioning task and an attention task, we examine how depressive symptoms impact learning and memory. 37 participants (22 male; mean age = 28.35 +/- 10.897) completed a depression questionnaire, followed by a reward conditioning VR task. Every four trials of the reward conditioning task, a neutral (i.e.,

nonvalenced) object would appear. Participants collected and later replaced the neutral objects in their original location (16 trials). After participants completed the task, they were asked a series of questions relating to the name and location of the object and the pattern of the rewards within the environment. Participants were divided into learners (n=22) and non-learners (n=15) based on whether they learned the reward contingencies. The means of HAM-D and BDI-II scores were lower in learners compared to non-learners. There was a significant main effect of zone ($F(1,35)=35.070$, $p< 0.001$). There was also a significant main effect of block ($F(3,33)=4.983$, $p=0.006$). This study did not assess any sex differences between the participants. Our results suggest that higher depressive scores could affect learning ability; however, a bigger sample and future analysis are needed. This pilot study is an important step in elucidating the impact of depressive symptoms on learning and memory.

Disclosures: K. Jordan: None. **C.A. Sharp:** None. **C. Marino:** None. **S. Bavdekar:** None. **T. Garg:** None. **J. Taylor:** None. **M. Halvorsen:** None. **E. Pineda:** None. **B. Suarez-Jimenez:** None.

Poster

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Human Navigation

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Program #/Poster #: PSTR426.19/V33

Topic: H.09. Spatial Navigation

Support: Prebys Foundation Award
University of San Diego Faculty Research Grant

Title: The effects of visual distractor cues on human solutions of the Traveling Salesperson Problem (TSP)

Authors: *R. BLASER, K. LEVY, T. OLSEN, **B. WEST**;
Univ. of San Diego, San Diego, CA

Abstract: The Traveling Salesperson Problem (TSP) is a combinatorial optimization problem originally of interest to mathematicians, but more recently used also in the context of cognitive and comparative psychology. Humans perform extremely well on spatial versions of this task, despite its mathematical complexity, making it an appealing tool for the study of spatial and mathematical cognition. Previously, we demonstrated that lesions of the hippocampus, and to a lesser extent, the medial entorhinal cortex (MEC), impair the performance of rats in a task analogous to the TSP. Lesions of the MEC primarily affected performance on spatial configurations that required a global strategy for efficient navigation, with little impairment on configurations for which a local strategy produced efficient performance. In the current study, we looked more closely at global vs. local strategy use in human participants. We presented participants with three versions of a computerized TSP; one that could be solved visually, one

with visual distractors to interfere with global cues, and one that included visual distractors and also memorization of targets. As expected, performance was best in the 'visual solution' condition, and impaired by visual distractors. Surprisingly, both reaction time and distance measures were impaired in the visual search condition compared to the memory condition.

Disclosures: R. Blaser: None. **K. Levy:** None. **T. Olsen:** None. **B. West:** None.

Poster

PSTR426

Human Navigation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR426.20/V34

Topic: H.09. Spatial Navigation

Support: NIH Grant

Title: Different task-related network configurations related to a 10 day cognitive training in navigation or episodic memory

Authors: *L. ZHENG[1], J. GARREN[1], Z. BOOGAART[2], A. MCAVAN[3], S. DONER[4], W. GROVES[5], E. YUKSEL[6], A. D. EKSTROM[1], S. WEISBERG[6];
[1]Univ. of Arizona, Tucson, AZ; [2]Univ. of Miami, Miami, FL; [3]Vanderbilt Univ., Nashville, TN; [4]Dept. of Psychology and Neurosci., Temple Univ., Philadelphia, PA; [5]the Univ. of Florida, Gainesville, FL; [6]Univ. of Florida, Gainesville, FL

Abstract: The neural basis of how new learning is integrated with existing schema (ways of organizing new knowledge), remains unclear. We tested the hypothesis that the encoding and retrieval of new episodic memories would recruit partially dissociable brain networks based on whether participants underwent training in mnemonic coding (a version of the method of loci) or spatial navigation. Alternatively, models predicting the fundamental importance of largely overlapping networks to both navigation and memory centered on the hippocampus would predict little to no differences. In two experimental groups (n=26 for each group), participants underwent a 10-day training program, with each session lasting 2 hours, dedicated to either enhancing navigation skills or mastering free recall techniques. A control group (n=13) did not receive any specific training; instead, they watched videos and documentaries related to navigation, memory, and cognition. Before and after the 10-day training period, all participants completed a source memory task while undergoing fMRI scanning. Preliminary analyses of brain-wide functional connectivity during the encoding phase of the source memory task showed a significant difference between free recall and navigation groups. Specifically, the free recall group exhibited a greater post-test increase in functional connectivity compared to the navigation group. This heightened connectivity was particularly pronounced in the parahippocampus for the free recall group, as evidenced by graph theory analysis showing an increased degree of connectivity. The free recall group also displayed a higher degree of connectivity to the left

hippocampus post-test compared to the navigation group. In contrast, the navigation training group showed a higher degree of connectivity to the precuneus compared to the free recall group. These preliminary observations suggest that distinct cognitive skill acquisition may lead to divergent network reconfigurations, potentially reflecting the unique specific cognitive demands of each skill.

Disclosures: L. Zheng: None. **J. Garren:** None. **Z. Boogaart:** None. **A. McAvan:** None. **S. Doner:** None. **W. Groves:** None. **E. Yuksel:** None. **A.D. Ekstrom:** None. **S. Weisberg:** None.

Poster

PSTR426

Human Navigation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR426.21/V35

Topic: H.09. Spatial Navigation

Title: Uncovering spatially selective neurons in the human prefrontal cortex

Authors: *A. CHAVEZ[1], A. WATROUS[1], E. BARTOLI[2], J. ADKINSON[1], R. MATHURA[3], S. A. SHETH[1], B. Y. HAYDEN[3];
[1]Neurosurg., Baylor Col. of Med., Houston, TX; [2]Dept. of Neurosurg., Baylor Col. of Med., Houston, TX; [3]Baylor Col. of Med., Houston, TX

Abstract: Cognitive maps, a mental representation of spatial relationships, have traditionally been most closely associated with the hippocampus. However, recent studies reveal that neocortical regions such as the anterior cingulate cortex (ACC), also contain map-like representations. We hypothesize that indeed there are neurons in the human prefrontal cortex that are spatially selective and that these neurons have features in common with place and head direction cells. Yet we may expect to observe functional differences in single neuron responses such as strength of tuning, robustness, and stability. To test this hypothesis, we will characterize single neuron responses in humans with indwelling microelectrodes in the anterior and posterior cingulate cortices, as well as the hippocampus, during goal directed virtual navigation. We will show results from six patients that span across several ages and from both genders. Our results show that neurons in the prefrontal cortex convey significant spatial information, after permutation testing, that is comparable to place and head direction cells. Similarly peak activity within discrete spatial bins (place or head rotation in the virtual arena) suggest that these neurons are preferentially responsive to these task features in the ACC, PCC, and hippocampus. This result is in alignment with theories of the prefrontal cortex as a hub for the abstraction of task relevant variables. While hippocampal place cells contain narrowly tuned place and head direction fields for given locations, prefrontal neurons begin to show a decrease in selectivity and instead show broader tuning. Establishing the functional differences of spatial tuning at the level

of single neurons across these brain regions is crucial for unraveling the organizing principles underlying spatial navigation.

Disclosures: A. Chavez: None. **A. Watrous:** None. **E. Bartoli:** None. **J. Adkinson:** None. **R. Mathura:** None. **S.A. Sheth:** None. **B.Y. Hayden:** None.

Poster

PSTR426

Human Navigation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR426.22/V36

Topic: H.09. Spatial Navigation

Support: UIC LAS CSSR Seed Grant

Title: Common representational geometry links mouse CA1 and human spatial memory in deformed environments

Authors: *S. PECIRNO[1], A. T. KEINATH[2];
[1]Univ. of Illinois at Chicago, Chicago, IL; [2]Psychology, Univ. of Illinois Chicago, Chicago, IL

Abstract: To understand the neural bases of human memory researchers often rely on inferences drawn from rodent models. This inference is sound only when mechanisms are conserved. Some evidence of cross-species conservation in the domain of spatial memory comes from deformation studies, where reshaping a familiar space impacts human spatial memory and mnemonic neural codes in the hippocampus and entorhinal cortex of rodents in qualitatively similar ways (O'Keefe & Burgess, 1996; Hartley, 2004; Keinath et al., 2018; Keinath et al., 2020; Bellmund et al., 2020). Though provocative, adjudicating between possible mechanistic differences requires going beyond qualitative similarity. Here, we build on this work by quantitatively comparing the impacts of nine parametric deformations on the representational geometry of mouse CA1 neural codes and human spatial memory. To do so, we leveraged untethered immersive virtual reality to teach human participants (n = 104) the locations of five objects in a 5 x 5 m square virtual space, and later asked them to replace each object in deformed versions of this space. We then compared the geometry of human spatial memory to that of mouse CA1 assayed in analogous deformations in our prior work (Lee, Keinath, et al., 2023). We find that deformations impact human spatial memory in a way which closely, but not absolutely, resembles their impacts on mouse CA1. Furthermore, we find that the geometry of CA1 cells with higher coding quality better resembles the geometry of human spatial memory, and that human participants with lower precision (but not accuracy) better resemble the geometry of mouse CA1. Finally, we show that the geometry of human spatial memory can be (to the resolution of the data) fully understood as a more change-resistant version of the geometry of mouse CA1. Follow-up experiments in which visual cues are occluded with fog during object replacement and in which the virtual space is doubled in size produced similar findings,

demonstrating that our results are not the product of visual cue use during recall or particular to the scale of the environment. Together, these results provide new, high-precision evidence of conserved mnemonic mechanisms across species from rodents to humans.

Disclosures: S. Pecirno: None. **A.T. Keinath:** None.

Poster

PSTR426

Human Navigation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR426.23/V37

Topic: H.09. Spatial Navigation

Support: CONAHCYT CVU 409838 scholarship to Cindy Oceánida Zurita Bautista

Title: Similarities in brain activity betewwn Open Eyes and Baseline condition in children during navigation in a virtual enironment.

Authors: *C. ZURITA BAUTISTA[1], I. G. GALÁN[2], Y. DEL RÍO-PORTILLA[3];
[1]UNAM Natl. Autonomous Univ. of Mexico, Mexico city, Mexico; [2]UNAM, Natl. Autonomous Univ. of Mexico, Mexico City, Mexico; [3]UNAM, México City, Mexico

Abstract: Lin et al. (2022) observed an increase in theta power in the right medial temporal region associated with movement. They highlighted the significance of optic flow, whose activity is manifested within the 1-8 Hz range, alongside other power in oscillations ranging from 2 to 7.21 Hz (delta-theta) in the frontal midline during physical movement (as opposed to standing still). They emphasize that delta-theta oscillations during navigation integrate motor, vestibular, proprioceptive, and visual information. Lin also highlights the role of optical flow during navigation, as previously reported in monkeys (Killian, Jutras, and Buffalo, 2012). Two sets of paradigms serve as baselines for brain activity during navigation: random navigation versus remaining stationary (no movement) in either a virtual or real environment (Cornwel et al., 2008; Pu et al., 2017). However, these paradigms have not been studied in children, whose maturation-related changes in brain activity need to be considered (Rowan and Tolunsky, 2004).We investigated scalp EEG activity in 57 children aged 6 to 11 years, dividing them into three groups: 6-7 years old, 8-9 years old, and 10-11 years old. We placed 19 electrodes according to the international 10-20 system. EEG activity was recorded during navigation in a virtual arena while participants traversed a virtual environment (prior to landmark encoding and recall conditions), with eyes open (EO), eyes closed (EC) during rest, and a baseline (B) condition. We analyzed age effects within the three groups described.We found nearly identical significant differences between EO and EC conditions, as well as EO and B conditions, in alpha absolute power ($p < .05$) for all age groups. In the 6-7-year-old group, both comparisons revealed differences in O2, T5, and T6; in the 8–9-year-old group, differences were observed in O2 in both comparisons; and in the 10–11-year-old group, differences were detected in P3, P4, Cz, and

Pz in both comparisons. Similar differences between Opened Eyes and Baseline (Random Navigation) suggest the role of optic flow in navigation, as proposed by Lin et al. (2022). Optical flow appears to be a factor that can influence brain electrical activity alongside other cognitive processes during navigation.

Disclosures: C. Zurita Bautista: None. **I.G. Galán:** None. **Y. del Río-Portilla:** None.

Poster

PSTR426

Human Navigation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR426.24/V38

Topic: H.09. Spatial Navigation

Title: Navigating the Translational Divide: A Cross-Species Approach to a Spatial Memory Task

Authors: *M. BORZELLO[1], N. ELLSWORTH[2], W. SUPIAN[2], A. A. CHIBA[3];
[1]Cognitive Sci., [2]Univ. of California San Diego, La Jolla, CA; [3]Cognitive Sci. and Program in Neurosci., UCSD, La Jolla, CA

Abstract: The continuous formation of new and distinct memories stands out as a critical capability observed across species. In the wild, an animal's ability to link subtle changes in an environment with food or danger can mean life or death. In our daily lives, we are compelled to acquire and retrieve distinct relationships and associations within our environments to achieve our goals, whether we are acquainting ourselves with the everchanging nature of an urban cityscape or something more quotidian such as finding your car in a parking lot. *How does the brain solve the problem of reducing interference between memories and does it do so in a similar fashion across different domains and mammalian species?* It has been hypothesized that our brain does this through pattern separation, the critical ability of the brain to disambiguate similar inputs into distinct outputs. To date, a highly pertinent question concerns whether similar behavioral motifs and organizing principles exist across rodents and humans? This is constrained, somewhat, by a limited ability to behaviorally probe rat and human subjects similarly or to fully understand the diverse ways each species solves spatial memory tasks. The first aim of the study was to develop an analogous spatial memory task in virtual reality (VR) in Unity, based on an experimental task that we previously ran in rats. We designed the present task as a translational tool for cross-species comparisons. The second aim was to examine behavioral pattern separation ability across different memory domains, to determine if this ability is domain specific in a neurotypical population. 230 subjects performed two pattern separation tasks- a virtual spatial pattern separation task that our lab developed and the validated mnemonic similarity task (MST task) which probes pattern separation in the visual domain, in addition to questionnaires regarding spatial abilities. We present results that examine the relationship between performance on the MST and our spatial VR task. Our findings demonstrate a strong

correlation between performance on the two behavioral pattern separation tasks and a comparable function of performance across separations (visual and spatial, respectively). To our knowledge, this is the first study to compare human behavioral pattern separation ability in visual and spatial domains, revealing a correspondence in the ability to disambiguate small differences across multiple domains.

Disclosures: M. Borzello: None. **N. Ellsworth:** None. **W. Supian:** None. **A.A. Chiba:** None.

Poster

PSTR426

Human Navigation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR426.25/W1

Topic: H.09. Spatial Navigation

Title: The role of implicit threat stimuli processing affects route learning in the virtual environment

Authors: *M. K. ASTHANA[1,2];
[1]Humanities & Social Sci., Indian Inst. of Technol. Roorkee, Roorkee, India; [2]Department of Design, Indian Institute of Tehcnology Roorkee, Roorkee, India

Abstract: The present study examined the impact of threat stimuli processing on route learning. Additionally, participants were subjected to threat and no-threat conditions during the route learning. We recruited forty undergraduates (12 females, mean age = 19.66; and 28 males, mean age = 19.39) from the Indian Institute of Technology Roorkee, India. All participants were randomly assigned to two groups, which further underwent learning of the short and long mazes. Group 1 (Image condition) received the images at junction points of the virtual route (Image condition), while Group 2 (No-image condition) images were not presented at the junction point. In the learning phase, participants learned the short and long routes. During the testing phase, the arrow cues were removed, accessing their learned representation of the maze. We measured the decision time at each 6 and 12 junction points and repeated trials during the testing phase. Our findings highlight that the participants were not distracted by the implicit threat stimuli during the 12 junctions in relation to the 6 junctions. Results suggest the attenuation of threat information processing during the route learning of 12-junction versus 6-junctions. It might be because of high cognitive demand during 12-junction versus 6-junctions. The study's findings hint at the dampening of threat response during the 12-junction condition versus the 6-junction condition, which is less demanding.

Disclosures: M.K. Asthana: None.

Poster

PSTR426

Human Navigation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR426.26/W2

Topic: H.09. Spatial Navigation

Support: National Institute on Aging R01 grant (1R01AG073250-01A1 to Thackery Brown)
Shurl and Kay Curci Foundation

Title: Route navigation interacts with environment geometry in distance error estimation

Authors: *J. LONG[1], E. HERRERA[2], Y. LI[1], T. I. BROWN[2];
[1]Georgia Inst. of Technol., Atlanta, GA; [2]Sch. of Psychology, Georgia Inst. of Technol., Atlanta, GA

Abstract: Current human research offers incomplete explanations for the impact of geometric information during navigation. Cognitive psychology theories indicate that geometric distinctiveness may help navigation. Rodent research contradicts this, since grid cell firing fields distort in the presence of geometrically distinct boundaries. Still, grid-cell based research of geometric information encoding in human navigation is sparse and typically focuses on single object memory. This study explores the role of route navigation (multiple objects in a fixed sequence) in the encoding of environment geometry. Participants completed an egocentric, virtual navigation task where they learned routes of six object locations in two square and two trapezoidal open-field environments. The presentation order of the environments was randomized. Participants' spatial memory of the object locations and sequences was then tested by navigating the same environments from an egocentric perspective once again. In this testing, participants were instructed to place the objects in the learned locations and order as accurately as possible. Distance error estimation was defined by the Euclidean distance between participant object locations and true object locations. Analyses revealed that distance error estimation depended on an interaction between geometry and an object's position in trapezoidal environments. Object distance error estimation was non-significantly better when in the broader half of the trapezoid compared to in the square. On the other hand, object distance error estimation was significantly worse when in the narrow half of the trapezoid compared to in the square. These results may offer insight into which mechanisms mediate whether geometric distinctiveness helps or harms route navigation. In the broader half of the trapezoid, the small amount of geometric distortion compared to geometric distinctiveness could aid navigation. In the narrow half of the trapezoid, the larger geometric distortion could overshadow any benefits of geometric distinctiveness, harming navigation. Such findings highlight the importance of investigating complex route navigation scenarios and contribute to our understanding of the mechanisms that mediate geometric information encoding in navigation.

Disclosures: J. Long: None. **E. Herrera:** None. **Y. Li:** None. **T.I. Brown:** None.

Poster

PSTR426

Human Navigation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR426.27/W3

Topic: H.09. Spatial Navigation

Support: NIH R01 AG073250-01

Title: The role of medial frontal and hippocampal regions, and cognitive aging, on interactions between new and old route memories in navigation performance

Authors: *P. MAXIM, T. I. BROWN;
Sch. of Psychology, Georgia Technol., Atlanta, GA

Abstract: Research has set out to test whether existing spatial knowledge can benefit novel learning in humans, and if there are similar neural characteristics during prospective planning and recall of memories related to existing spatial knowledge. Furthermore, findings suggest aging negatively impacts flexible navigation performance, perhaps in part due to memory integration and route memory deficits. Thus, it is imperative we understand how memory influences navigation strategies and what factors lead to navigation differences as we age. The present study tests 25 healthy young adults (YA: 18-35) and 25 healthy older adults (OA: 65-80) across two days in a virtual navigation task, and uses fMRI to test complementary views of how the medial temporal lobe and medial prefrontal cortex contribute to route planning and navigation from prior knowledge. Participants are trained on specific paths through six virtual towns, and after 24 hours are tested on memory of the trained routes during fMRI. They are also tasked with navigating to novel goals in the environments using any strategy (unbound to the now-familiar route [FR]). Some trials are designed so that the optimal shortcut follows the same direction as the FR (forward shortcut), while other trials require the participant to travel backward relative to the FR direction (backward shortcut). Behavioral results show that YAs follow closer to the optimal route for forward shortcuts. But during backward shortcut trials, when given a choice between a) backtracking or shortcutting in a “conflicting” direction with the FR vs. b) traveling along the FR direction, YAs show a significant bias toward more closely following the FR than the optimal shortcut. The OAs however, show similar strategies and performance across forward and backward trials, taking fewer FRs compared to the YAs on backward shortcut trials. These early findings suggest that route familiarity and directional conflict with prior experiences may be important moderators of past evidence that aging leads to decreased flexibility/increased route-based navigation. YA fMRI data reveal 1) representational similarity between current and past routes show divergent relationships to navigator performance in different subdivisions of mPFC. 2) There is broad agreement between when and how the hippocampus and mPFC are engaged for task stages, represent environments, and track participant differences. Further analysis of OA fMRI data will help unpack the neural bases of

how aging may impact how individuals execute navigational strategies and which neural factors play a role in the different strategies implicated during prospective planning and *en route* behaviors.

Disclosures: P. Maxim: None. **T.I. Brown:** None.

Poster

PSTR426

Human Navigation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR426.28/W4

Topic: H.09. Spatial Navigation

Support: NSF Graduate Research Fellowship Program

Title: Investigating aging-related deficits in human spatial navigation through naturalistic navigation ability and reference frame utilization

Authors: *Y. BASSIL[1], A. KANUKOLANU[3], E. FUNDERBURG[5], E. CUI[1], A. ARUNKUMAR[4], A. PELTON[4], M. R. BORICH[2];
[1]Rehabil. Med., [2]Emory Univ., Atlanta, GA; [4]Col. of Sci., [3]Georgia Inst. of Technol., Atlanta, GA; [5]Emory University, Col. of Arts and Sci., Westerville, OH

Abstract: One of the earliest indicators of aging-related cognitive decline in older adults (OAs) is impaired *spatial navigation ability*. Specifically, OAs demonstrate deficits in utilizing *allocentric* (world-centered) information and rely on *egocentric* (body-centered) cues during navigation, resulting in *reference frame (RF) bias.* Though RF bias is observed in clinical populations as a behavioral symptom of dementia, it may also serve as an early predictor of the onset and development of neurodegenerative disorders throughout aging. Therefore, the overarching aim of this project is to investigate behavioral markers and neurological mechanisms of RF bias in healthy aging. Towards this aim, this study utilized a novel, naturalistic, city-like, virtual reality maze (NavCity), paired with a NavCity Allocentric Representation Assessment (NARA), to quantify naturalistic navigation ability, effects of navigation training after repeated NavCity exposure, and allocentric RF formation after navigation. Our central hypothesis is that, compared to younger adults (YAs), OAs will show lower navigation ability, less improvement in navigation performance after NavCity exposure, and lower allocentric RF formation.
To test our hypotheses, YAs (N = 26; 18-35 years) and OAs (N = 21; 60+ years) completed 3 blocks of locating 8 target buildings in NavCity and NARA. NavCity outcomes included orientation time, navigation time, distance traveled, speed, dwell time, and teleportations. Linear mixed models were run on each outcome to determine effects of age, block, target, and their interactions. NARA scores and associations to NavCity performance were analyzed using t-tests and Pearson's correlations.
Results revealed significant effects of age, block, and target on all outcomes (all $p < .05$), where

OA performance was lower than YAs, block 3 performance was higher than block 1, and varied patterns of target effects on performance were observed. Dwell time was the only outcome that showed significant effects across all variables and interactions ($p < .05$). The rate of improvement across blocks was similar between age groups ($p > .05$). OAs exhibited lower NARA scores than YAs ($p < .001$) and NARA scores were positively correlated with navigation outcomes across age groups ($p < .001$).
Findings reveal aging-related reductions in navigation performance that varied by target and outcome. However, change in performance with repeated exposure was not significantly affected by aging. Aging effects on allocentric RF formation were also associated with navigation performance. Immediate next steps will investigate neural circuit mechanisms of aging-related effects on navigation ability.

Disclosures: Y. Bassil: None. **A. Kanukolanu:** None. **E. Funderburg:** None. **E. Cui:** None. **A. Arunkumar:** None. **A. Pelton:** None. **M.R. Borich:** None.

Poster

PSTR426

Human Navigation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR426.29/W5

Topic: H.09. Spatial Navigation

Support: NSF Graduate Fellowship Program

Title: Effects of demographic factors on spatial navigation ability in aging

Authors: *E. FUNDERBURG[1], Y. BASSIL[2], E. CUI[3], A. PELTON[4], A. ARUNKUMAR[4], M. R. BORICH[5];
[1]Emory Univ., Col. of Arts and Sci., Atlanta, GA; [2]Emory Univ., Atlanta, GA; [3]Rehabil., Emory Univ., Atlanta, GA; [4]Georgia Inst. of Technol., Atlanta, GA; [5]Dept. of Rehabil. Med., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: As the global population ages, one crucial health issue to address is the prevalence of aging-related cognitive decline. One potential early indicator of aging-related cognitive decline is reduced spatial navigation ability, typically observed in the increased reliance on person-centered, egocentric reference frames (ERFs) and decreased utilization of world-centered, allocentric reference frames (ARFs) in older adults (OAs), relative to young adults (YAs). This RF bias in OAs is hypothesized to result from aging-related atrophy in the hippocampus (HPC), which functions in the utilization of ARFs. This reduced reliance on the HPC may prompt increased reliance on the posterior parietal cortex (PPC), which functions in the utilization of ERFs. Therefore, the overarching aim of this project is to characterize behavioral correlates of aging-related RF bias and relevant associations with HPC and PPC activation.
To study behavioral aging-related deficits in RF utilization, our lab has utilized a novel,

naturalistic, city-like, virtual reality (VR) maze ("NavCity"), as well as a traditional, computerized, seated Y-maze task, to quantify navigation ability. While prior literature has demonstrated effects of demographic factors (e.g., gender, handedness) and lifestyle (e.g., exercise, sleep quality) on navigation performance, the influence of these factors and their interactions with aging on NavCity and Y-maze performance in our study remains unclear. Our central hypotheses of this specific study include that lower navigational ability will be observed in OAs, compared to YAs, as well as in women, individuals with left/mixed handedness, lower prior VR experience, higher VR-related sickness, lower self-reported navigation ability, and lower sleep quality.
To test our hypotheses, YAs (N = 15; 18-35 years) and OAs (N = 11; 60+ years) completed a computerized Y-maze task and 3 repetitions of NavCity. Group differences in primary outcomes were assessed with independent parametric and non-parametric tests.
Only a significant effect of age was observed, as OAs exhibited decreased average speed in NavCity ($p < 0.001$) and increased ERF utilization in the Y-maze ($p = 0.047$). We hypothesize that the lack of significant associations with other analyzed demographic factors results from sample heterogeneity and VR familiarization trial usage.
Immediate next steps include looking at neural correlates of aging-related changes in RF utilization and navigation performance through use of task-based fMRI to analyze HPC and PPC activation during completion of the Y-maze. We also aim to assess PPC → HPC effective connectivity using concurrent TMS-fMRI.

Disclosures: E. Funderburg: None. **Y. Bassil:** None. **E. Cui:** None. **A. Pelton:** None. **A. Arunkumar:** None. **M.R. Borich:** None.

Poster

PSTR427

Human Relational and Spatial Learning

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR427.01/W6

Topic: H.10. Human Learning and Cognition

Support: Swiss National Science Foundation 100019_165481

Title: Why do individuals with Williams syndrome or Down syndrome fail the Weather Prediction Task?

Authors: E. BOCHUD-FRAGNIÈRE[1], P. LAVENEX[1], ***P. BANTA LAVENEX**[2];
[1]Inst. of Psychology, Univ. of Lausanne, Lausanne, Switzerland; [2]Fac. of Psychology, UniDistance Suisse, Brig, Switzerland

Abstract: Williams syndrome (WS) and Down syndrome (DS) are two neurodevelopmental disorders with distinct genetic origins characterized by mild to moderate intellectual disability. Individuals with WS or DS exhibit impaired hippocampus-dependent place learning and

enhanced striatum-dependent spatial response learning. Here, we used the Weather Prediction Task (WPT), which can be solved using hippocampus- or striatum-dependent learning strategies, to determine whether individuals with WS or DS exhibit similar profiles outside the spatial domain. Only 10% of individuals with WS or DS solved the WPT. We further assessed whether a concurrent memory task could promote reliance on procedural learning to solve the WPT in individuals with WS but found that the concurrent task did not improve performance. To understand how the probabilistic cue-outcome associations influences WPT performance, and whether individuals with WS or DS can ignore distractors, we assessed performance using a visual learning task with differing reward contingencies, and a modified WPT with unpredictive cues. Both probabilistic feedback and distractors negatively impacted the performance of individuals with WS or DS. These findings are consistent with deficits in hippocampus-dependent learning and executive functions, and reveal the importance of congruent feedback and the minimization of distractors to optimize learning in these two populations. @font-face {font-family:"Cambria Math"; panose-1:2 4 5 3 5 4 6 3 2 4; mso-font-charset:0; mso-generic-font-family:roman; mso-font-pitch:variable; mso-font-signature:-536870145 1107305727 0 0 415 0;}p.MsoNormal, li.MsoNormal, div.MsoNormal {mso-style-unhide:no; mso-style-qformat:yes; mso-style-parent:""; margin:0cm; mso-pagination:widow-orphan; font-size:12.0pt; font-family:"Times New Roman",serif; mso-fareast-font-family:"Times New Roman"; mso-ansi-language:FR-CH; mso-fareast-language:FR;}.MsoChpDefault {mso-style-type:export-only; mso-default-props:yes; mso-ascii-font-family:Aptos; mso-ascii-theme-font:minor-latin; mso-fareast-font-family:Aptos; mso-fareast-theme-font:minor-latin; mso-hansi-font-family:Aptos; mso-hansi-theme-font:minor-latin; mso-bidi-font-family:"Times New Roman"; mso-bidi-theme-font:minor-bidi; mso-fareast-language:EN-US;}div.WordSection1 {page:WordSection1;}

Disclosures: E. Bochud-Fragnière: None. **P. Lavenex:** None. **P. Banta Lavenex:** None.

Poster

PSTR427

Human Relational and Spatial Learning

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR427.02/W7

Topic: H.10. Human Learning and Cognition

Title: Drawing Memory Away: 4 Hour Fasted Blood Draw Diminishes Proceeding Relational Memory in Children.

Authors: *I. E. MANAVBASI[1], L. ROSOK[2], C. N. CANNAVALE[1], C. ROMÁN[1], M. PASCUAL-ABREU[1], C. KINDER[1], S. KEYE[3], N. A. KHAN[4], J. KIM[1];
[1]Univ. of Illinois, Urbana, IL; [2]Neurosci. Grad. Program, Univ. of Illinois Urbana-Champaign, Urbana, IL; [3]Kinesiology and Community Hlth., Univ. of Illinois Urbana-Champaign, Urbana, IL; [4]Hlth. and Kinesiology, Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: Hippocampal dependent relational memory is thought to be particularly susceptible to adverse states. Nonetheless, no study has reported on the impact of adverse states on relational memory in children. We aimed to explore how a 4-hour fasted blood draw procedure would impact proceeding spatial reconstruction task performance among school-aged children. We hypothesized that despite the provisions of snacks afterwards, the blood draw would adversely impact proceeding spatial reconstruction performance. Cross sectional analyses were conducted using secondary data among children aged 7-13 (n=75, M=10.92±0.27). An electroencephalography-compatible modified Eriksen Flanker task was used to assess attentional inhibition on Visit 1. The P3b event related potential was measured from the PZ electrode to assess attentional resource allocation (amplitude) and processing speed (latency). A 4-hour fasted blood draw was conducted on the second Visit. A spatial reconstruction task was completed to assess relational memory either on Visit 2 proceeding the 4-hour fasted blood draw (n=37) for the blood draw group, or on Visit 1 (n=38) for the non-blood draw group. Everyday Object Relational Memory (EORM) paradigm was administered only to the blood draw group on Visit 3 as a separate measure of relational memory. An independent sample t-test revealed significant differences in spatial reconstruction performance, object-location binding (OLB) between the blood draw (M=1.28±1.14) and the non-blood draw (M=2.66±0.57) groups [$t(73) = 6.66$, $p<0.001$]. There were no significant age differences [$t(73) = 1.54$, $p = 0.128$]. However, the performance of 23 subjects in the blood draw condition and 0 in the non-blood draw condition were indistinguishable from random arrays (at-chance). The OLB of the remaining 14 subjects (above-chance) in the blood draw group (M=2.46±1.05) was not significantly different from the non-blood draw group (M=2.66±=0.57) [$t(50) = 0.844$ $p = 0.403$]. Moreover, EORM accuracy between at-chance (M=40.27±10.70) and above-chance (M=34.63±12.69) subjects in the blood draw group were not significantly different [$t(21) = 1.13$, $p =0.271$]. No differences in Flanker outcomes between at-chance and above-chance performers were present either. Subjects who completed the 4-hour fasted blood draw preceding the spatial reconstruction task exhibited selectively reduced spatial reconstruction performance compared to the non-blood draw group. However, there were no differences in attentional inhibition and non-spatial relational memory between individuals whose spatial reconstruction performance were at-chance or above-chance following the blood draw.

Disclosures: I.E. Manavbasi: None. **L. Rosok:** None. **C.N. Cannavale:** None. **C. Román:** None. **M. Pascual-Abreu:** None. **C. Kinder:** None. **S. Keye:** None. **N.A. Khan:** None. **J. Kim:** None.

Poster

PSTR427

Human Relational and Spatial Learning

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR427.03/W8

Topic: H.10. Human Learning and Cognition

Support: Gallaudet University Seed Funding

Title: Eeg insights into embodied stem learning through virtual reality among deaf and hard-of-hearing asl users

Authors: *C. LEANNAH[1], L. C. QUANDT[2];
[1]Gallaudet Univ., Washington, DC; [2]Educational Neurosci., Gallaudet Univ., Washington, DC

Abstract: Research shows that hands-on STEM learning has embodied learning benefits, as demonstrated by increased sensorimotor cortex activation. Learning in virtual reality (VR) increases immersion and interactivity, which parallels recent research findings on embodied STEM learning. However, little is known about the impact of the experience of being deaf/hard-of-hearing (DHH) and using a signed language (i.e., American Sign Language, ASL) on embodied learning in VR. DHH ASL users who use ASL, a highly embodied visuospatial language, show stronger sensorimotor responses than hearing non-signers. Our study explores the far-reaching benefits of learning spatial STEM content in VR.
Our ongoing experiment uses a 2 (groups: VR, Video) x 2 (time point: Pre-Learning and Post-Learning) design to investigate behavioral accuracy responses and the sensorimotor neural impacts of learning chemistry among DHH ASL users. Participants engaged in either an interactive VR-based or passive video-based chemistry lesson. We assessed the effects of these environments on learning gains and sensorimotor neural processing using behavioral and EEG time-frequency analyses. While both groups showed similar accuracy in their chemistry reaction balancing behavioral knowledge responses, the VR group exhibited higher sensorimotor engagement as indicated by mu rhythm desynchronization. These results support the hypothesis that interactive VR can enhance embodied STEM learning processes compared to passive video watching.
Our behavioral and EEG time-frequency analyses compared the impact of different learning environments on sensorimotor neural processing, shedding light on embodied learning processes. Studying how DHH ASL users benefit from STEM learning in VR provides insights into comparisons between using technology for interactive and passive learning. Our findings underscore the effectiveness of VR as an educational tool that leverages the visuospatial strengths of DHH learners, supporting their embodied learning processes. This contributes to educational neuroscience by highlighting how immersive learning environments can promote inclusive and effective educational practices.

Disclosures: C. Leannah: None. **L.C. Quandt:** None.

Poster

PSTR427

Human Relational and Spatial Learning

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR427.04/W9

Topic: H.10. Human Learning and Cognition

Title: Narrative of the Cities: Help the Palace Form a Better Memory

Authors: *S. NASIRI;
Georgia Inst. of Technol., Atlanta, GA

Abstract: Abstract:
This research aims to investigate the effects of augmented reality (AR) technology combined with narrative associative techniques on the spatial memory of older adults aged between 55 to 70. Specifically, the study seeks to determine whether associating narratives and characters to environments enhances spatial memory in comparison to traditional navigation methods, and whether the familiarity or unfamiliarity of objects within these narratives influences memory retention differently. The research comprises four groups of older adults: one navigating without any technology or added narrative, the second utilizing AR goggles with directional arrows only, the third employing AR goggles with randomly placed objects but no narrative, and the fourth engaging with AR goggles featuring objects embedded within a structured narrative. Additionally, narratives will be categorized into familiar and unfamiliar contexts to discern potential differences in memory enhancement. We will also be tracking their brain interaction with fNIRS device to see any potential considerable brain activities. By exploring these variables, the study aims to shed light on the efficacy of storytelling and object association in enhancing spatial memory among older adults. Findings from this research will contribute to designing age-friendly cities by leveraging narratives and technology to improve spatial navigation for older populations, thereby enhancing their quality of life and independence. This study by reversing the traditional well-known method of “memory palace” in which we have items to recall, and we associate them with an imaginary environment to remember. Here we have the environment with which we associate items, characters, and stories in novel approach and techniques in urban design and technology integration for memory enhancement. This will result in heathier environment and life for aging adults and therefore a more hopeful future for all.

Disclosures: S. Nasiri: None.

Poster

PSTR427

Human Relational and Spatial Learning

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR427.05/W10

Topic: H.10. Human Learning and Cognition

Support: ’The Adaptive Mind’ funded by the Hessian Ministry for Higher Education, Research, Science and the Arts.

Title: Navigating virtual environments: learning, adapting, and minimizing risk

Authors: *B. KRETZMEYER[1], M. MCMANUS[1], C. A. ROTHKOPF[2], K. FIEHLER[1]; [1]Justus Liebig Univ. Giessen, Giessen, Germany; [2]Tech. Univ. of Darmstadt, Darmstadt, Germany

Abstract: Detecting and learning the statistical regularities of an environment is an important tool for efficient route planning and facilitates adaptive behavior to sudden changes. For instance, more efficient pathways can be chosen in advance and gaze can be guided predictively to the most relevant locations, thereby minimizing the need for large movement adjustments if obstacles appear. Using a virtual reality experiment, we investigated how humans learn environmental statistics and then adjust their gaze allocation and movement decisions accordingly. Participants navigated a virtual museum, tasked with finding the shortest path to one of two exit doors while avoiding a suddenly appearing virtual museum guest blocking either the left or right path. We manipulated the expected reward of a path by varying obstacle frequency: one path had a high frequency (90% probability of path blocking), while the other had a low frequency (10%) across all 20 trials of an experimental block. In control blocks, path blocking occurred equiprobably. If participants learn and capitalize on the statistical regularity in the world (obstacle appearance), they should predictively adjust their eye and body movements towards the less frequently blocked path in later trials of a block. We found that only around one-third of participants showed the anticipated predictive adjustments. Further analysis revealed that many of the participants who did not exhibit predictive adjustments refrained from choosing a path at the beginning of the trial. Instead, they walked in a straight line until obstacle appearance, delaying their decision until the last moment. While this approach resulted in greater adjustments upon obstacle appearance compared to scenarios where participants had committed to the correct pathway earlier, it minimized the risk of needing to make even larger movement adjustments if the initially chosen path was blocked. We hypothesized that this behavior reflects a deliberate strategy to minimize risk rather than a lack of learning capacity. To explore this, in a follow-up experiment, we eliminated the opportunity for participants to employ this strategy by separating the pathways right at the start of the room, forcing earlier decisions. Now, a majority of participants consistently selected the path presumed to be free, however, roughly one-third still chose the wrong path frequently in later trials. While our study demonstrates the human ability to learn and leverage statistical regularities in virtual navigation, our findings also suggest differences in learning capacity and strategies, with some participants minimizing the risk of major adjustments by delaying decisions.

Disclosures: B. Kretzmeyer: None. **M. McManus:** None. **C.A. Rothkopf:** None. **K. Fiehler:** None.

Poster

PSTR427

Human Relational and Spatial Learning

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

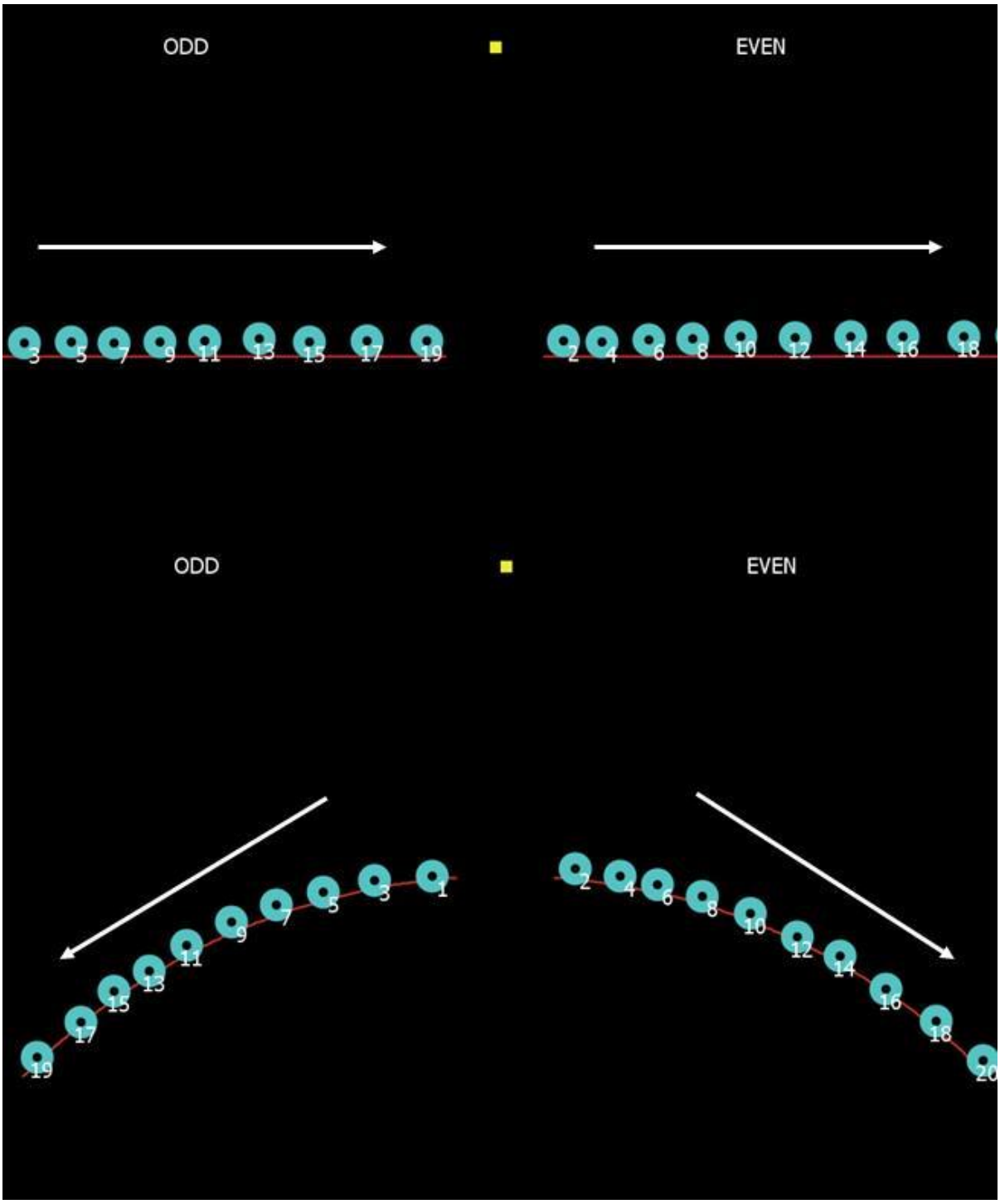
Program #/Poster #: PSTR427.06/W11

Topic: H.10. Human Learning and Cognition

Title: Mapping numerical sequences in linear and curvilinear space

Authors: *L. F. SCHETTINO[1], M. BYRNE[1], A. BUDAYR[2], H. BYRNES[1];
[1]Psychology, Lafayette Col., Easton, PA; [2]Lafayette Col., Easton, PA

Abstract: There is strong evidence that human participants organize numerical magnitudes in space with a bias that allocates smaller values to the left and larger values to the right in a one-dimensional spatial arrangement commonly called a Mental Number Line (MNL). The organization of values in 2D or 3D arrangements is less well understood, though the available evidence suggests that smaller numbers are associated with the left-, down- and near-dimensions. During a typical experiment, participants are asked to categorize numerical symbols by parity while they are presented one at a time. It is not clear, however, how participants would arrange the symbols as a spatial sequence while the values are simultaneously present. In this study, we asked participants (N=20) to organize numerical tokens as sequences on a computer screen. Participants dragged even or odd numbered tokens to a path on each side of the screen using a computer mouse and placing them in a manner that seemed most 'natural' to them. Half of the participants placed the numerical tokens on linear paths while the other half placed them on curvilinear paths. Our results showed that when the shape of the path on which the participants placed the tokens was linear, the preferred organization was sequential from left to right (100% of participants), as predicted by the MNL. However, when the path was curvilinear, the preferred organization was a mirror sequence with the lowest values at the periphery and the larger values at the center or vice versa (70% of participants) ($\chi^2(1)=10.77$, $p<0.001$, $V=0.73$). These data point to a possible interaction between a culturally-learned sequence positioning which follows the typical MNL arrangement and a tendency to organize sequences as mirrored patterns. While the available evidence suggests that when placed on a 2D pattern lower values are usually placed at lower or near positions, our results suggest that the arrangement of sequences in curvilinear space may be guided by a mirror symmetry.



Disclosures: L.F. Schettino: None. **M. Byrne:** None. **A. Budayr:** None. **H. Byrnes:** None.

Poster

PSTR427

Human Relational and Spatial Learning

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR427.07/W12

Topic: H.10. Human Learning and Cognition

Support: PAPIIT-UNAM: IN221324
CONACYT: 263377

Title: Effects of music on spatial ability and the underlying neural dynamics

Authors: *B. GARCÍA-GUDIÑO[1], L. OCHOA GONZÁLEZ[2], I. ABREGO-ISLAS[2], C. J. MONTES RODRIGUEZ[3], Z. MUNOZ-TORRES[4];
[1]Facultad de Psicología; Ctr. de Ciencias de la Complejidad, Univ. Nacional Autonoma de Mexico, Ciudad de México, Mexico; [2]Univ. Nacional Autonoma de Mexico, Mexico City, Mexico; [3]Ctr. de Ciencias de la Complejidad, UNAM, CDMX, Mexico; [4]Ctr. for the Sci. of Complexity, Fac. of Psychology, Univ. Nacional Autonoma de Mexico, Mexico City, Mexico

Abstract: Previous research has shown that particular musical stimuli have an impact on spatial abilities. Although most studies have presented the musical stimulus before performing the task, the brain activity underlying musical exposure during task performance is unknown. In the present study, we investigated changes in neural dynamics during the performance of a spatial reasoning task in healthy subjects while listening to and after listening to Mozart's (K. 448) or Wagner's music (Lohengrin). To achieve this objective, electroencephalography (EEG) was used to record the brain electrical activity of 20 healthy participants (13 women, 7 men) between 21 to 30 years old, right-handed, with normal vision and hearing. Exclusion criteria included mood disorders, addictions, or medications that altered nervous system function. Fast Fourier Transform was used to extract power spectral density (PSD) hz by hz. Participants performed a computer version of the paper-folding and cutting subtest of the Stanford-Binet intelligence scale during and after listening to the sonata K. 448 or Wagner´s music. Preliminary results showed that participants' reaction times were significantly longer for Wagner's than Mozart's music, regardless of the presentation (before or during the task). EEG results showed a higher PSD when the music was presented during the task than before. Higher PSD was consistently observed in Wagner than Mozart conditions. While listening to Wagner before the task required greater slow-frequency power in right fronto-occipital regions, listening to Wagner versus Mozart during the task primarily recruited the left posterior hemisphere and frontal regions at fast frequencies. The brain networks involved and affected by music are related to attention and spatial processing, suggesting a compensatory effect reflected in longer reaction times and higher PSD observed in Wagner conditions. The results of this study provide information about the neurophysiological correlates underlying the music effect on spatial abilities.

Disclosures: B. García-Gudiño: None. **L. Ochoa González:** None. **I. Abrego-Islas:** None. **C.J. Montes Rodriguez:** None. **Z. Munoz-Torres:** None.

Poster

PSTR427

Human Relational and Spatial Learning

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR427.08/W13

Topic: H.10. Human Learning and Cognition

Title: Visual statistical learning is associated with changes in low-dimensional cortical and subcortical architecture

Authors: *K. ROWCHAN[1], D. GALE[2], Q. NICK[1,3], J. P. GALLIVAN[2,4,5], J. D. WAMMES[1,3]; [1]Psychology, Queen's Univ., Kingston, ON, Canada; [2]Psychology, Queens Univ., Kingston, ON, Canada; [3]Centre for Neuroscience Studies, Queen's University, Kingston, ON, Canada; [4]Psychology, Queen's University, Kingston, ON, Canada; [5]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada

Abstract: Our ability to automatically learn environmental regularities over time and use this information to make predictions about the world is central to human cognition and behavior. While prior work investigating the neural basis of this statistical learning (SL) process initially emphasized the role of the cortex, later work has suggested that the medial temporal lobe and hippocampus play a critical roles in guiding SL. While these results are compelling, recent work shows that despite bilateral hippocampal damage, patients still show evidence of SL, and in some cases, outperform controls, calling into question whether the hippocampus is necessary for SL, or simply learns in parallel via different mechanisms than the cortex. Therefore, the relative contribution of cortical and subcortical regions during SL, and the ways in which they coordinate their activity over time, remain unclear. Here, using functional MRI, we measured human brain activity (N = 33) during a classic visual SL task, whereby individuals implicitly learned to associate pairs of images embedded within a larger sequence. By projecting patterns of cortical and subcortical functional connectivity onto a low-dimensional manifold space, we found that SL was associated with changes along a single neural dimension, describing connectivity across the visual-parietal and perirhinal cortex. We found that during learning, regions within the higher-order visual cortex significantly expanded along this dimension, reflecting both their increased functional segregation from other networks, and increased within-network integration. Simultaneously, we found that regions within the parietal cortex, belonging to the dorsal attention network (DAN), contracted along this dimension, reflecting their increased integration with higher-order transmodal cortex. Notably, once SL was interrupted, we found that subcortical regions that despite not initially showing learning effects, the perirhinal and entorhinal cortex now contracted along this dimension, reflecting their increased integration with the default mode and DAN, and decreased covariance with the visual cortex. While prior work on the neural foundations of SL differentially suggest that either cortical or subcortical networks underlie SL, our findings propose an integrative view, suggesting that it is the interactions within and between these networks that drives differential roles in learning and eventual updating when faced with prediction error. Together, our results not only have implications for our current understanding of SL, but also for studying whole-brain interactions that contribute to learning and behaviour, in general.

Disclosures: K. Rowchan: None. **D. Gale:** A. Employment/Salary (full or part-time):; Voxel AI. **Q. Nick:** None. **J.P. Gallivan:** A. Employment/Salary (full or part-time):; Voxel AI. **J.D. Wammes:** None.

Poster

PSTR427

Human Relational and Spatial Learning

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR427.09/

Topic: H.10. Human Learning and Cognition

Support: NIH Grant K08MH121775
NIH Grant R01MH130068

Title: Multiple levels of abstract learning occur simultaneously and are supported by distinct neural systems

Authors: *D. L. KIMMEL[1], K. L. STACHENFELD[2,3], N. KRIEGESKORTE[1], S. FUSI[1], C. D. SALZMAN[1], D. SHOHAMY[1];
[1]Columbia Univ., New York, NY; [2]Google DeepMind, New York, NY; [3]Columbia University, New York, NY

Abstract: A long-standing question in cognitive neuroscience is how we abstract a general rule or concept from a set of experiences and generalize it to novel situations to support inference. Abstraction and generalization arise from learning processes operating at multiple levels simultaneously: how behavioral states are related in the current situation ("within-context learning"), how these relationships change over multiple situations ("across-context learning"), and how to apply this structural knowledge to novel situations ("structure learning"). Yet typically these processes are studied separately in discrete phases. Here we asked how the processes of abstract learning occur simultaneously, separably contribute to decision-making, and are supported by distinct neural systems. We developed a novel task in which human participants learned the correct response and outcome contingencies for a set of stimuli (i.e., within-context learning). Unbeknownst to participants, the contingencies depended on two latent contexts that alternated in blocks of trials. Participants learned and exploited the context-dependent structure: a change in one stimulus was sufficient to update choices for the remaining stimuli (i.e., across-context learning). Across sessions, they transferred meta-knowledge of the task structure to new instances of the task with novel stimuli (i.e., structure learning). To disentangle the contribution of each learning process, we extended the successor representation, a predictive model of temporal abstraction previously applied to planning and navigation. For each unique combination of contingencies (i.e., "state"), the model learned the long-run occupancy of future states given the prior state, and thereby could infer the current state and its correct response. The state relationships were learned via reinforcement learning in which the

prediction error (PE) for state occupancy was factorized into terms for within-context, across-context, and structure learning. The model reproduced the observed learning dynamics at multiple timescales. Moreover, the composition of the PE evolved with experience: within-context learning was overtaken by structure learning. As predicted, distinct neural systems supported the different learning processes. Structure learning correlated with BOLD activity in hippocampus and orbitofrontal cortex, regions implicated in learning state relationships and representing behaviorally relevant states, respectively. In summary, we show how the different levels of abstract learning co-occur, evolve over time, and are supported by distinct neural systems.

Disclosures: D.L. Kimmel: None. **K.L. Stachenfeld:** None. **N. Kriegeskorte:** None. **S. Fusi:** None. **C.D. Salzman:** None. **D. Shohamy:** None.

Poster

PSTR427

Human Relational and Spatial Learning

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR427.10/W15

Topic: H.10. Human Learning and Cognition

Support: Max Planck Society
Humboldt Research Fellowship for postdoctoral researchers

Title: Structural organization of multiple sources of information for efficient encoding in working memory

Authors: *Q. HUANG[1], C. F. DOELLER[1,2];
[1]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany; [2]Kavli Inst. for Systems Neuroscience, NTNU, Trondheim, Norway

Abstract: Working memory (WM) is a core cognitive function to flexibly inform and guide future behavior, with its capacity constraining various cognitive abilities. Previous empirical and modelling studies have implied that the human brain may compress or organize multiple sources of information based on their relational regularities, or underlying structure. However, empirical evidence is scarce for how the brain represents structural information in WM and for how it spontaneously leverages this information to organize the storage of multiple items in the service of efficient encoding. Recent developments in cognitive neuroscience suggest that cognitive maps may provide a general framework for organizing information in different tasks and across various domains. Here, we developed a novel experimental WM paradigm in combination with MEG recordings to examine the neural mechanisms supporting efficient information storage by leveraging the underlying task structure. Participants were asked to memorize a sequence of gratings varying on two continuous dimensions: orientation and frequency. Each stimulus could therefore be described within a two-dimensional feature space. Crucially, we manipulated the

consistency of directional information defined in the two-dimensional feature space between items in the sequence. We observed that information was more precisely stored in a consistent direction compared to an inconsistent direction condition. Furthermore, directional information was reactivated during memory retention in the consistent direction condition, while this was not the case in inconsistent direction condition. Instead, item-by-item sequential reactivation is observed in the inconsistent direction condition. These preliminary findings reveal that abstract information of the underlying task structure is encoded in the brain and is leading to a behavioral advantage, possibly by facilitating the compression of multiple sources of information in working memory.

Disclosures: Q. Huang: None. **C.F. Doeller:** None.

Poster

PSTR427

Human Relational and Spatial Learning

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR427.11/W16

Topic: H.10. Human Learning and Cognition

Title: The relation between cognitive maps and cognitive abilities

Authors: *R. TENDERRA, S. THEVES;
Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: Psychometric research has revealed a hierarchical factor structure of cognitive abilities underlying correlations in performance across diverse tasks. A longstanding debate concerns the correspondence of these latent factors to biological variables. Previous studies localized correlates in brain structure, connectivity, and activation levels, whereas the mechanisms by which neural information processing may contribute remained unexplored. In this study, we set out to examine the relationship between cognitive performance across diverse tasks and representational properties of the hippocampal-entorhinal system (‘cognitive maps’), which in recent years have been suggested to support relational processing and reasoning in a domain-general manner. To this end, we collected standardized cognitive tests as well as behavioral and fMRI data of140 participants during mnemonic cognitive tasks and analyzed neural task representations with respect to performance differences. Preliminary results suggest a specific link between relational representations in the hippocampal system and interindividual differences in general reasoning ability. These findings offer initial empirical support for a link between neural representational mechanisms related to relational reasoning and measures of general cognitive performance.

Disclosures: R. Tenderra: None. **S. Theves:** None.

Poster

PSTR427

Human Relational and Spatial Learning

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Program #/Poster #: PSTR427.12/W17

Topic: H.10. Human Learning and Cognition

Support: Max Planck Society
International Max Planck Research School on Neuroscience of Communication: Function, Structure, and Plasticity (IMPRS NeuroCom)

Title: Spatial Cognition Box: an open-source toolkit for generating egocentric and allocentric neural representations to integrate with models and experiments

Authors: *A. ZABOLOTNII[1], A. BICANSKI[2], C. F. DOELLER[3,4];
[1]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany; [2]Psychology, Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany; [3]Dept. of Psychology, Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany; [4]Kavli Institute for Systems Neuroscience, Center for Neural Computation, The Egil and Pauline Braathen and Fred Kavli Center for Cortical Microcircuits, Jebsen Center for Alzheimer's Disease, Norwegian University of Science and Technology, Trondheim, Norway

Abstract: The development of large-scale, dynamic models that accurately reflect the complexity of spatial cognition remains a significant challenge. We present an open-source, extendable Python toolkit to model spatial cognition in the framework established by the so-called BB model (Bicanski & Burgess 2018) and its predecessors - called the Spatial Cognition Box (SCB). The SCB is designed to generate egocentric and allocentric spatial cell responses and their systems-level interaction, in interactive environments of arbitrary shape and object composition. Recall of spatial snapshots and replay of trajectories, as well as mental navigation can be triggered at will. The SCB Toolkit thus offers a flexible and user-friendly way to generate neural responses to parallel behavioral experiments or for integration with other computational models. Finally, the SCB can easily be integrated with other toolkits such as RatInABox (T. George et. al., 2024). The toolkit features tutorials and comprehensive documentation, and we endeavour to make it an adaptable tool for both experimentalists and modelers to advance the understanding of spatial cognition.

Disclosures: A. Zabolotnii: None. **A. Bicanski:** None. **C.F. Doeller:** None.

Poster

PSTR427

Human Relational and Spatial Learning

Location: MCP Hall A

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Program #/Poster #: PSTR427.13/W18

Topic: H.10. Human Learning and Cognition

Support: ERC Starting Grant NOAM
Max Planck Society

Title: Foraging in conceptual spaces: neurophysiological mechanisms of mental search in semantic memory

Authors: *S. VIGANÒ[1,2], G. GIARI[3], R. MAI[4], C. F. DOELLER[1,5], R. BOTTINI[3];
[1]Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany; [2]Center for Mind/Brain Sciences (CIMeC), University of Trento, Rovereto, Italy; [3]Ctr. for Mind/Brain Sci. (CIMeC), Univ. of Trento, Rovereto, Italy; [4]"Claudio Munari" Ctr. for Epilepsy and Parkinson Surgery,, Niguarda Ca' Granda Hosp., Milan, Italy; [5]Kavli Institute for Systems Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway

Abstract: Neuronal recordings and cognitive neuroimaging have highlighted the hippocampal formation in the medial temporal lobe (MTL) as a key region for representing relational knowledge of both spatial information and abstract concepts in memory. However, how do we search and access stored knowledge? During navigation, when searching for goal locations, the hippocampal formation displays rhythmic oscillatory activity, particularly in the theta band (3-9 Hz). The theta rhythm plays a crucial role in spatial memory more generally: for instance, it conveys information about traveled distance and velocity, and has been suggested to be involved in the formation of grid fields. Here we asked if this physiological signature of hippocampal engagement during physical exploration also extends to mental exploration of abstract spaces, such as when recalling concepts from memory.
To answer this question we used stereo-EEG to record local field potentials from the MTL of epileptic patients while they performed simple categorical verbal fluency tasks, randomly "foraging" for concepts from different categories (animals, professions, or famous cities). Preliminary results from 11 patients indicate that, in the period preceding the utterance of a word (-1000 ms to word onset), theta power in MTL was significantly higher than during or after word pronunciation. This effect was independent of the semantic category that was mentally explored, and was more pronounced in the hippocampus and entorhinal cortex than the parahippocampal or lateral temporal cortices. To investigate the possibility that theta frequency conveys information about the explored semantic space, we used linear mixed models to test whether theta power was modulated by semantic distances between words (modeled as FastText linguistic vectors). We observed significant modulation from -1000 to -600 ms relative to word onset when considering high-dimensional semantic distances. In contrast, low-dimensional semantic distances more strongly modulated theta power in the time interval from -300 to -100 ms. Interestingly, time-frequency analysis also revealed an unexpected significant increase in the beta band (21-32 Hz) around -600 to -500 ms to word onset, which role has yet to be understood. Although preliminary (data collection and analyses are currently ongoing), these results suggest that physiological signatures of hippocampal activity during physical exploration might also extend to mental exploration of abstract spaces, and potentially reveal novel mechanisms underlying the access of conceptual information from memory.

Disclosures: S. Viganò: None. **G. Giari:** None. **R. Mai:** None. **C.F. Doeller:** None. **R. Bottini:** None.

Poster

PSTR427

Human Relational and Spatial Learning

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR427.14/W19

Topic: H.10. Human Learning and Cognition

Support: Max Planck Society

Title: Exploring Task Structure and Training Regimes in Continual Learning

Authors: *N. MENGHI[1], S. VIGANÒ[1], C. F. DOELLER[1,2];
[1]Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany; [2]Kavli Institute for System Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway

Abstract: Humans possess an exceptional ability to learn and adapt across various tasks, showing resilience against interference and forgetting. We can effortlessly navigate big US cities while retaining spatial memory of small Italian towns, and acquire new languages without losing our native tongue. Understanding this "continual learning" is vital for both cognitive science and research on artificial intelligence, especially concerning the problem of "catastrophic forgetting" in neural networks. Yet, the exact neurocognitive mechanisms underlying this ability remain unresolved. Previous research has shown that the specific curriculum humans follow while acquiring new information plays an important role in the learning process. Blocked training regimes have been shown to be more beneficial than interleaved ones, preventing interference between different and orthogonal tasks, potentially because these regimes facilitate factorization into non-interfering subcomponents. But what happens when tasks share similar structures? In such instances, the difficulties encountered with interleaved training regimes should disappear or potentially reverse, as the system can now capitalize on the similarities between subcomponents to transfer knowledge between tasks instead of putting effort into factorization to protect them from interference. In the current study, we independently manipulated task structures and training regimes to characterize learning and generalization in a multitask learning experiment. To this end, we created two experimental conditions (between participants) in which a spatial and a conceptual task, under different training regimes, either shared the same underlying structure or not. Participants learned to separately associate spatial or conceptual features with specific outcomes. Subsequently, we evaluated participants' performance using stimuli encountered during training as well as novel stimuli. Using a combination of behavioural and magnetoencephalography (MEG) measures, we characterized the existence of a lower dimensional map-like representation of knowledge, where information can be generalized and transferred within and between tasks. Behaviourally, we found that learning performance

changed based on the training regime and interacted with the structure similarity between tasks. Neurally, we used representation similarity analysis (RSA) and identified the temporal dynamics of task representations and compression. Our findings shed light on the role of task structures and training regimes in how knowledge is represented and shared between different tasks.

Disclosures: N. Menghi: None. **S. Viganò:** None. **C.F. Doeller:** None.

Poster

PSTR427

Human Relational and Spatial Learning

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR427.15/W20

Topic: H.10. Human Learning and Cognition

Title: Prior knowledge and memory encoding: investigating the influence of congruency and incongruency on learning

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Abstract: Encoding new memories takes place against the backdrop of a rich library of informationacquired through one's life. Several studies show that prior knowledge, such as schemas,strengthens encoding and accelerates recall of new memories that are in agreement with it(congruent), while others show the opposite pattern where prediction violation (related toinformation being incongruent with a schema) facilitates learning. To reconcile thecontradictory findings in these two lines of research, a recent framework, the schema-linkedinteraction between the medial temporal and medial prefrontal regions (SLIMM model),postulates that memory shows a non-linear, U-shaped function with degrees of congruencyto prior information. In other words, highly congruent and highly incongruent informationwith a schema benefit the process of consolidation during learning. However, the SLIMMmodel remains under scrutiny since empirical evidence is scarce and not sufficient tosupport its hypotheses yet. Furthermore, the neural underpinnings of such learningprocesses remain unknown. While some models suggest a trade-off between the medialprefrontal cortex (mPFC) and the medial temporal lobe (MTL) for congruent andincongruent effects respectively (e.g. SLIMM), other models predict an essential role of MTLstructures in encoding information congruent to existing knowledge structures. In thisstudy, we use behavioural methods as well as neuroimaging (fMRI) to understand whetherand how the representation of prior knowledge

enhance encoding and retrieval of newevents. We developed a novel spatial schema paradigm, which compares three conditionswith varying degrees of congruency to previous knowledge and test the seeminglycontradictory behavioural findings in the literature. Our results demonstrate a mnemonicadvantage for congruent events, while incongruent events and those lacking a strong priorschema exhibit a disadvantage, suggesting that reaffirming expectations facilitates learning.In the concurrent fMRI study, we directly compare learning systems in the brain thatsupport learning under certain (congruent) and uncertain (incongruent) conditions andinvestigate the formation and update of schema representations with newly acquiredinformation. This study could lead to a better understanding towards a refinedneuroscientific model of how brain networks interact to successfully integrate newinformation with previous knowledge schema.**Key terms:** *learning, prior knowledge, memory, hippocampus, fMRI, schema, knowledgestructures, generalisation, inference, prediction error, machine learning*

Disclosures: S. Elnagar: None. **C.F. Doeller:** None. **N. Menghi:** None. **A. Greve:** None.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.01/W21

Topic: I.02. Systems Biology and Bioinformatics

Support: U01MH130881
UG3MH120094
1R01NS100908-01
P51-OD011132

Title: Molecular Diversity of Globus Pallidus in Non-Human Primates

Authors: *J. HE[1], O. R. BRULL[1], R. BHIK-GHANIE[1], A. C. BOSTAN[1], A. R. PFENNING[2], A. GALVAN[3], W. R. STAUFFER[1];
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Abstract: Understanding the cellular architecture and projection patterns of basal ganglia nuclei is crucial for elucidating reward-based learning and the pathophysiology of motor, cognitive, and affective disorders. The external (GPe) and internal (GPi) segments of the globus pallidus (GP) act as major relay and processing centers in regulating motor function and cognitive processes within the basal ganglia circuitry of primates. Here, we aimed to elucidate the molecular diversity and projection patterns of GP neurons in non-human primates. We used single nucleus RNA sequencing to identify distinct neuron types within the GPe and GPi of three rhesus macaques. We performed a comparative analysis of our data with an existing rodent dataset and

found several conserved cell types, including PVALB, FOXP2, and CHAT-positive clusters. Utilizing fluorescent in situ hybridization and the advanced Xenium spatiomics platform, we mapped rostro-caudal distribution of neuron types in the GP and quantified their relative abundance in two rhesus macaques. In two different animals, we injected into the subthalamic nucleus (STN) an AAV-retro encoding GFP to trace and identify the cellular identities of STN-projection neurons in the GPe. These results will enhance our understanding of pallidal circuits in the primate basal ganglia and will help us understand similarities and differences across species. Moreover, this data may offer novel insights into the neuroanatomical substrates of motor control, cognitive functions, and potentially guide targeted therapeutic strategies.

Disclosures: J. He: None. **O.R. Brull:** None. **R. Bhik-Ghanie:** None. **A.C. Bostan:** None. **A.R. Pfenning:** None. **A. Galvan:** None. **W.R. Stauffer:** None.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.02/W22

Topic: I.02. Systems Biology and Bioinformatics

Support: NSF 1948181

Title: The OpenBehavior Project: A database and dissemination platform for open-source tools used for behavioral neuroscience research

Authors: *K. CHAVEZ LOPEZ[1], J. PALMER[2], S. R. WHITE[3], L. AMARANTE[4], J. A. FRIE[5], S. BRADLEY[6], J. Y. KHOKHAR[7], A. V. KRAVITZ[8], M. LAUBACH[2];
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Abstract: The OpenBehavior Project champions the use of open-source tools in behavioral neuroscience research. Since 2016, we have facilitated the dissemination and adoption of these tools through various initiatives. We maintain a comprehensive, searchable database of open-source tools with unique identifiers (RRIDs) for easy citation. Additionally, we host a repository containing raw animal behavior videos and validated open-source designs for 3D-printed objects used in neuroscience research. Our efforts extend beyond resource sharing to include educational workshops on video analysis methods and Arduino-based microcontrollers. Importantly, we recently launched the "Setups and Protocols" initiative, which aims to extract and compile detailed descriptions of novel research tools and analysis pipelines from existing publications. This initiative relies on community collaboration to unlock the potential of these often-overlooked resources within the wider research community. We invite researchers to join us in

promoting open-source tools and methodologies that can advance the field of behavioral neuroscience.

Disclosures: K. Chavez Lopez: None. **J. Palmer:** None. **S.R. White:** None. **L. Amarante:** None. **J.A. Frie:** None. **S. Bradley:** None. **J.Y. Khokhar:** None. **A.V. Kravitz:** None. **M. Laubach:** None.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.03/W23

Topic: I.02. Systems Biology and Bioinformatics

Support: NLM Grant T15LM007442

Title: Identification of cell type trajectories during embryonic neurodevelopment linked to pediatric brain tumor progression using single cell transcriptomics and natural language progression

Authors: *A. RAJENDRAN[1], S. S. PATTWELL[2];
[1]Biomed. Informatics and Med. Educ., Univ. of Washington Sch. of Med., Seattle, WA; [2]Ben Towne Ctr. for Childhood Cancer Res., Seattle Children's Res. Inst., Seattle, WA

Abstract: Human brain development involves a highly intricate choreography of molecular processes guiding the expansion, differentiation, and orientation of hundreds of diverse cell types. Aberrations during any point of this process can lead to detrimental downstream effects, including pediatric brain tumors. These embryonic origins between initiation and progression of these tumors are not fully understood yet for many pediatric brain cancer types. In addition, the genetic mechanisms involved in fetal and embryonic brain development still require additional modeling especially with the advent of big data and single cell technologies. We hypothesize that expanding on existing neurodevelopmental and pediatric brain cancer atlases using single cell RNA sequencing (scRNAseq) integrative approaches will allow us to 1- create a comprehensive cellular meta-analysis of human neurodevelopment and 2- identify the neurodevelopmental genetic programs involved in pediatric brain cancer progression.Here, we use conventional and newly adapted approaches for scRNAseq data analysis---proposing new methods for cell identification and gene module creation rooted in probabilistic topic models and information theory. We use scRNAseq in mouse and in human to create a large neurodevelopmental atlas. The datasets are individually analyzed using natural language processing techniques and conventional scRNAseq data analysis approaches to mark transcriptomic programs and cell lineages across developmental time. These transcriptomic programs and cellular lineages are integrated with pediatric brain tumor datasets to identify brain tumor initiating cell types and genesets. These analyses are anticipated to contribute to our understanding of cellular and

genetic dynamics involved in in utero brain development and uncover novel insight into the origins of pediatric cancer.

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

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.04/W24

Topic: I.02. Systems Biology and Bioinformatics

Title: Exploring the gene regulatory network of the mouse DRG for analgesic strategies in chronic pain

Authors: *D. KRAUTER[1], P. ERNFORS[2];
[1]MBB, Karolinska Institutet, Solna, Sweden; [2]Karolinska Institutet, Stockholm, Sweden

Abstract: Sensory neurons allow us to sense our environment. We can distinguish between pleasant stimuli such as the warmth of the sun on our skin or a hug from a friend and painful stimuli like when a hand is placed on a hot stove or stabbed by a sharp object. Under normal circumstances, pain is beneficial as a protective mechanism from dangers in the environment. However, under pathological conditions, malfunctioning sensory neurons are the cause of chronic pain which leads to significant distress, reduced quality of life and societal consequences. Pain itself is a result of the activation of molecularly unique sensory neuron types that form assemblies of different active neuronal types, located in the dorsal root ganglion (DRG). Nowadays we only have very limited understanding of which subtypes of sensory neurons are responsible for the various chronic pain conditions hampering development of specific and effective treatments for pain relief. Here, we used single nuclei multiome sequencing to profile the transcriptome (RNA) and accessible chromatin (ATAC) of mouse DRG neurons, identifying 17 neuronal clusters and cell-type specific differentially accessible chromatin regions. Further we explored the utilization of enhancers elements using non-viral gene delivery via lipid nanoparticles in order to develop tools to specifically target the pain causing neurons in chronic pain conditions. This application is timely, since technologies for identifying accessible chromatin in single cells and deep learning methods to design optimal cell type-specific enhancers are just emerging, opening for discoveries previously unreachable. These new deep insights into the cellular and molecular basis of chronic pain will be enabling for highly needed new therapeutic options to treat pain.

Disclosures: D. Krauter: None.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.05/W25

Topic: I.02. Systems Biology and Bioinformatics

Title: Multi-omic analysis of brain tissue reveals comprehensive insights through the integration of spatial gene expression with highly scaled single-cell RNA and epigenetic data

Authors: M. RAY[1], R. CHEN[1], H. WANG[1], S. KHARE[2], D. SKINNER[2], B. BIDDY[2], F. SCHLESINGER[2], M. NAKAMOTO[2], *G. EMANUEL[1], J. HE[1];
[1]Vizgen, Cambridge, MA; [2]Scale Biosci., San Diego, CA

Abstract: Single-cell technologies have transformed our understanding of cellular mechanisms, particularly through techniques like single-cell RNA sequencing (scRNA-seq) and single-cell methylation (scMET) profiling. However, while scRNA-seq and scMET offer deep insights into gene expression and regulation, they lack spatial information crucial for understanding cellular organization and interactions. Conversely, spatial technologies provide spatial context but have limitations in gene or methylation site coverage. By integrating spatial and single-cell genomic data, we can bridge this gap and gain comprehensive, multi-modal insights into complex tissues. In this study, the integration of single-cell genomic data was performed using the ScaleBio Single Cell RNA Sequencing Kit, the ScaleBio Single Cell Methylation Kit, and the Vizgen MERSCOPE® Spatial Platform. Serial brain slices from three distinct regions of a mouse brain were collected. 10 µm sections were spatially profiled using Vizgen's MERSCOPE Platform and a 500-gene pan-neuron panel, while 300 µm slices underwent single-cell transcriptome-wide RNA and methylation profiling. The outputs from both platforms were then integrated and analyzed.

Our analysis revealed high-quality data from both the ScaleBio scRNA-seq and MERSCOPE spatial transcriptomics platforms. Integration of the spatial and single-cell datasets using overlapping genes found both platforms allowed for accurate assessment. Notably, mapping of the clusters identified in the scRNA dataset based on this integration revealed distinct spatial locations of these clusters. Moreover, we observed high similarity between gene expression observed in the MERSCOPE data and the imputed location of the same genes in the scRNA data. Additionally, the imputed spatial location of genes exclusively found in the ScaleBio scRNA dataset showed a strong correlation with the Allen Brain Atlas, enabling spatial profiling of genes absent in the spatial panel. Finally, the single-cell methylation data was examined using differentially methylated regions to identify various cell types in the data. The genome-wide scMet profiles were mapped onto spatial transcriptome data, revealing the underlying epigenomic states of these populations.

Overall, our study demonstrates the value of integrating spatial gene expression, scRNA-seq and scMETdata for comprehensive spatial genomic analysis. Together, these modalities offer a holistic view of the spatial organization of gene expression and regulation within intricate neuronal tissues.

Disclosures: M. Ray: A. Employment/Salary (full or part-time):; Vizgen. **R. Chen:** A. Employment/Salary (full or part-time):; Vizgen. **H. wang:** A. Employment/Salary (full or part-time):; Vizgen. **S. Khare:** A. Employment/Salary (full or part-time):; Scale Biosciences. **D. Skinner:** A. Employment/Salary (full or part-time):; Scale Biosciences. **B. Biddy:** A. Employment/Salary (full or part-time):; Scale Biosciences. **F. Schlesinger:** A. Employment/Salary (full or part-time):; Scale Biosciences. **M. Nakamoto:** A. Employment/Salary (full or part-time):; Scale Biosciences. **G. Emanuel:** A. Employment/Salary (full or part-time):; Vizgen. **J. He:** A. Employment/Salary (full or part-time):; Vizgen.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.06/W26

Topic: I.02. Systems Biology and Bioinformatics

Support: Mitacs
Kapoose Creek Bio

Title: Behavioural profiling of fungal secondary metabolites for neuroactive compound discovery

Authors: *E. D. BROWN;
McMaster Univ., Hamilton, ON, Canada

Abstract: Behavioural profiling of fungal secondary metabolites for neuroactive compound discovery
Shawn French[1], Telmah Lluka[1], Julia Nacaratto[1], Michael Ranieri[1], André E.X. Brown[2], and Eric D. Brown[1,3]
[1]McMaster University, Hamilton, Canada;[2]Imperial College London, London, United Kingdom;[3]Kapoose Creek Bio, Vancouver, Canada
Medicines derived from natural products have unmatched structural and functional diversity, refined over millions of years of evolutionary trial-and-error. While fungi have seen therapeutic use across human history in traditional medicines, in modern drug discovery these microbes are relatively underexplored. We have assembled a fractionated fungal extract library, with fungi foraged largely from a remote and temperate rainforest (British Columbia, Canada). Fungal metabolites were extracted and purified using flash chromatography, yielding thousands of testable fractions. Using this unique library, we are able to identify neuroactive fungal metabolites in high-throughput, using a behavioural assay with the model organism *Caenorhabditis elegans.* Videos are acquired in 96-well plates using a custom imaging system to capture behavioural responses to drug treatment. Resulting neuroactive or behavioural phenotypes are quantified using Tierpsy Tracker, an open-source software that tracks individual

and multi-worm behavioural phenotypes such as swimming dynamics, morphology, and trajectory. Comparing these phenotypes to a training set of known *C. elegans* phenotypes enables rapid detection of neuroactive compounds. Supervised and unsupervised machine learning methods create a functional map of neuroactive drugs to understand the activity and mechanism of neuroactive fungal metabolites. This high-throughput platform is an exciting opportunity to discover novel neuroactive natural products derived from fungi, using a whole-animal behavioural model.

Disclosures: E.D. Brown: A. Employment/Salary (full or part-time):; Kapoose Creek Bio.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.07/W27

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant

Title: Development of PIASO, a single cell omics analysis toolkit, and establishment of an enhancer-based toolkit to target and manipulate distinct interneuron populations

Authors: *M. DAI[1,2], J. WU[3,4], G. J. FISHELL[3,4];
[1]Broad Inst. of MIT and Harvard, Cambridge, MA; [2]Department of Neurobiology, Harvard Medical School, Boston, MA; [3]Dept. of Neurobio., Harvard Med. Sch., Boston, MA; [4]Broad Institute of MIT and Harvard, Cambridge, MA

Abstract: Enhancers are cis-acting DNA elements that regulate gene transcription and are crucial regulators of spatial-temporal gene expression. Cell-type specific enhancers are critical to establishing and maintaining cell identity in multicellular organisms and identifying these enhancers is key to understanding cellular diversity. Recent developments in single-cell technologies have revolutionized our understanding of cellular diversity. The judicious use of single cell RNA and chromatin methods provides the fundamental information needed for the identification of cell-type specific enhancers. Yet, the accurate and efficient integration of data remains challenging. To address this, we developed PIASO (Precise Integrative Analysis of Single-cell Omics), an optimized toolkit with several newly designed algorithms. Distinguishing features include efficient data preprocessing and gene activity inference of single-cell ATAC-seq (scATAC-seq) data, improved peak calling to identify cell type-specific enhancers, and optimized integration of single-cell RNA-seq (scRNA-seq) and scATAC-seq data. The brain contains numerous cell types, making it a perfect system to study cellular diversity. We used PIASO to analyze scATAC-seq and scRNA-seq data acquired from mouse cortical interneurons to identify cell-type specific enhancers for different interneuron subtypes. We then incorporated these enhancers into recombinant adeno-associated virus (rAAV) vectors to validate their

activity and specificity. We identified a panel of enhancers that are highly selective for distinct interneuron populations and demonstrated that PIASO outperforms other methods in identifying cell-type specific enhancers with high success rate. We also built a rAAV toolkit by combining cell-type specific enhancers with different effectors (fluorescent reporters, opto- and chemo-genetic tools, gCaMP, mono-synaptic rabies, etc). This toolkit allows one to target, manipulate, observe activity and trace the connectivity of specific neuronal subtypes. Thus, we present here a powerful set of tools to study cortical interneurons and the neural circuits to which they contribute. Moreover, our enhancer identification and validation strategies provide the potential to develop more precise therapeutic tools for complex diseases.

Disclosures: M. Dai: None. **J. Wu:** None. **G.J. Fishell:** None.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.08/W28

Topic: I.02. Systems Biology and Bioinformatics

Support: NIMH grant MH108053-01

Title: Brain-wide analysis of rodent brains using standardized mouse and rat reference atlases

Authors: *N. O'CONNOR[1], B. EASTWOOD[1], P. ANGSTMAN[1], C. S. GERFEN[1], C. R. GERFEN[2], J. GLASER[1];
[1]MBF Biosci., Williston, VT; [2]LNP NIMH, Bethesda, MD

Abstract: Molecular neuroanatomical methods have expanded the ability to map connections and activity of neuron subtypes and circuits in whole-brain experimental models. When performed on mouse and rat brains that have been registered to a reference atlas, these analyses reveal details about the functional organization of brain circuits related to behavior and pathologies that are comparable across animals, experiments, and laboratories.
Here we present advances to our research on reconstructing whole slide images of mouse and rat brains from sections labeled with histochemical techniques into whole-brain 3D image volumes with anatomic constrains imposed by the Allen Mouse Brain Common Coordinate Framework (CCF, for mouse), or the Waxholm Rat Brain Atlas (for rat). This advancement results in a compiled section brain image volume that is closer in shape to the in vivo brain. Once images are registered to a reference space, cellular populations and expression intensities (e.g. for cFos) are quantified for each brain region.
We also present mapping tools for registering intact brain volumes imaged with light sheet microscopy. Intact mouse or rat brain volumes imaged from cleared brain tissues by light sheet microscopy methods can be registered to the Allen CCF or Waxholm Rat Brain Atlas using linear and nonlinear registration methods.

We also present an neuroscience research study of whole brain analysis of connectivity using trans-synaptic rabies labeling of neurons. For this analysis, whole slide images of coronal brain sections were reconstructed into a 3D volume, labeled neurons were automatically marked using a neural network, and brain sections and detected neurons were registered to the CCF. The number of labeled neurons in each of the 2500 brain structures in the CCF were calculated, allowing for comparative quantitative analysis between mice. Similar results are presented for rat brain volumes expressing cFos. We also present the results of registering mouse brain volumes from light sheet imaging to the CCF.

Disclosures: N. O'Connor: A. Employment/Salary (full or part-time):; MBF Bioscience. **B. Eastwood:** A. Employment/Salary (full or part-time):; MBF Bioscience. **P. Angstman:** A. Employment/Salary (full or part-time):; MBF Bioscience. **C.S. Gerfen:** A. Employment/Salary (full or part-time):; MBF Bioscience. **C.R. Gerfen:** None. **J. Glaser:** A. Employment/Salary (full or part-time):; MBF Bioscience.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.09/W29

Topic: I.02. Systems Biology and Bioinformatics

Support: Schram Stiftung (T0287/35359/2020)
Deutsche Forschungsgemeinschaft (DFG) grant (FO 1342/1-3)

Title: Deciphering splicing and proteostasis alterations in the aging mouse brain

Authors: *N. HEMANDHAR KUMAR[1], V. KLUEVER[1], M. CORREA MARRERO[2,3], A. ORI[4], E. F. FORNASIERO[1,5];
[1]Inst. für Neuro- und Sinnesphysiologie, Univ. Med. Ctr. Göttingen, Göttingen, Germany; [2]Dept. of Biol., Inst. of Mol. Systems Biol., Zurich, Switzerland; [3]SIB Swiss Inst. of Bioinformatics, Lausanne, Switzerland; [4]Leibniz Inst. of Aging, Jena, Germany; [5]Dept. of Life Sci., Univ. of Trieste, Trieste, Italy

Abstract: Brain aging is a multifaceted process characterized by a number of alterations including changes in mRNA and protein levels, leading to functional impairment and susceptibility to neurodegenerative diseases. Despite the recent advances in omics technologies, the intricate molecular interplay between different molecular layers during brain aging remains poorly understood. To address this gap, we measured gene expression, protein abundance, and protein turnover profiles in the brains of young-adult, middle and aged mice. We collected data from wild-type mice and mouse models relevant for the study of brain aging and organized the results in a searchable proteo-transcriptomic atlas to facilitate comprehensive studies of brain aging. Here, we focus on the changes observed in physiological aging and mouse models

considering in parallel mRNA and protein levels changes. Our analysis reveals specific changes in key pathways related to mitochondria, ribosomal activity, and neuronal function. Furthermore, the integration of mRNA and protein datasets allowed to identify novel age-specific proteoforms. Our findings highlight distinct trajectories for mRNA and protein changes during aging and indicate possible incomplete compensatory mechanisms that are activated during aging, which could be exploited for anti-aging interventions.

Disclosures: N. Hemandhar Kumar: None. **V. Kluever:** None. **M. Correa Marrero:** None. **A. Ori:** None. **E.F. Fornasiero:** None.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.10/W30

Topic: I.02. Systems Biology and Bioinformatics

Title: Spatial proteomic analysis of immune cells in alzheimer's disease human brain using multiplexed imaging and AI-assisted phenotyping

Authors: *A. BOSE[1], R. CHO[2], S. STRUBLE[1], G. SPANG[2], R. HEIL-CHAPDELAINE[1], N. FERNANDEZ DIAZ GRANADOS[1], V. AGRAWAL[1];
[1]Leica Microsystems, Waltham, MA; [2]Cell Signaling Technol., Danvers, MA

Abstract: Alzheimer's disease (AD) is a genetic and sporadic neurodegenerative disease and a common cause of age-related dementia characterized by progressive memory loss. Pathological hallmarks in AD include the presence of plaques and neurofibrillary tangles composed of β-amyloid and hyperphosphorylated tau, respectively. The role of the innate and adaptive immune response in AD is poorly understood, including T cells, which can infiltrate the brain and either mitigate or augment AD neuropathogenesis. Presence of immune cells support a neuroimmune axis of AD which may reveal the interplay of innate and adaptive immune systems. Neuroimmune interactions establish disease heterogeneity in the etiology and pathogenesis of AD. The Cell DIVE Multiplexed Imaging Solution, in combination with IF/IHC-validated antibodies from Cell Signaling Technology (CST), can be used to computationally examine resident and infiltrating immune cells, surrounding pathological hallmarks in AD. Segmentation and clustering analysis can identify spatially co-localized populations of cells, including subpopulations of microglia and T-cells defined by specific disease-associated microglia markers and T-cell markers, respectively. CST's broad portfolio of Cell DIVE validated antibodies enables profiling of immune cell populations in the context of human AD tissue. Here we demonstrate multiplexed Cell DIVE imaging using a novel CST panel and discuss the complexities of AD neuropathology highlighting immune signaling and their interactions in the human AD brain. We examine the spatial distribution of immune cells in AD and the

mechanisms by which immune cells influence AD neuropathology. Cell type specific markers, combined with multiplexed tissue imaging and AI-guided spatial analysis, powered by Aivia, will provide a new approach to understand the existing challenges and help predicting immune-based therapies for treating and preventing AD.

Disclosures: A. Bose: A. Employment/Salary (full or part-time):; Leica Microsystems. **R. Cho:** A. Employment/Salary (full or part-time):; Cell Signaling Technology. **S. Struble:** A. Employment/Salary (full or part-time):; Leica Microsystems. **G. Spang:** A. Employment/Salary (full or part-time):; Cell Signaling Technology. **R. Heil-Chapdelaine:** A. Employment/Salary (full or part-time):; Leica Microsystems. **N. Fernandez Diaz Granados:** A. Employment/Salary (full or part-time):; Leica Microsystems. **V. Agrawal:** A. Employment/Salary (full or part-time):; Leica Microsystems.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.11/W31

Topic: I.02. Systems Biology and Bioinformatics
UG3

Title: Signatures of DNA Damage and Epigenetic Erosion in Opioid Use Disorder

Authors: *C. SRINIVASAN[1], B. N. PHAN[2], C. FU[3], M. KUPPE[4], M. L. SENEY[5], A. R. PFENNING[6], R. W. LOGAN[3];
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Abstract: Opioid abuse and overdose are an increasingly major public health concern. The cellular diversity of brain tissue complicates studying the molecular basis of opioid use disorder (OUD). Using same cell, single nuclei RNA-sequencing and ATAC-sequencing in OUD subjects and unaffected comparisons, we identified canonical cell populations and less abundant cell subtypes in human striatum. Genetic risk variants associated with addiction behaviors including OUD were significantly enriched in putative *cis*-regulatory elements of medium spiny neuron (MSN) subtypes. Using gene set enrichment analysis, we found pathways related to neurodegeneration, interferon response, and DNA damage were significantly altered in MSNs of individuals with OUD. Increased levels of DNA damage over time can result in a loss of epigenetic information over time. From integratively analyzing the transcriptional and open chromatin measurements, we found substantial signatures of “epigenetic erosion” in OUD

subjects. In summary, our results suggest chronic opioid use increases levels of DNA damage, which in turn may produce broad patterns of epigenetic erosion in MSNs.

Disclosures: C. Srinivasan: None. **B.N. Phan:** None. **C. Fu:** None. **M. Kuppe:** None. **M.L. Seney:** None. **A.R. Pfenning:** F. Consulting Fees (e.g., advisory boards); Advisory Board Member of Avista Therapeautics. Other; Founder of Snail Biosciences. **R.W. Logan:** None.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.12/W32

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: funded by Standard Biotools

Title: Whole Slide Imaging modes for Imaging Mass Cytometry reveal cellular and structural heterogeneity of mouse brain tissues

Authors: Q. RAZA[1], **N. ZABINYAKOV**[1], *T. D. PFISTER[1], N. PARSOTAM[1], D. HOWELL[2], L. LIM[1], C. LOH[1], J. CHWEE[1];
[1]Standard BioTools, Markham, ON, Canada; [2]Standard BioTools, San Francisco, CA

Abstract: Mouse models are widely utilized for the study of brain development, functionality and disease-formation mechanisms, helping address the difficulties related to studying the human brain. The mouse brain can be regarded as a miniaturized model of the human brain, permitting visualization of whole tissues to assess the spatial relationship between adjacent cells or tissue compartments. Imaging Mass Cytometry™ (IMC™) is capable of quantitative evaluation of the brain's protein composition, including diseased tumor microenvironments (TMEs), while eliminating the complications of autofluorescence, tissue degradation and spectral overlap. The Hyperion XTi™ Imaging System utilizes IMC technology to simultaneously assess more than 40 individual markers in any type of tissue. We demonstrate the application of IMC using a 40-marker panel composed of the Maxpar OnDemand™ Mouse Immuno-Oncology IMC Panel Kit and the Maxpar® Neuro Phenotyping IMC Panel Kit on whole mouse embryo, normal brain and glioblastoma (GBM) tissue. We performed imaging using three features of Hyperion™ XTi. Preview Mode was applied to rapidly screen entire brain sections for marker expression signatures associated with various immuno-oncology processes. This enabled biomarker-guided selection of areas in tumor tissue that were imaged using Cell Mode and analyzed using single-cell analysis (SCA). Tissue Mode (TM) was applied to perform a whole slide scan of mouse brain tissues that were quantified using pixel-clustering analysis to unravel the composition of the TME. Using TM, we were able to successfully visualize the entire sagittal section of normal mouse embryo and adult brain as well as GBM tissue. Pixel-clustering analysis on TM data provided quantitative spatial expression patterns of structural and immune markers across the

whole tissue. In embryonic and adult brain tissue, we visualized the highly organized structure of the organs and detected neurons, oligodendrocytes, vascular-adjacent astrocytes and extracellular matrix. In GBM tissue, necrotic cores, areas with high immune infiltration, extracellular matrix deposits and activated tumor cells were detected. SCA of GBM tissue demonstrated expansive vascularization, replicating Olig2+ cells, activation of Ras signaling and high abundance of infiltrating immune cells. Overall, we demonstrate the successful application of multiple IMC modes and highlight the power of this technology to simultaneously explore multiple biological outputs to better understand the TME of brain-specific mouse tissues. For Research Use Only. Not for use in diagnostic procedures.

Disclosures: Q. Raza: A. Employment/Salary (full or part-time):; Standard Biotools. 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.; Standard Biotools. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Standard Biotools. **N. Zabinyakov:** A. Employment/Salary (full or part-time):; Standard Biotools. 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.; Standard Biotools. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Standard Biotools. **T.D. Pfister:** A. Employment/Salary (full or part-time):; Standard BioTools. 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.; Standard BioTools. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Standard BioTools. **N. Parsotam:** A. Employment/Salary (full or part-time):; Standard Biotools. 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.; Standard Biotools. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Standard Biotools. **D. Howell:** A. Employment/Salary (full or part-time):; Standard Biotools. 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.; Standard Biotools. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Standard Biotools. **L. Lim:** A. Employment/Salary (full or part-time):; Standard Biotools. 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.; Standard Biotools. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Standard Biotools. **C. Loh:** A. Employment/Salary (full or part-time):; Standard Biotools. 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.; Standard Biotools. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding

diversified mutual funds); Standard Biotools. **J. Chwee:** A. Employment/Salary (full or part-time):; Standard Biotools. 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.; Standard Biotools. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Standard Biotools.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.13/W33

Topic: I.02. Systems Biology and Bioinformatics

Support: Author's research is funded by a company that produces a product or service related to the work being reported.

Title: Imaging Mass Cytometry Using Whole Slide Imaging Quantitatively Resolves Spatial Cellular Heterogeneity in Neurodegenerative Brain Pathology

Authors: *N. ZABINYAKOV[1], Q. RAZA[1], T. D. PFISTER[1], D. HOWELL[2], N. PARSOTAM[1], C. LOH[1], L. LIM[1], J. PEMBERTON[1], J. CHWEE[1];
[1]Standard BioTools, Markham, ON, Canada; [2]Standard BioTools, San Francisco, CA

Abstract: Neurodegenerative diseases (NDs) such as Parkinson's disease (PD), Alzheimer's disease (AD) and multiple sclerosis (MS) represent the most prominent contributors to the global ND population. Understanding the etiology of these diseases is a major challenge and requires deciphering the complex spatial biological processes that cause deterioration of neuronal tissue, such as formation of protein aggregates and tangles, infiltration of immune cells and myelin loss. Imaging Mass Cytometry™ (IMC™) is a spatial biology technology that facilitates comprehensive visualization of the features of ND. Unlike cyclic fluorescent methods, IMC can reveal the distribution of 40-plus distinct protein markers simultaneously without tissue degradation and autofluorescence artifacts observed in the brain. IMC offers various whole slide imaging (WSI) modes that range from visualization of an entire tissue section to single-cell assessment, permitting in-depth exploration of tissue heterogeneity. We report the application of IMC WSI modes with three specific 41-marker panels tailored to AD, PD or MS. Tissue Mode (TM) was used to scan the entire tissue followed by pixel-clustering analysis to uncover the spatial distribution of all markers at a tissue level. Preview Mode (PM) was applied to sample the entire tissue at low resolution, informing selection of areas for high-resolution Cell Mode (CM) imaging on the same slide. PM in combination with ND panels allowed whole tissue visualization of the main protein contributors to disease pathology: amyloid precursor protein in amyloid plaques; Tau in tangles of AD; p-αSynuclein in Lewy bodies and Lewy neurites of PD;

and large demyelinated lesions of MS. PM facilitated marker-guided ROI selection for subsequent imaging with CM on the same slide. Single-cell analysis of CM revealed a small population of neurons in the periphery of large aggregates in AD in less than 2% of all cells, expressing high levels APP and pTau; a distribution of infiltrated neutrophils with cytotoxic properties in PD; and three distinct populations of cells associated with lesions in MS. Pixel-clustering analysis of TM images offered a complementary perspective, unveiling two distinct types of amyloid aggregates in AD, widely spread Lewy bodies and Lewy neurites in PD and three clusters with varying levels of MOG, neurofilament and MBP expression in MS, suggesting a progressive loss of myelin in the vicinity of a lesion. Overall, the data indicates the complexity of the disease for each case, which could have been underrepresented if low-plex visualization techniques were used. For Research Use Only. Not for use in diagnostic procedures.

Disclosures: N. Zabinyakov: A. Employment/Salary (full or part-time):; Full-time employee of Standard BioTools. 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.; Author's research is funded by a company that produces a product or service related to the work being reported.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Author has ownership interest with Standard BioTools. **Q. Raza:** A. Employment/Salary (full or part-time):; Full-time employee of Standard BioTools. 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.; Author's research is funded by a company that produces a product or service related to the work being reported.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Author has ownership interest with Standard BioTools. **T.D. Pfister:** A. Employment/Salary (full or part-time):; Full-time employee of Standard BioTools. 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.; Author's research is funded by a company that produces a product or service related to the work being reported.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Author has ownership interest with Standard BioTools. **D. Howell:** A. Employment/Salary (full or part-time):; Full-time employee of Standard BioTools. 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.; Author's research is funded by a company that produces a product or service related to the work being reported.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Author has ownership interest with Standard BioTools. **N. Parsotam:** A. Employment/Salary (full or part-time):; Full-time employee of Standard BioTools. 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.; Author's research is funded by a company that produces a product or service related to the work being reported.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent

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Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.14/W34

Topic: I.02. Systems Biology and Bioinformatics

Support: Author's research is funded by a company that produces a product or service related to the work being reported.

Title: Whole Slide Imaging Modes for Imaging Mass Cytometry Quantitively Resolve Spatially Extensive Cellular Heterogeneity in Human Gliomas

Authors: *J. PEMBERTON[1], N. ZABINYAKOV[2], Q. RAZA[3], T. D. PFISTER[4], N. PARSOTAM[3], D. HOWELL[5], L. LIM[3], C. LOH[6];
[1]Standard Biotools Inc., Markham, ON, Canada; [2]StandardBioTools, Markham, ON, Canada; [3]Standard Biotools Inc, Markham, ON, Canada; [4]Standard BioTools (formerly Fluidigm), Markham, ON, Canada; [5]Standard Biotools Inc, San Francisco, CA; [6]Standard BioTools, Markham, ON, Canada

Abstract: Gliomas, the most common neoplasms of the central nervous system, are complex forms of cancer that are challenging to diagnose and treat. Median survival rate is just over one year after diagnosis for primary glioblastoma (GBM), the most malignant type. Hallmark features of GBM include necrosis, hemorrhage and pseudopalisades, making it a highly phenotypically heterogeneous disease requiring further investigation. Spatial identification of the cellular composition of GBM is vital for interpretation of disease origin, progression and treatment. Imaging Mass Cytometry™ (IMC™) is a spatial biology technology that facilitates comprehensive visualization of normal, diseased and cancerous tissues. Unlike cyclic fluorescent methods, IMC can reveal the distribution of 40-plus distinct protein markers simultaneously without tissue degradation and autofluorescence artifacts observed in the brain. IMC offers various whole slide imaging modes that range from visualization of an entire tissue section to single-cell assessment, permitting in-depth exploration of tissue heterogeneity. A 41-marker neuro-oncology IMC antibody panel was used to determine the cellular and structural landscape of the brain tumor microenvironment. We applied the panel on a tissue microarray (TMA) containing dozens of human glioma cores and performed imaging using the Hyperion XTi™ Imaging System, which has whole slide scanning capabilities. Tissue Mode (TM) was used to scan the entire slide followed by pixel-clustering analysis to uncover the spatial distribution of relevant markers at a tissue level. Preview Mode was applied to sample the entire tissue at low resolution, informing selection of areas for subsequent high-resolution Cell Mode imaging on the same slide. Using TM, we successfully mapped the spatial location of neurons, astrocytes, microglia and oligodendrocytes in TMA cores. Additionally, we identified glioma subtypes based on their marker expression patterns. Various tumor niches, infiltrating cells, activated microglia, clinically relevant prognostic survival markers and extensive structural tumor scaffolds were detected across all TMA cores. Subsequent single-cell analysis of selected regions of interest provided a quantitative assessment of the cellular composition of glioma samples. Striking cellular and protein heterogeneity was observed between the acquired cores, indicating the complexity of the disease for each case. This complexity could have been underrepresented if low-plex visualization techniques were used. For research purpose only. Not for use in diagnostic procedures.

Disclosures: J. Pemberton: A. Employment/Salary (full or part-time):; Full-time employee of Standard BioTools. 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.; Author's research is funded by a company that produces a product or service related to the work being reported.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Author has ownership interest with Standard

BioTools. **N. Zabinyakov:** A. Employment/Salary (full or part-time):; Full-time employee of Standard BioTools. 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.; Author's research is funded by a company that produces a product or service related to the work being reported.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Author has ownership interest with Standard BioTools. **Q. Raza:** A. Employment/Salary (full or part-time):; Full-time employee of Standard BioTools. 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.; Author's research is funded by a company that produces a product or service related to the work being reported.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Author has ownership interest with Standard BioTools. **T.D. Pfister:** A. Employment/Salary (full or part-time):; Full-time employee of Standard BioTools. 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.; Author's research is funded by a company that produces a product or service related to the work being reported.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Author has ownership interest with Standard BioTools. **N. Parsotam:** A. Employment/Salary (full or part-time):; Full-time employee of Standard BioTools. 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.; Author's research is funded by a company that produces a product or service related to the work being reported.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Author has ownership interest with Standard BioTools. **D. Howell:** A. Employment/Salary (full or part-time):; Full-time employee of Standard BioTools. 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.; Author's research is funded by a company that produces a product or service related to the work being reported.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Author has ownership interest with Standard BioTools. **L. Lim:** A. Employment/Salary (full or part-time):; Full-time employee of Standard BioTools. 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.; Author's research is funded by a company that produces a product or service related to the work being reported.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Author has ownership interest with Standard BioTools. **C. Loh:** A. Employment/Salary (full or part-time):; Full-time employee of Standard BioTools. 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.; Author's research is funded

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Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.15/W35

Topic: I.02. Systems Biology and Bioinformatics

Support: U54AG065187
U54AG065181

Title: Bioinformatic Resources Provided By TREAT-AD

Authors: *S. KEEGAN[1], G. CARY[1], J. C. WILEY[2], R. K. TRIPATHY[3], R. R. BUTLER III[4], K. LEAL[5], Y. LI[6], A. I. LEVEY[7], A. K. GREENWOOD[5], G. W. CARTER[1];
[1]The Jackson Lab., Bar Harbor, ME; [2]Univ. Washington, Seattle, WA; [3]Computat. Sci., The Jackson Lab. for Genomic Med., Farmington, CT; [4]Neurol. & Neurolog. Sci., Stanford Univ., Stanford, CA; [5]Sage Bionetworks, Seattle, WA; [6]The Jackson Lab., Farmington, CT; [7]Emory Univ., Atlanta, GA

Abstract: Background: Alzheimer’s disease (AD) therapeutics have largely been unsuccessful in alleviating disease burden in those afflicted by the disease. The TREAT-AD Consortium is an international group of academic researchers dedicated to identifying novel molecular targets for AD from underexplored areas of disease linked pathology.
Method: An expertly curated collection of 19 biological domains, which describe functional aspects of endophenotypes of AD using manually curated Gene Ontology Terms are defined. These large biological domain networks are split further into subdomain networks using the same process. Knowledge Graphs (KGs) where the nodes are genes and the edges are protein-protein interactions depicting these biological and sub domains have been created. The TREAT-AD Target Risk Score provides a multi-omic score to each gene in the network. A weighted Key Driver Analysis is conducted on each network to determine causal nodes in the network. A Graph Neural Network trained on clinical omics samples investigates the importance of each gene as they relate to clinical phenotypes.
Result: A collection of bioinformatic pipelines have been created to organize Alzheimer's Disease data and arrange it in a way that can be computationally analyzed in order to identify novel hypotheses areas. Network interactions between biological domains indicate how endophenotypes of the disease are dependent on one another. The subdomains of the larger biological domains provide insight on the biological processes driving those interactions. These

pipelines help prioritize therapeutic targets, not only for internal use, but all of the methods and results are public for community use. Based on these analyses, novel disease modifying hypotheses are proposed and sent to experimental groups for validation.
Conclusion: Alzheimer's Disease biology can be subdivided into networks which represent processes that are notable to AD patients. Genes from these networks are prioritized to identify candidate therapeutic targets that are well situated to affect the network biology. TREAT-AD investigators will validate these targets in cell models and generate experimental resources to support further target development.

Disclosures: S. Keegan: None. **G. Cary:** None. **J.C. Wiley:** None. **R.K. Tripathy:** None. **R.R. Butler:** None. **K. Leal:** None. **Y. Li:** None. **A.I. Levey:** None. **A.K. Greenwood:** None. **G.W. Carter:** None.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.16/W36

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant AG046170
NIH Grant RF1AG074010

Title: Dysregulation of Musicality Genes in Alzheimer's Disease

Authors: P. ZHANG[1,2], G. YU[3], A. THORPE[3], M. WANG[4], ***B. ZHANG**[3];
[1]Special Music Sch., New York, NY; [2]Precollege, The Juilliard Sch., New York, NY; [4]Genet. and Genomic Sci., [3]Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Recent studies show that exposure to music benefits the cognitive function and social-emotional wellbeing of Alzheimer's disease (AD) patients. Music therapy alleviates symptoms including memory loss, delirium, and agitation, and thus, is a promising non-pharmaceutical approach to managing AD. However, little is known about the molecular mechanisms of music's effect on brain functions. This study aims to systematically examine dysregulation of over 50 musicality genes in AD that were recently identified by genome-wide association study (GWAS), given the potential of music in treating dementia and other neurological disorders. Large-scale bulk RNA-seq gene expression data from seven brain regions including the parahippocampal gyrus (PHG), the frontal pole, the inferior frontal gyrus, the superior temporal gyrus, the dorsolateral prefrontal cortex (DLPFC), the cerebellum, and the temporal cortex, as well as single nucleus RNA-seq (snRNA-seq) data from the DLPFC were used to investigate expression changes and network rewiring of musicality genes between AD and control samples. Differentially expressed genes (DEGs) were identified by T-test and linear regression, and significant gene-gene correlations were determined using Spearman's correlation analysis. An

AD relevance score was calculated for each gene by summing the significance levels in all the seven brain regions. The musicality genes most significantly dysregulated in AD include *HERC1*, *NLK, AKAP6, CCSER1*, *TSSC1*, and *NGEF*. *HERC1*, a ubiquitin protein ligase, is involved in Class I MHC mediated antigen processing and presentation and innate immune system while *NGEF* (neuronal guanine nucleotide exchange factor) was recently found to be involved in regulating short-/intermediate-term memory. For each brain region, we also constructed a coexpression network from gene-gene correlations. Strikingly, we found that the network connectivity of musicality genes was significantly correlated with their AD relevance scores. The analysis of the snRNA-seq data in the DLPFC further revealed that many musicality genes exhibited cell-type specific differential expression between AD and control in several brain cell types such as excitatory neurons and oligodendrocytes. This study has revealed the dysregulation of various musicality genes in AD and a strong correlation between their network connectivity and the degree of dysregulation in the brain. Such findings establish a solid foundation for investigating the molecular mechanisms of dysregulated musicality in AD, ultimately contributing to the development of music therapy modalities specifically tailored to AD.

Disclosures: P. Zhang: None. **G. Yu:** None. **A. Thorpe:** None. **M. Wang:** None. **B. Zhang:** None.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.17/W37

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant U01MH130907

Title: Bridging circuit connectivity and function in mouse primary visual cortex

Authors: *J. AMAN[1], O. ZOBEIRI[2], M. M. TAKENO[3], M. A. BUICE[4], C. M. SCHNEIDER-MIZELL[4], F. C. COLLMAN[4], N. M. DA COSTA[4], A. ARKHIPOV[1];
[2]Biomed. Engin., [1]Allen Inst., Seattle, WA; [3]Neural Coding, [4]Allen Inst. For Brain Sci., Seattle, WA

Abstract: A key question in neuroscience is whether neurons with distinct functional activity share morphological or connectivity properties. We link EM-reconstructed connectivity of excitatory neurons in mouse V1 with their functional responses to visual stimuli. We use an Allen Institute EM dataset (similar to the recent MICrONS dataset) with co-registered functional activity from excitatory neurons in L2/3, L4 and L5. A Generalized Linear Model (GLM) is used to predict cell responses to visual stimulus features and behavioral state. We use this model to identify clusters of excitatory cells with similar responsiveness to visual stimuli and arousal. We

then map co-registered cells to EM morphological classes identified by clustering on anatomical features. Further, we test whether neurons with similar visual tuning receive similar patterns of synaptic inputs or project to the same target cells.

Disclosures: J. Aman: None. **O. Zobeiri:** None. **M.M. Takeno:** None. **M.A. Buice:** None. **C.M. Schneider-Mizell:** None. **F.C. Collman:** None. **N.M. da Costa:** None. **A. Arkhipov:** None.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.18/W38

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant U01MH130907

Title: Bridging Function and Transcriptomics: Visual Responses of Diverse Cell Classes in the Mouse Visual Cortex

Authors: *O. ZOBEIRI[1], J. AMAN[1], H. SCHRYVER[2], M. DAVIS[1], J. KIM[1], S. MCCULLOCH[1], A. LEON[3], S. CALDEJON[1], A. WILLIFORD[1], S. M. SEID[4], S. R. OLSEN[1], P. A. GROBLEWSKI[5], M. E. GARRETT[6], A. ARKHIPOV[1];
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Abstract: The brain's intricate functions arise from diverse populations of neurons, each with unique genetic profiles and potential roles in neural circuits. A central question in neuroscience is how these diverse cell types contribute to the broader functional properties of neural circuits. To decipher this complex interplay, we are investigating how different neuron types in the visual cortex contribute to processing visual information and generating behavior. We present mice with different visual stimuli, such as moving gratings and natural movies, while monitoring their neuronal activity. These experiments employ multiplane two-photon calcium imaging in vivo to record neuronal responses and post-hoc Hybridization Chain Reaction (HCR) mFISH to analyze gene expression at single-cell resolution. This allows us to categorize neurons into distinct classes and subclasses based on their transcriptomic profiles. By incorporating information about the animal's locomotion, face movement, and pupil dilation, we use artificial neural networks and statistical models to analyze how visual and non-visual factors influence neuronal responses. Our findings reveal that over half of the neurons respond to visual and/or non-visual stimuli, with inhibitory neurons exhibiting a higher responsiveness. To understand how different neurons respond to visual stimuli, we employed generative models. These models helped us identify the preferred stimuli for each neuron, uncovering what kind of visual input excites them the most.

We then combined this information with the neurons' specific response characteristics to group them into distinct functional clusters. While certain functional differences exist between transcriptomic classes and subclasses, we also observe significant heterogeneity within each group. This heterogeneity suggests a complex relationship between cell type and function, opening exciting avenues for further investigation. By exploring whether this functional diversity corresponds to even finer-grained transcriptomic differences, we can gain a deeper understanding of how various cell types contribute to the intricate computations underlying visual processing and its interaction with behavioral signals in the visual cortex.

Disclosures: O. Zobeiri: None. **J. Aman:** None. **H. Schryver:** None. **M. Davis:** None. **J. Kim:** None. **S. McCulloch:** None. **A. Leon:** None. **S. Caldejon:** None. **A. Williford:** None. **S.M. Seid:** None. **S.R. Olsen:** None. **P.A. Groblewski:** None. **M.E. Garrett:** None. **A. Arkhipov:** None.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.19/X1

Topic: I.02. Systems Biology and Bioinformatics

Support: U01MH130907

Title: Bridging function and transcriptomics: Mapping cell types in the mouse visual cortex

Authors: *H. SCHRYVER[1], C. BERRY[1], S. MCCULLOCH[1], C. LAITON[1], O. ZOBEIRI[1], J. KIM[1], E. PETERSON[1], C. BONATTO PAESE[1], M. TAORMINA[1], S. CALDEJON[1], A. WILLIFORD[1], J. A. MILLER[2], J. WATERS[3], T. WANG[1], J. BAKA[1], K. SVOBODA[4], S. R. OLSEN[1], M. E. GARRETT[5], A. ARKHIPOV[1];
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Abstract: The relationship between the transcriptomic identity of neurons and their functional properties is a crucial area of research for understanding how functional roles come about and are implicated in neuronal circuit activities and computations. Further, the importance of cell type identities and influence in processes like sensory processing and learning remains elusive. We aim to improve the understanding of multiple cell types at the fine-grained level and their association with functional types of neuronal activity in vivo in mouse primary visual cortex (V1). In our study, we utilized multiplexed fluorescence in situ hybridization (mFISH) to delineate cell populations into transcriptomic types. Drawing upon data from the Allen Institute, we designed gene panels to discern cell types using a minimal set of genes. We implemented a standardized analysis pipeline where lightsheet-imaged tissue fluorescent spots in multiple

channels were associated with segmented cells that were registered across rounds of hybridization and imaging. We validated our approach by comparing expression patterns to other Allen Institute datasets, including scRNAseq and MERFISH datasets, as well as other datasets measuring relative expression patterns in mouse V1. We applied quality-control metrics to derive a cell-by-gene table comprised of high-quality cells. We mapped gene expression to assign cell type identities and found that we could identify various subpopulations, including subclasses and further hierarchical subdivisions of cell types. We are connecting the observed cell populations with those recorded via two-photon calcium imaging in vivo. This cell co-registration will allow us to examine the relationship between transcriptomically defined cells and visual responses, as well as neural activity during behavioral tasks. Moving forward, we aim to further refine our analysis pipeline for standardized and reproducible creation of cell-by-gene tables and cell type mapping to further illuminate the role of cell types and their function.

Disclosures: H. Schryver: None. **C. Berry:** None. **S. McCulloch:** None. **C. Laiton:** None. **O. Zobeiri:** None. **J. Kim:** None. **E. Peterson:** None. **C. Bonatto Paese:** None. **M. Taormina:** None. **S. Caldejon:** None. **A. Williford:** None. **J.A. Miller:** None. **J. Waters:** None. **T. Wang:** None. **J. Baka:** None. **K. Svoboda:** None. **S.R. Olsen:** None. **M.E. Garrett:** None. **A. Arkhipov:** None.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.20/X2

Topic: I.02. Systems Biology and Bioinformatics

Title: Accurate estimation of neural interactions provides evidence in favor of hierarchical predictive processing

Authors: *K. KHALVATI, S. DURAND, B. HARDCASTLE, H. BELSKI, H. CABASCO, R. GILLIS, G. R. HELLER, H. LOEFFLER, T. RAMIREZ, S. MIHALAS, J. H. SIEGLE, S. R. OLSEN, M. A. BUICE;
Allen Inst., Seattle, WA

Abstract: According to the predictive processing hypothesis, perception is a hierarchical inference process where bottom-up connections convey sensory information while top-down connections transmit the prior/prediction of the brain's internal model about the upcoming sensory information (Rao & Ballard, 1999; Singer, 2021). Validating this theory requires measuring the information flow among different brain regions across the sensory hierarchy. This comes with enormous technological and computational challenges, including simultaneous recording from multiple areas and accurate estimation of information flow between areas. As a result, this hypothesis has not yet been adequately tested in experiment settings. For the first

time, we thoroughly investigated the predictive processing hypothesis by utilizing multi-region recording with Neuropixel probes and developing a method for accurately estimating information flow between different areas. More specifically, we recorded from 6 visual regions of 14 mice simultaneously during passive viewing of various natural video clips, each of which repeated 25 times. In addition, we utilized deep Bayesian networks to estimate the neural population correlations accurately and efficiently (Khalvati et al., 2023). Then, we measured functional connectivity and information flow between two visual areas based on the relationship of their population correlations with each other. Finally, we compared our measurement with the anatomical connections obtained via viral traces by the Allen Institute (Harris et al., 2019). Our measurements and results strongly supported the predictive processing hypothesis. The flow of information reflected the bottom-up anatomical connections immediately after the change of scenes. On the other hand, the information flow among pairs of visual regions was highly correlated with top-down anatomical connections when there was not much change in the natural videos, and the next frame was predictable. We reproduced our results on the publicly available Allen Institute Visual Brain Observatory dataset during three different stimuli of natural videos, low-contrast drifting gratings and high-contrast drifting gratings. Overall, all of our results on two different experiments performed on 14 and 26 mice, respectively, and on four different stimulus sets, strongly favor the predictive processing hypothesis.

Disclosures: K. Khalvati: None. **S. Durand:** None. **B. Hardcastle:** None. **H. Belski:** None. **H. Cabasco:** None. **R. Gillis:** None. **G.R. Heller:** None. **H. Loeffler:** None. **T. Ramirez:** None. **S. Mihalas:** None. **J.H. Siegle:** None. **S.R. Olsen:** None. **M.A. Buice:** None.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.21/X3

Topic: I.02. Systems Biology and Bioinformatics

Support: JST ERATO grant number JPMJER2001
the Science and Technology Platform Program for Advanced Biological Medicine (AMED/MEXT to H.R.U.)
grant-in-aid for scientific research (S) (H.R.U., grant number JP18H05270)
MEXT Quantum Leap Flagship Program (MEXT QLEAP) (to H.R.U., grant number JPMXS0120330644)
grant-in-aid for scientific research (C) (to K.M., grant number 20K06885)
a Grant-in-Aid from the Human Frontier Science Program (to H.R.U.)
JST [Moonshot R&D] (to K.M., grant number JPMJMS2023)

Title: Evaluation of drug effects on central nervous system at the whole-brain scale using CUBIC

Authors: *F. KINOSHITA[1,2], K. YAMASHITA[3], S. YOSHIDA[4], T. T. MITANI[1,2], K. MATSUMOTO[4], R. YAMADA[3], H. R. UEDA[3,5];
[1]Osaka Univ., Suita, Japan; [2]RIKEN Center for Biosystems Dynamics Research, Osaka, Japan; [3]RIKEN Ctr. for Biosystems Dynamics Res., Osaka, Japan; [4]the Univ. of Tokyo, Tokyo, Japan; [5]the University of Tokyo, Tokyo, Japan

Abstract: Psychoactive and anti-epileptic drugs affect the central nervous system. However, the impact of these drugs on brain activity has not been extensively evaluated. In this study, we developed an analyzing pipeline for whole-brain scale data and assessed the effects of 17 drugs (anti-schizophrenia: 3, anti-epileptic: 4, stimulant: 2, anti-depression: 4, anti-Alzheimer's disease and parkinsonism: 3, anti-anxiety: 1) on c-Fos expression at the whole-brain scale. Mice were divided into drug-administered treatment group and control group (n= 6 in each group). Whole brains of mice were cleared and analyzed by using CUBIC (clear, unobstructed brain imaging cocktails and computational analysis). Nuclear staining and immunostaining of c-Fos were conducted in the clearing process and 3D image stacks of whole brains were obtained using a laboratory-made light-sheet fluorescence microscope. c-Fos-positive cells were detected and these coordinates were obtained in raw images using a laboratory-made cell detection algorithm. We used image normalization methods, ANTs (advanced normalization tools), to compare images between samples. After individual brain samples were registered to CUBIC-R+ reference atlas using images of nuclear staining, images of c-Fos signals and detected c-Fos-positive cell coordinates in each sample were transformed to the CUBIC-R+ Atlas space. Then, all cells were given anatomical region IDs (following the Allen Brain Atlas CCF v.3). The number of cells and cell density, and the intensity of images and its density were calculated and statistically analyzed between groups per region or voxel unit. Furthermore, we transformed the voxel-level data to the Allen Brain Atlas CCF v.3 space and created an open-source database so that anyone can access and refer to our results.In the caffeine or risperidone-administered group, a significant increase in c-Fos expression was observed mainly in the cortex. Based on the patterns of significant increase or decrease in the brain regions, we classified the effects of drugs into several categories such as cortical patterns or subcortical patterns etc. and discussed whether these patterns can be pharmacologically explained by the mechanism of action of drugs. Our methods and results reveal the effects of drugs on the central nervous system at the whole-brain scale and provide a database that can be integrated with previous data about cell types, transcriptome or connectivity.

Disclosures: F. Kinoshita: None. **K. Yamashita:** None. **S. Yoshida:** None. **T.T. Mitani:** None. **K. Matsumoto:** None. **R. Yamada:** None. **H.R. Ueda:** None.

Poster

PSTR428

Systems Biology and Multiomics Approaches

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR428.22/X4

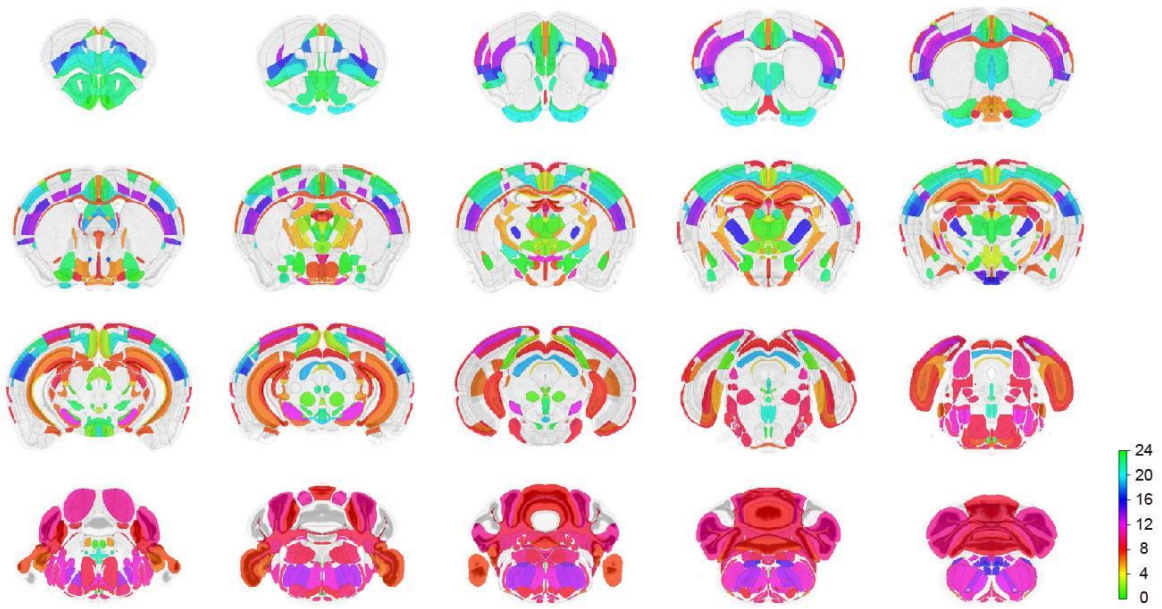
Topic: I.02. Systems Biology and Bioinformatics

Support: JST ERATO grant number JPMJER2001
the Science and Technology Platform Program for Advanced Biological Medicine (AMED/MEXT to H.R.U.)
JSPS KAKENHI grant-in-aid for scientific research (S) (H.R.U., grant number JP18H05270)
JSPS KAKENHI grant-in-aid for scientific research (C) (to K.M., grant number 20K06885)
a Grant-in-Aid from the Human Frontier Science Program (to H.R.U.)
JST [Moonshot R&D] (to K.M., grant number JPMJMS2023)
MEXT Quantum Leap Flagship Program (MEXT QLEAP) (to H.R.U., grant number JPMXS0120330644)

Title: Whole brain activity atlas of circadian rhythm: comprehensive quantitative analysis using CUBIC

Authors: *K. YAMASHITA[1,2], F. L. KINOSHITA[1,2], S. Y. YOSHIDA[1,3], K. MATSUMOTO[1,3], R. G. YAMADA[1,4], H. R. UEDA[1,2,3,4];
[1]RIKEN Lab. for Synthetic Biol., Suita-shi, Osaka, Japan; [2]Graduate School of Medicine, Osaka University, Suita-shi, Osaka, Japan; [3]Graduate School of Medicine, University of Tokyo, Tokyo, Japan; [4]Institute of Life Science, Kurume University, Kurume-shi, Fukuoka, Japan

Abstract: The circadian clock drives oscillations in diverse tissues and organs in mammals, allowing an organism to adapt its physiology in anticipation of the day-night transition. The suprachiasmatic nucleus (SCN), a region in the hypothalamus, is the well-known coordinator of the peripheral clocks. While the circadian activity of the SCN has been extensively investigated, studies on circadian rhythms in other brain regions are limited. Establishing a comprehensive map of the circadian rhythms of neuronal activity across the whole brain is essential to investigate region-specific functions at different times of the day. We selected c-Fos protein as a neuronal activity marker to explore these rhythms. *c-fos* is one of the immediate early genes (IEGs) widely expressed throughout the brain, unlike other IEGs such as *Arc* which are concentrated in specific regions in the cortex and hippocampus. To enable a comprehensive assessment of c-Fos expression, we first developed stable techniques for 3D c-Fos immunostaining, 3D imaging, and quantitative analysis by using the CUBIC (Clear, Unobstructed Brain Imaging Cocktails and Computational Analysis) method. Then, we investigated the circadian changes of intrinsic c-Fos expression across all brain regions. We placed mice in the dark-dark (DD) condition and sampled their brains every four hours over two days. We used CUBIC to perform whole-brain c-Fos immunostaining and image the samples by light-sheet microscopy, and used CUBIC Atlas to quantify cells in each brain region. We found the circadian changes of c-Fos expression not only in the SCN but also in other various brain regions. Our findings revealed that regions across the entire brain exhibit distinct circadian phases. For instance, even within the cortex, areas were identified with different phases. These findings lead to the creation of a whole-brain activity atlas of circadian rhythms and a comprehensive description of the temporal dynamics of neural activities across diverse brain regions, establishing a foundation for the research community for precise brain studies.



Disclosures: K. Yamashita: None. **F.L. Kinoshita:** None. **S.Y. Yoshida:** A. Employment/Salary (full or part-time):; CUBICStars. **K. Matsumoto:** A. Employment/Salary (full or part-time):; CUBICStars. **R.G. Yamada:** None. **H.R. Ueda:** A. Employment/Salary (full or part-time):; CUBICStars.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.01/X5

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Establishing humanising drug discovery research with integration of automation workflows

Authors: *D. KUMAR[1], E. ROSETHORNE[1], D. SWIFT[1], P. GYASI-ANTWI[1], H. SHARPLIN[2], J. TILMAN[2], D. WALLBANK[2], J. BHAGWAN[2], S. VYAS[2], T. PHILLIPS[1], N. MIRZA[1];
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Abstract: A current lack of physiologically relevant *in vitro cell* disease models has led to high attrition rates of novel therapies progressing through clinical trials. In recent years, the use of induced pluripotent stem cells (iPSCs) derived from patients has improved effective drug screening for human genetic diseases. However, there are challenges in using iPSCs derived cell types for screening programmes, such as scalability, purity of cell populations that robustly maintain disease phenotype, and variability in data across labs. We have developed a High Content Imaging (HCI) phagocytosis assay using automation systems to culture iPSC derived microglia (iMGL), increase assay throughput, and demonstrate consistent functionality which is necessary for screening and identifying compounds which might be good chemistry start points for projects focussed on neuroinflammation. Human iMGL were dispensed into 384-well imaging plates using the Multidrop™ Combi Reagent Dispenser and matured over 7 days with frequent media changes using the Integra VIAFLO. Compounds were prepared using Echo Acoustic Liquid Handlers and dispensed onto cells using the Biomek NX^{P} Automated Workstation. Cells were then treated with pHrodo Red-labelled Amyloid-β 1-42 (Aβ) to stimulate phagocytosis, after which time they were fixed and stained with Hoechst to identify nuclei, and specific antibodies to a range of microglial markers. Cells were imaged using the ImageXpress Confocal HCI System and automated plate handling. Phagocytosis was quantified using a Cell Scoring algorithm in MetaXpress6 to identify cells positive for pHrodo-Aβ near the nucleus. Mosaic was used for compound tracking and Genedata for data analysis. To characterise the consistency of iMGL populations, we evaluated the expression of the microglial markers transmembrane protein 119 (TMEM119), purinergic P2Y12 receptor and triggering receptor expressed on myeloid cells 2 (TREM2). A comparison between manual and automated assays confirmed that there was no significant difference in the number or morphology of cells, the expression of microglia markers using either approach. Similarly, there was no significant difference in the percentage of phagocytosis positive iMGL, which was approximately 50 % for both assay formats. In conclusion we have established an automation process to support culture of iPSC derived cells and a HCI phenotypic assay to both screen for novel compounds that augment phagocytosis and explore iMGL cell biology. These processes can be adapted to a range of different assay formats suitable for neurodegenerative disease research and will allow us to accelerate human neuroscience drug discovery.

Disclosures: D. Kumar: None. **E. Rosethorne:** None. **D. Swift:** None. **P. Gyasi-Antwi:** None. **H. Sharplin:** None. **J. Tilman:** None. **D. Wallbank:** None. **J. Bhagwan:** None. **S. Vyas:** None. **T. Phillips:** None. **N. Mirza:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.02/X6

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Reporter cell line expressing small chaperonins and beta-amyloid. A tool to develop new pharmacotherapies in Alzheimer's disease.

Authors: S. PAPASERGI[1], **C. FERRARO**[1], P. SALADINO[2], M. MACRÌ[3], G. BRUNACCI[4], F. DI GAUDIO[5], *C. CASCIO[6];
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Abstract: Alzheimer’s disease (AD) is the most common type of dementia, characterized clinically by a slow progressive decline in cognitive function. AD represents one of the major concerns for public health in the 21 st century. This neurological disorder is considered a misfolding disease, characterized by extracellular deposits of amorphous aggregates of beta amyloid (Aβ) indicated as Aβ plaques one of the hallmarks of the disease. To date, no treatments are available to cure the AD in terms of blocking or slowing down the progression. Several clinical trials designed to beta amyloid clearance or reduce its levels have been unsuccessful. Recent findings have reconsidered the target of treatment by focusing on proper folding of Aβ and allowing it to exert physiological effects. The proteins folding correlate with their soluble state. Proteins folding is maintained by the network of molecular chaperonins or heat shock proteins (HSPs). In this contest, the small HSPs (sHSPs or HSPBs) are considered sentinel proteins that are early involved during the processing of misfolded proteins, preventing further misfolding, blocking aggregation and driving the activity of the HSP network. In our work we generated a reporter human cell line(s) as a tool, engineered to monitor, in living condition the interactions between a sHSP and Aβ. Specifically, in the reporter system the CDS (CoDing Sequence) of two subunits of luciferase are to be cloned in frames with the CDS of Aβ and sHSP(s).The system it has been designed to respond by an enhanced light emission in function of an increased sHSP(s)/Aβ interaction The sHSP(s)/A-beta interaction it has been analyzed and optimized at an early stage by transient transfection on plasmid-type vectors and translated, and successively, on lentiviral vector to allow transgenic and stable production of sHSP(s)/A-beta reporter system in the selected cell target. Therefore, the reporter cell lines obtained could be the first tool capable of easily following the sHSP/A-beta interaction and could be used, through a simple comparison of the level of light emitted, to screen libraries of drug molecules and physiological stimuli (e.g. example, hormones, cytokines, chemokines), nutraceutical compounds, random peptide libraries.

Disclosures: S. papasergi: None. **C. Ferraro:** None. **P. saladino:** None. **M. Macrì:** None. **G. Brunacci:** None. **F. Di gaudio:** None. **C. Cascio:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.03/X7

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Characterizing the neuroinflammatory profile in neurodegenerative conditions using rapid, multiplexed assays with enhanced sensitivity

Authors: *G. KHAYRULLINA, M. ABDEL-GHANI, T. PARIRA, D. TSELENCHUK, T. BARVIR, M. SAMAROO, J. SLEZAK, C. SHAW, G. GALEN, M. MORELLI, P. KRAI, S. HARKINS, J. DEBAD, J. WOHLSTADTER;
Meso Scale Diagnostics, Rockville, MD

Abstract: Neurological disorders affect approximately 15% of the world's population, with neurodegenerative conditions representing the most significant burden. Complex interactions between lymphocytes and resident cells in the central nervous system (CNS) can determine the progression and severity of symptoms. Excessive or dysregulated expression of cytokines released by these immune cells may lead to neuronal cell death and synaptic plasticity dysfunction. Subsequent impacts on learning, memory, and other cognitive deficits pose significant challenges to healthcare systems, highlighting the need for better tools to understand their pathogenesis. However, sample volumes are limited, and there is a dearth of assay methods that provide the required sensitivity for detecting analytes during the pre-symptomatic phase. Moreover, the need for higher sensitivity assays to correlate animal models to human disease remains unmet.

A one-incubation assay protocol using the MESO SCALE DISCOVERY electrochemiluminescent (ECL) platform provides a faster, simpler workflow for detecting biomarkers of interest. Using this platform, we examined circulating levels of GM-CSF, Granzyme B, IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, IL-17A, Perforin, and TNF-α in samples with Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, as well as expression profiles in human glioblastoma cells and rodent samples. The addition of a proprietary signal enhancement process complemented the platform, resulting in an average three-fold increase in the number of samples within the detection limits across all analytes. The increase in sensitivity provides the ability for higher dilution, thus preserving valuable samples. This holds particular importance for rodent studies due to the limited quantity of material that researchers can extract from a single animal. Initial testing showed distinct concentrations of markers associated with individual diseases, suggesting unique phenotypic profiles for each condition.

Our data successfully show a reliable multiplex solution with ECL detection and accelerated time-to-result on the MSD platform, offering increased sensitivity and throughput without the need for large sample volumes. Identifying the immune landscape and onset of cell death pathways using such tools can improve our understanding of disease progression.

Disclosures: G. Khayrullina: A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **M. Abdel-Ghani:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **T. Parira:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **D. Tselenchuk:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **T. Barvir:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **M. Samaroo:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **J. Slezak:** A. Employment/Salary (full or part-time):; Meso Scale

Diagnostics, LLC. **C. Shaw:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **G. Galen:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **M. Morelli:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **P. Krai:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **S. Harkins:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **J. Debad:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC. **J. Wohlstadter:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics, LLC..

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.04/X8

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: ECL-based immunoassay for markers of neurodegeneration in rodent models

Authors: C. DEMOS[1], N. PADMANABHAN[1], M. STENGELIN[1], ***S. B. HARKINS**[2], A. MATHEW[1], G. SIGAL[1], J. N. WOHLSTADTER[1];
[1]Meso Scale Discovery, Rockville, MD; [2]R&D, Meso Scale Diagnostics, Rockville, MD

Abstract: Animal models of neurodegenerative disease (ND) are a critical component of mechanistic, diagnostic, and therapeutic research. Monitoring biomarkers is key to recapitulating human disease pathology in preclinical research. Large quantities of tissue samples cannot be collected from small animals such as mice and rats, thus the ability to multiplex measurements with a small sample volume is advantageous. Here, we demonstrate both that a multiplexed assay for human glial fibrillary acidic protein (GFAP), neurofilament light (Nf-L), and total Tau (tTau) cross-reacts with the mouse and rat analogues of these biomarkers and that the assays are suitable for measuring mouse and rat samples ranging from healthy controls to various models of ND. The MSD S-PLEX Neurology Panel 1 kit uses ultrasensitive electrochemiluminescence immunoassay technology to measure GFAP, Nf-L, and tTau simultaneously in a 96-well plate format with standard liquid handling techniques. Serum and plasma samples from healthy mice and rats (n=5 each) were tested up to a 5-fold dilution, and brain lysates were tested from 32-100,000 pg/mL of total brain protein. Common mouse models of Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease were tested to determine detectability of GFAP, Nf-L, and tTau. The quantifiable range was 0.24-1,420 pg/mL for GFAP, 0.98-5,270 pg/mL for Nf-L and 0.025-782 pg/mL for tTau based on calibration with the human proteins. GFAP, Nf-L, and tTau were detectable in all serum and plasma samples after a 2-fold dilution, requiring only 12.5µL of neat sample per determination (for all three markers). All analytes were detectable in mouse and rat brain lysates tested at 160 pg/mL of total brain protein, and GFAP and Nf-L were further detectable in lysates tested at 32 pg/mL. Linear dilution was observed in

serum and mouse samples for all analytes in mice and for GFAP and tTau in rats, with average recoveries of 92-118% for 5-fold dilutions. In brain lysates, all analytes diluted linearly from 100,000 to 32 pg/mL of total brain protein, with average recovery of 88% for GFAP, 86% for Nf-L, and 92% for tTau. We measured biomarkers in serum, plasma, cerebrospinal fluid, brain, and spinal cord from mouse ND models, including tauopathy models expressing human tau transgenes. GFAP, Nf-L, and tTau were found in varying amounts dependent on the mouse line. Mouse and rat cross-reactivity with the ultrasensitive multiplexed human panel is sufficient to measure GFAP, Nf-L, and tTau in healthy mouse and rat samples, as well as in samples from several popular ND mouse models. This provides an advanced tool for preclinical research in the ever-expanding menu of rodent ND models.

Disclosures: C. Demos: A. Employment/Salary (full or part-time):; Meso Scale Diagnostics. **N. Padmanabhan:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics. **M. Stengelin:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics. **S.B. Harkins:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics. **A. Mathew:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics. **G. Sigal:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics. **J.N. Wohlstadter:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.05/X9

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Off-target gaze information potentially reflects subjects' cognitive functions and enhances the scoring accuracy of an eye-tracking-based cognitive assessment.

Authors: *S. TESHIROGI[1,3], A. OYAMA[3], T. NAKAJIMA[1,3], S. YAMAMOTO[1,3], Y. ITO[3,2], R. MORISHITA[2], S. TAKEDA[3,2];
[1]Geriatric and Gen. Med., [2]Clin. Gene Therapy, Osaka Univ., Suita, Osaka, Japan; [3]Osaka Psychiatric Res. Ctr., Osaka Psychiatric Med. Ctr., Hirakata, Osaka, Japan

Abstract: Background Effective preventive interventions against dementia require screening methods that can detect cognitive decline at an early stage. We previously developed an eye-tracking-based cognitive assessment (ETCA) as a new modality for cognitive testing and reported its clinical validity and utility in dementia screening (Oyama et al., *Scientific Reports,* 2019). By combining eye-tracking technology with task movies, the ETCA quantitatively evaluates a subject's cognitive functions in about three minutes. We have reported that the percentage of the subject's viewing time spent in the correct region of interest (ROI) correlates

with scores of neuropsychological tests such as the Mini-Mental State Examination (MMSE). However, the extent of a subject's cognitive impairment may also be reflected in eye movement information outside the correct ROIs (i.e., irrelevant ROIs). Utilizing this information could potentially improve the scoring accuracy of the ETCA. **Objective** This study explored the significance of the viewing time spent in the irrelevant ROIs in the ETCA and its impact on cognitive function evaluation. We also attempted to improve the scoring accuracy of the ETCA using this feature. **Design** The study cohort included 56 individuals with a mean age of 78.4 (SD 7.6) who were recruited from the memory clinic at Osaka University Hospital. All participants completed both the ETCA and MMSE on the same day. The ETCA's task movie used in this study included attention, memory, and visuospatial function tasks. The percentages of viewing time spent in correct and irrelevant ROIs were measured based on gaze plot data. **Results** The mean percentage of viewing time in the correct ROIs positively correlated with the MMSE scores ($r = 0.739$, $p < 0.001$), replicating the results of our previous study. The mean percentage of viewing time in the irrelevant ROIs showed a significant negative correlation with the MMSE scores ($r = -0.676$, $p < 0.001$). Incorporating the viewing time on the irrelevant ROIs into the scoring increased the correlation coefficient with MMSE ($r = 0.776$). **Conclusion** Gaze information on the irrelevant ROIs can reflect a subject's cognitive function and potentially enhance the scoring accuracy of the ETCA.

Disclosures: S. Teshirogi: None. **A. Oyama:** None. **T. Nakajima:** None. **S. Yamamoto:** None. **Y. Ito:** None. **R. Morishita:** None. **S. Takeda:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.06/X10

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: CPRIT Scholar in Cancer Research RR180012
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NIH U24LM013755
NIH U01CA274576

NIH U54HG012510
NSF 2124789

Title: Advancing alzheimer's disease assessment with fine-tuned large language models

Authors: *Y.-C. HSU, X. JIANG, K. ZHANG;
McWilliams Sch. of Biomed. Informatics, UT Hlth. Houston, Houston, TX

Abstract: Early and accurate assessment of cognitive decline in Alzheimer's Disease (AD) patients is crucial for effective treatment planning and disease management. Assessing cognitive impairment traditionally relies on clinical evaluations and neuropsychological tests, although these methods can be subject to errors and subjectivity. Recent advances in artificial intelligence show promise in understanding clinical data comprehensively to enhance disease status detection in cognitive disorders. This study explores the feasibility of using large language models (LLMs), which are capable of handling long text inputs and enable the extraction of comprehensive knowledge from these tests. By fine-tuning with prompting techniques, we aim to improve the classification performance and explainability of cognitive status associated with Alzheimer's disease. We utilized data from 1,699 subjects who underwent cognitive score tests covering various aspects, including memory, language, orientation, and daily functioning, from the Alzheimer's Disease Neuroimaging Initiative (ADNI). We fine-tuned the pre-trained LLMs with prompts that contained enriched information from cognitive scores using supervised fine-tuning (SFT), aiming to refine the LLMs' ability to categorize cognitive states. We evaluated the fine-tuned LLMs' performance on classifying the subject's cognitive status with different prompting strategies, including basic question-answer (QA), chain-of-thoughts (COT), and tree-of-thoughts (TOT). Our preliminary results indicate that fine-tuned LLMs exhibit superior classification performance compared to traditional machine learning and deep learning classifiers. Our fine-tuned Mistral-7B model achieved 83.82% classification accuracy, and fine-tuned GPT-3.5 Turbo achieved 82.06%. XGBoost and TabPFN achieved 81.76% and 79.11%, respectively. Fine-tuned LLMs could further generate detailed reasoning and explainability with advanced prompting techniques. In conclusion, we showed that fine-tuned LLMs with strategic prompting could leverage information across cognitive score tests to improve the classification performance of disease status and model transparency. Our future work will expand the dataset, refine prompts based on clinical feedback, and further explore the alignment techniques to improve our model performance and explainability.

Disclosures: Y. Hsu: None. **X. Jiang:** None. **K. Zhang:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.07/X11

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Optimization of intrathecal injection procedure for improved delivery of ASO- or AAV-based therapeutics in a preclinical mouse model

Authors: *T. S. CHOWDHURY[1], Y. LUO[2], J. DION[2], S. HANA[2], M. ZHANG[2], J.-C. LO[3]; [1]Neuromuscular and Muscle Dis., Biogen, Cambridge, MA; [3]Neuromuscular and Muscle Dis., [2]Biogen Inc., Cambridge, MA

Abstract: Administration of drugs into the cerebrospinal fluid (CSF) of rodents is challenging. Intrathecal (IT) injection of drugs in the central nervous system (CNS) for gene therapy and antisense therapy has become a preferred method of drug administration due to its noninvasiveness, good target engagement, and specificity. For IT delivery of drugs in rats, it is common to perform intrathecal catheterization that allows sustained administration of drugs. Mice, due to their relatively smaller intraspinal space, are more prone to adverse effects of catheterization such as injury, paralysis, and infection than rats. Here, we have used acute needle puncture in mice to introduce drugs intrathecally. However, this method of delivery requires technical finesse for success and reproducibility in small animals. Using IT injection in a mouse model of Superoxide Dismutase 1 (SOD1) ALS, we demonstrate successful delivery of adeno-associated viruses (AAV) or antisense oligonucleotides (ASO) to target SOD1 gene and alleviate disease phenotype. As this method is analogous to techniques used for drug delivery into the CSF in clinical studies, the technique can be used for single or repeated injections to gain confidence in preclinical studies requiring direct administration of therapeutics into the intrathecal space.

Disclosures: T.S. Chowdhury: A. Employment/Salary (full or part-time):; Biogen Inc. **Y. Luo:** A. Employment/Salary (full or part-time):; Biogen Inc. **J. Dion:** A. Employment/Salary (full or part-time):; Biogen Inc. **S. Hana:** A. Employment/Salary (full or part-time):; Biogen Inc. **M. zhang:** A. Employment/Salary (full or part-time):; Biogen Inc. **J. Lo:** A. Employment/Salary (full or part-time):; Biogen Inc..

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.08/X12

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Development of coumarin-eugenol hybrids as a new class of dual targeting multifunctional anti-Alzheimer’s agents acting via simultaneous amyloid beta aggregation and selective acetylcholinesterase inhibition

Authors: *A. SINGH, P. BEDI;
Dept. of Pharmaceut. Sci., Guru Nanak Dev Univ., Amritsar, Amritsar, India

Abstract: Alzheimer's disease is a neurodegenerative disorder and the most common cause of dementia among elderly people resulting in impaired memory, thinking, and behaviour of the patients. Elevated acetylcholinesterase (AChE) and aggregation of insoluble amyloid beta oligomers ($A\beta_{1-42}$) to form senile plaques as well as reactive oxygen species are primary targets of Alzheimer's disease. Simultaneous inhibition of these targets can act as the optimum strategy for the effective management and hindering the progression of this disease. Previously developed dual-acting AChE and $A\beta_{1-42}$ aggregation inhibitors by our group (*Bioorg. Med. Chem. Lett.* 2020, 30 (20), 127477) emerged as highly selective and competitive AChE inhibitors (IC_{50} = 0.059 ± 0.006 µM) as over butyrylcholinesterase (BuChE: IC_{50} > 10 µM) with metal chelating properties against the metals (Fe, Cu and Zn ions) that are known to promote $A\beta_{1-42}$ aggregation in the brain of Alzheimer's disease patients but exhibited lower $A\beta_{1-42}$ aggregation inhibition (34.26 ± 1.97 % at 50 µM) capabilities. Taking the lead from previous outcomes and utilizing combined molecular hybridization and fragment-based drug design (FBDD) approaches, a new series of hybrid molecules was designed inspired by the natural products, coumarin and eugenol. Among the developed hybrid molecules, hybrid molecule AS15 showed selective AChE inhibition (IC_{50} = 0.047 ± 0.004 µM) over butyrylcholinesterase (BuChE: $IC_{50} \geq 10$ µM) with desired $A\beta_{1-42}$ aggregation inhibition (72.21 ± 2.11 % at 50 µM). In addition, AS15 showed protective effects against DNA damage by hydroxyl radicals originating from hydrogen peroxide. Molecular docking and molecular dynamic simulation studies further confirmed the favourable interactions and binding stability of AS15 with both AChE and Aβ1-42 monomers. AS15 possesses an LD_{50} value of 50 mg/kg with mild salivation at the dose level of 300 mg/kg (OECD 423 guidelines) and showed significant improvements in memory and learning behaviour in scopolamine-induced cognition impairment mice-based animal models (Y-maze test and Morris water maze test) for behavioural analysis. Overall outcomes represented AS15 as a potential preclinical multifunctional candidate for the efficient management of Alzheimer's disease and also act as a premium hit lead for further development of potent and safer multifunctional anti-Alzheimer's agents. However, there is still room for further improvements in the chemical structure of AS15 for further enhancement in the $A\beta_{1-42}$ aggregation inhibition along with keeping the selectivity of AChE over BuChE.

Disclosures: A. Singh: None. **P. Bedi:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.09/X13

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: A unique platform to demonstrate TREM2/DAP12 signaling cascade activation for neuroinflammatory disease

Authors: A. SHEEHAN, A. BROWN, S. FALCON, ***O. J. KELADA**;
Revvity Inc., Hopkinton, MA

Abstract: Objective and rationale: The triggering receptor expressed on myeloid cells-2 (TREM2), is one of the most important targets in the neuroinflammatory disease space. TREM2 signals through its association with co-receptor DNAX-activating protein (DAP12) to trigger intracellular signal transduction. Mutations in TREM2 and DAP12 have been associated with Nasu-Hakola disease which is characterized by dementia. Additionally, different variants of TREM2 have been linked to an increased risk for late-onset Alzheimer's disease. Stimulation of TREM2 results in phosphorylation of tyrosine residues in the ITAM motif of DAP12 which recruits the spleen tyrosine kinase (SYK). Therefore, the study of TREM2/DAP12/SYK cascade activation is of great interest for therapeutic discovery programs and the study of neurodegenerative diseases.
Methods: THP1 cells were prepared at 600K/mL and treated with 35 ng/mL TGFβ overnight in RPMI /10% FBS. Cells were washed with HBSS and seeded at 2x10^6 cells/mL in 96 well plates in HBSS/ 0.1% BSA. To measure TREM2/DAP12 complex and TREM2 & DAP12 aggregation, control lysates were prepared from THP1 cells and treated with 5μM TREM2 activator for 30 minutes and lysed with 5X SureFire Ultra Lysis Buffer. Lysate was serially diluted and assayed for TREM2/DAP12 Complex, TREM2 aggregate and DAP12 aggregate. For TREM2 activator dose response studies, cells were treated with various concentrations of TREM2 activator for 10 minutes and lysed in 5X SureFire Ultra Lysis Buffer. To measure DAP12 and SYK phosphorylation, cells were treated (or untreated) with 5 μg/mL of a TREM2 Antibody or 5μM TREM2 activator for 5 mins.
Results: TREM2/DAP12 complex formation, DAP12 (Y91) & SYK (Y525/526) phosphorylation and TREM2 and DAP12 aggregation all occur in a dose-dependent manner while DAP12 and TREM2 Total levels remain unchanged upon TREM2 activator treatment. Results from THP1 lysate dilutions show robust signal:noise windows for TREM/DAP12 complex, TREM2 aggregate, DAP12 aggregate to be 93, 85 and 55 for 40,000 cells per datapoint, respectively. For 5,000 cells per datapoint the results were 27, 22 and 14, respectively.
Conclusion: This study highlights the ability to provide enhanced signal windows for kinase programs and specifically measure TREM2 activation, with assays developed for TREM2/DAP12 complex, phosphorylated DAP12 (Y91), phosphorylated SYK (Y525/526), TREM2 aggregate & DAP12 aggregate. Herein we have developed a potent, rapid, and novel platform for the study and development of TREM2-based therapeutic strategies.

Disclosures: A. Sheehan: A. Employment/Salary (full or part-time):; Abcam Inc. **A. Brown:** A. Employment/Salary (full or part-time):; Abcam Inc. **S. Falcon:** A. Employment/Salary (full or part-time):; Abcam Inc. **O.J. Kelada:** A. Employment/Salary (full or part-time):; Revvity Inc.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.10/X14

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Machine learning-informed digital measures improve the sensitivity and translational relevance of in vivo neurodegenerative disease models

Authors: P. B. MARTIN[1], ***N. BRATCHER-PETERSEN**[2], B. BERRIDGE[3], S. CICIOTTE[4], A. E. CLIPPERTON-ALLEN[5], M. ELLIS[4], T. ROBERTSON[7], M. RUIDIAZ[7], M. C. SAUL[6], C. M. LUTZ[4];
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Abstract: Neurodegenerative diseases pose significant global health challenges, affecting millions of individuals worldwide. Amyotrophic Lateral Sclerosis (ALS) is a progressive disorder impacting motor neurons in the spinal cord and brain, leading to muscle weakness, and loss of motor control. The average life expectancy following a diagnosis is only 2-5 years. These statistics underscore the urgency and importance of studying ALS to understand the mechanisms of neurodegeneration and develop effective therapeutic strategies.Many of the current in vivo readouts used to assess neurodegeneration are subjective, time-consuming, and labor intensive. Rapid advances in sensor technologies and computational capabilities provide a unique opportunity to enhance the value of animal studies. Complementing standard measures with continuous measures of behavior and physiology would provide a more dynamic, biologically-, and clinically relevant characterization of disease progression and therapeutic effects. Here we used the B6.Cg SOD1-G93A model of ALS and the B6.Cg-*Pvalb*$^{tm1(cre)Arbr}$ *Fxn*^{em2Lutzy} *Fxn*$^{em2.1Lutzy}$/J model of Friedreich's Ataxia to assess machine-learning informed digital measures compared to standard measures of disease progression including body weight, rotarod, neuroscore, compound muscle action potential (CMAP), and neurofilament light chain (NfL) in home cages outfitted with computer vision cameras with infrared detection capabilities allowing continuous monitoring of socially housed individual animals throughout both light and dark cycles over 28 weeks. In both models, disease phenotype was identified earlier using digital measures compared to most standard endpoints. Additional insights were observed when evaluating sleep behavior.This innovative approach, demonstrated through objective and quantitative longitudinal assessment of digital measures of animal behavior, could provide valuable insights into neurodegenerative disease and contribute to the development of effective interventions. Our work has the potential to significantly advance our understanding of neurodegenerative disease and pave the way for novel therapeutic strategies, thereby addressing a critical need in global health.

Disclosures: P.B. Martin: None. **N. Bratcher-Petersen:** None. **B. Berridge:** None. **S. Ciciotte:** None. **A.E. Clipperton-Allen:** None. **M. Ellis:** None. **T. Robertson:** None. **M. Ruidiaz:** None. **M.C. Saul:** None. **C.M. Lutz:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.11/X15

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: A pilot study of m-space mapping for classification and rating of movements in an ice dance fatigue model of movement degeneration

Authors: *A. YING[1], S. YING[2], Y. TARUI[3], A. JOYCE[4], A. JOYCE[4], V. YE[5], C. JOYCE[4], M. BRAMANTE[6], S. H. YING[7];
[1]DeeperEdge, Cambridge, MA; [2]Res. Intern, DeeperEdge, Cambridge, MA; [3]DeeperEdge, Newton, MA; [4]DeeperEdge, Mansfield, MA; [5]DeeperEdge, Westwood, MA; [6]Cambridge Col., Boston, MA; [7]Neurol., MEEI, Cambridge, MA

Abstract: Background: Currently, there is a need for a method that can accurately measure and rate movements for clinical management and clinical trials. This pilot study proposes a method using ice dance movements as a model system, with the aim of developing a metric for movement degeneration. This could potentially provide a more objective and quantifiable way to assess and track the progression of neurodegenerative movement disorders. This pilot study tests two hypotheses: 1) when movements are mapped to dimensionally reduced “movement space” (m-space), distinct steps cluster together, and 2) the location of a given movement in m-space correlates with movement quality.
Methods: Individual dance steps, using 17 Noitom Perception Neuron inertial measurement unit (IMU) sensors, were represented by the first three components from principal component analysis (PCA). Hotelling’s T-squared statistic was used to compare individual steps to each other and compare different fatigue groupings. Student’s T was used to compare early vs late expert ratings of the IJS Skating Skills component score (SSCS). Pearson’s R was used to compare SSCS to a step’s location in step-specific PCA space.
Results: A single skater performed 4 sets of 10 patterns of the Dutch Waltz. Using Hotelling's T-squared statistic, after correction for multiple comparisons, then rightward/leftward/straight step groupings were significantly different, and individual steps were significantly different from each other ($p<0.001$), with the exception of duplicated steps. PCA coordinates and SSCS for early/late sets for each step were significantly different ($p<0.001$). SSCS’s correlated with PCA coordinates according to the appropriate PC dimension ($R>0.7$, $p<0.05$).
Conclusion: In this proof-of-concept pilot study, mapping of step data to m-space separated individual steps, and location in m-space correlated with movement quality, suggesting that m-space mapping merits further investigation as a movement degeneration metric.

Disclosures: A. Ying: None. **S. Ying:** None. **Y. Tarui:** None. **A. Joyce:** None. **A. Joyce:** None. **V. Ye:** None. **C. Joyce:** None. **M. Bramante:** None. **S.H. Ying:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.12/X16

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Developing new biomarkers using temporal profiles of non-evolved home cage behaviours

Authors: R. SILLITO[1], R. S. BAINS[2], S. WELLS[2], ***D. ARMSTRONG**[3];
[1]Actual Analytics Ltd, Edinburgh, United Kingdom; [2]Mary Lyon Ctr. at MRC Harwell, Oxfordshire, Harwell, United Kingdom; [3]Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: Home Cage Monitoring systems provide a rich longitudinal behavioral profile of laboratory animals (rat and mice) in small social groups. The approach is naturally focused on measuring non-evoked behaviors (although there are some exceptions) and these are typically captured day and night for prolonged periods, in the order of a few days but sometimes up to several weeks. The exact suite of measurements obtains varies but typically include behaviors such as social interactions, horizontal (locomotion) and vertical (climbing/rearing) activity, sleep, social interactions, drinking, eating and temperature. Different genetic strains show quite characteristic temporal profiles that can vary with age, with sex and notably, with time of day. We constructed temporal profiles of mouse and rat strains commonly used in laboratory research which capture the normal variance in behaviors at specific times of the day. From these profiles we are discriminate and quantify deviations from these profiles in individual animals. We show that the sensitivity is particularly high when we select specific times of day; with the later few (2-3) hours of the dark phase often particularly sensitive in picking up deviations from the controls. Using previously published datasets we are able to define new behavioral biomarkers that can be used to identify early phenotypes in a range of situations including genetic models for neurodegeneration where we see changes in a range of locomotor measurements, both horizontal and vertical at various times in the dark phase (for example, in a mouse model of Huntingdon's Disease). We have also shown measurable effects during safety pharmacology assessment in rats. These deviations from normal behaviors can be used to identify outliers within control populations where we see naturally hyperactive and hypoactive individuals. Ongoing research suggests a combination of these behaviors can be used to assemble a composite measure for general welfare. Although sample sizes are very small, there are indications that a combination of suppressed locomotion and temperature profiles is highly predictive of subsequent welfare interventions.

Disclosures: R. Sillito: A. Employment/Salary (full or part-time):; Actual Analytics Ltd, Edinburgh. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Actual Analytics Ltd, Edinburgh. **R.S. Bains:** None. **S. Wells:** None. **D. Armstrong:** A. Employment/Salary (full or part-time):; Actual Analytics Ltd, Edinburgh, UK. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Actual Analytics Ltd, Edinburgh, UK.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.13/X17

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Multipurpose neuromodulation treatment of cognitive symptoms in patients with Myalgic encephalomyelitis/chronic fatigue syndrome.

Authors: *M. FIOLETOVA[1], I. ROZENFELD[2], P. DE FINA[3];
[1]Nova Southeastern Univ. Col. of Osteo. Med., Davie, FL; [2]Inst. for Neuro-Immune Med., Nova Southeastern Univ., Davie, FL; [3]Intl. Inst. Brain Enhancement, Delray Beach, FL

Abstract: Myalgic encephalomyelitis/chronic fatigue syndrome (ME-CFS) is a chronic, disabling illness that is poorly understood without any existing FDA-approved treatment, and therefore often undiagnosed in patients. Our study focuses on potential treatments for ME-CFS. In this study, we observed a patient, a 61-year-old man, who had been diagnosed with post-viral ME-CFS with severe complaints regarding his cognitive abilities. His relevant psycho-social history includes past substance abuse, mild traumatic brain injury, and the Alzheimer's disease gene present in the family tree. Initial neurological evaluation revealed below-average visual memory in terms of initial learning trials, delayed recall, and below-average performance on the executive functioning test involving abstract reasoning and feedback utilization. The default mode network showed 9% hyper-connectivity and 8% hypo-connectivity in different zones which can also contribute to impaired cognition and memory. To improve his symptoms, the patient underwent a multipurpose neuromodulation procedure such as quantitative electroencephalography (qEEG)-guided neurofeedback and transcranial direct current stimulation to improve memory and reduce anxiety. In addition, hyperbaric oxygen therapy (HBOT) is used to reduce neuro-inflammatory processes and help regenerate neuronal activity. Neuro photo-biomodulation (N-PBM) and tri-modal cranial-electrical stimulation (T-CES) help increase alpha signals and improve mitochondrial functions for energy and better sleep patterns. The effects of this treatment were measured by a series of cognitive assessments, which came back with impressive results. According to the Wisconsin Card Scoring Test, after only one

month of treatment, he was in the 95th percentile conceptual level of responses compared to 70% in the past, and 90th percentile of total errors compared to 73% in the past. According to the Integrated Visual and Auditory 2 detailed report, the patient has remarkably improved auditory, visual, and conceptual responses since the first treatment. In conclusion, our findings demonstrate potential treatment modalities such as HBOT, N-PBM, and T-CES for patients with ME-CFS who demonstrate improvement in auditory, visual, and conceptual responses. These results suggest a promising future approach to identifying ME-CFS and treating it to improve cognitive functions in similar patient populations.

Disclosures: M. Fioletova: None. **I. Rozenfeld:** None. **P. De Fina:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.14/X18

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Development of an in vitro assessment platform for drug-induced neuron degeneration based on morphological deep learning using cultured neurons in a microphysiological system

Authors: X. HAN[1], *N. MATSUDA[2], M. YAMANAKA[3], I. SUZUKI[4];
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Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is a major common adverse event associated with neurological abnormalities, while an accurate assessment is essential to improve knowledge about CIPN incidence. Microphysiological system (MPS) is an in vitro culture technology that reproduces the physiological microenvironment and functionality of humans, and is expected to be applied for evaluating drug efficiency/toxicity. In this study, an in vitro MPS device was developed for compartmentalized co-culture of different types of neurons, and drug-induced neurotoxicity was measured by morphological analysis. This MPS device could separate the cell body and neurites, so that elongated neurites morphology can be analyzed alone. COP (Cyclo olefin polymer), which has excellent observability and low drug adsorption, is used as the resin material, and the bottom surface is created thin and flat enough for a clear view by microscope. Next, human iPSC derived sensory neurons were cultured in the device coated with Poly-L-lysine and Laminin. After culturing with a specific medium containing insulin, neurites grew sufficiently to occupy almost the whole microfluidic channel area, and the axon elongated unidirectional along the horizontal direction. Successful culture of sensory neurons with separating neurites growth were achieved in the MPS for longer than 8 weeks.

After administration of several typical anti-cancer drugs, peripheral neurotoxicity was predicted by a deep learning AI trained with morphological image datasets on both soma and axonal area. After training, AIs were capable of accurately detecting the peripheral neurotoxicity of compounds at low concentrations and within 24 h post exposure. By integrating the results from both soma and axonal AIs, it became feasible to predict the MoA of compounds that induce CIPN.Furthermore, drug-induced neurotoxicity in CNS neurons was also measured using cultured human iPSC derived cortical neuron on the current MPS. After administration of different types of Amyloid β, the toxic effects were evaluated by training two AI models on axonal and PSD-95 images. And the combined results indicated that Amyloid β1-42 and 1-40, but not 1-28, induced neuron degeneration, which is close to clinical reports.Taken together, it suggests that the present MPS combined with morphological deep learning is a useful platform for in vitro neurotoxicity assessment.

Disclosures: X. Han: None. **N. Matsuda:** None. **M. Yamanaka:** None. **I. Suzuki:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.15/X20

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: RF1 AG071805
U01 AG032969
R01 CA172546
P01 CA186866
R01 AG072599
R56 AG061869
R01 AG067598
R01 AG074004
R56 AG072599
P30 CA08748
S.S. would like to acknowledge funding support from BrightFocus Foundation (Award ID: A2022020F)

Title: Illuminating Epichaperomes at the Single-Cell Level: Tools and Methods for Studying Neurodegenerative Disorders

Authors: *S. BAY[1], C. DIGWAL[1], A. RODILLA[1], S. SHARMA[1], A. STANISAVLJEVIC[3], A. RODINA[1], A. ATTARAN[6], T. ROYCHOWDHURY[1], K. PARIKH[1], E. TOTH[1], P. PANCHAL[1], E. ROSIEK[1], C. PASALA[1], O. ARANCIO[7], P. E. FRASER[8], M. J. ALLDRED[4], M. A.

PRADO[9], S. D. GINSBERG[5], G. CHIOSIS[2];
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Abstract: Stressors associated with disease trigger the remodeling of protein-protein interaction networks, converting chaperones into epichaperomes. These structures are long-lived assemblies and disease-associated pathologic scaffolds comprising tightly bound chaperones, co-chaperones, and other factors. Unlike chaperones, which are ubiquitous proteins functioning through dynamic one-on-one complexes, epichaperomes act as pathologic scaffolds, leading to improper interactions and organization of thousands of proteins inside cells. This negatively impacts neuronal function, including synaptic plasticity, cell-to-cell communication, protein translation, cell cycle re-entry, axon guidance, metabolic processes, and inflammation, ultimately resulting in network-wide dysfunction and cognitive decline. Notably, the ability of epichaperome disruptors to reverse disease-related phenotypes highlights their critical role in regulating functions underlying disease pathology, suggesting a novel therapeutic approach. To deepen our understanding of epichaperomes in neurodegenerative disorders and gain important mechanistic insights into their context-dependent composition, structure, and function, we developed chemical probes and methods suitable for use in confocal and single-molecule super-resolution imaging approaches. As a demonstration of the utility of these probes in investigating biology, we characterized an epichaperome click probe along with a relevant negative control. Our study includes high-resolution imaging of epichaperomes at the single-cell level in a transgenic mouse model. We demonstrate the successful detection of cell-specific vulnerability to epichaperome formation using the click probe, highlighting its potential as a valuable tool for dissecting the intricate cellular responses underlying neurodegenerative diseases. The probe shows promise for understanding epichaperome formation, composition, and localization in different biological contexts, representing a significant step towards realizing its potential for diagnosing and treating neurodegenerative disorders. Importantly, the versatility of this click probe allows for multiplexing with antibodies against specific disease markers, offering the opportunity to investigate intricate mechanistic details.

Disclosures: S. Bay: None. **C. Digwal:** None. **A. Rodilla:** None. **S. Sharma:** None. **A. Stanisavljevic:** None. **A. Rodina:** None. **A. Attaran:** None. **T. Roychowdhury:** None. **K. Parikh:** None. **E. Toth:** None. **P. Panchal:** None. **E. Rosiek:** None. **C. Pasala:** None. **O. Arancio:** None. **P.E. Fraser:** None. **M.J. Alldred:** None. **M.A. Prado:** None. **S.D. Ginsberg:** None. **G. Chiosis:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.16/X21

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Using microfluidics for culturing ex-vivo brain tissue slices and investigating Alzheimer's disease-associated proteins

Authors: *V. NORMAN[1], R. A. PROSSER[2];
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Abstract: Microfluidic devices are useful tools for investigating cellular physiology but adapting them to investigate brain tissue slices is a challenge, given the high metabolic demands of neuronal tissue. Nevertheless, addressing this challenge will significantly enhance our ability to investigate neuronal function outside of the animal under conditions where local brain structure and cell networking remain intact. A microfluidic device that allows multiple time-stamped assessments of neuronal responses in acute brain slices would be especially useful to investigate complex issues such as the neurochemical changes associated with neurodegenerative diseases. Together with our collaborators, we are developing a novel microfluidic device to maintain healthy *ex vivo* brain tissue slices for multiple hours while assessing responsiveness through real-time fluorescent imaging, quantifying time-stamped chemical releasates, and monitoring overall health with end-of-experiment histological assessments. We are specifically interested in studying Alzheimer's disease, a progressive and irreversible brain disorder that is currently the leading cause of dementia. The accumulation of amyloid-beta (AB) and tau peptides is thought to create a toxic environment that leads to the hallmark memory loss associated with Alzheimer's disease, but the initial effects of these peptides on brain functioning are not known. Currently, we are optimizing our microfluidic device utility by comparing different perfusion pause times, fluorescent imaging parameters, and intracellular calcium indicators. Next, to investigate the initial effects of AB and tau on neuronal function, we are maintaining brain slices containing the entorhinal cortex in our microfluidic device and exposing them to different peptide combinations. Changes in intracellular calcium in response to peptide exposure are being assessed using the calcium indicator, Calbryte 520 AM. Releasates from the slices are being collected for offline analyses. Changes in tissue health are being assessed through histological analysis of propidium iodide uptake. Should we determine that the peptides are significantly increasing cell death and damage, we will expand our histological analyses to determine what cell types are most affected by these Alzheimer's disease-associated peptides. The results of these experiments will not only demonstrate the usefulness of our novel microfluidic device but will also provide critical new insights regarding whether AB and/or tau accumulation may be an early event in Alzheimer's disease progression.

Disclosures: V. Norman: None. **R.A. Prosser:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.17/X22

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: CARNOT

Title: Monitoring Parkinson's disease based on intracerebral biosensor implants

Authors: *M. BRUN-COSME-BRUNY, P. MAILLEY, I. MUÑOZ VELASCO, Y. R. THOMAS, C. PERNEL, P. BLEUET, N. TORRES;
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Abstract: Sensors play an essential role in healthcare by enabling the detection of pathological markers. This assists in the diagnosis, treatment, and monitoring of diseases. However, a gap exists when considering sensors integrated into brain implantable devices. Few of them are capable of sensing changes in their environment that could lead to adjustments in the delivered therapy. This could be crucial in established technologies like deep brain stimulation (DBS) and also in emerging technologies, such as photobiomodulation (PBM), used in Parkinson's disease (PD) to slow down neural degeneration. PBM has shown efficacy to slow down neurodegeneration in both cellular and animal models of PD. It involves optically stimulating specific regions of the brain to act on mitochondrial activity. Optical stimulation comes from a tunable light source transmitted through implanted optical fibers. Years of research led to the initiation of a world-first clinical trial at Clinatec, with 5 patients implanted at that time [https://classic.clinicaltrials.gov/ct2/show/NCT04261569]. Currently, however, to adjust the stimulation parameters, the protocol requires regular burdensome monitoring of the patient, focusing on the quantification of dopamine concentration by PET tomography, which necessarily involves irradiation. Hence the need to develop a cerebral detection system capable of monitoring PD progression. In this context, we considered two parallel exploratory detection approaches. The first one is to use backscattered light to derive specific PD biomarkers. We successfully determined optical reflectance properties for both brain tissue models and anatomical brain specimens using endoscopy. In particular, we were able to obtain a clear optical signature of midbrain, which is promising for long-term monitoring of the disease. A second strategy is the electrochemical monitoring of organic molecules involved in PD. Two anatomical sites, the striatum and the ventricle, are known to have different concentration ranges, micromolar and nanomolar, which led us to consider two voltammetric analysis alternatives. The use of diamond microelectrodes provides nanomolar detection limit in phantom solutions, encouraging further studies with cellular (midbrain organoids) and animal (mini-pig) models. For both options, the development of diagnostic implants based on these biomarkers is crucial, as it would enable to refine the optical parameters of photobiomodulation in real time. Such an approach could also be extended to other brain implants (i.e. DBS) in order to fine-tune the settings of electrical parameters and support pharmacological therapy.

Disclosures: M. Brun-Cosme-Bruny: None. **P. mailley:** None. **I. Muñoz Velasco:** None. **Y.R. Thomas:** None. **C. Pernel:** None. **P. Bleuet:** None. **N. Torres:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.18/X23

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: University of Minnesota
Minnesota State Legislature

Title: Visual detection of misfolded alpha-synuclein and prions via capillary-based quaking-induced conversion assay (Cap-QuIC)

Authors: *H. JEONG[1], P. CHRISTENSON[1,2], H. AHN[1], M. LI[1,2], G. ROWDEN[2,3], R. SHOEMAKER[2,3], P. LARSEN[2,3], H. PARK[1], S.-H. OH[1,2];
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Abstract: Neurodegenerative diseases, such as Parkinson’s disease, pose a significant global health challenge. The prevalence of neurodegenerative diseases is predicted to greatly escalate in the coming decades, making the need for effective diagnostics increasingly urgent. While Real-Time Quaking-Induced Conversion (RT-QuIC) has shown promise in diagnosing neurodegenerative diseases by targeting misfolded proteins, its reliance on costly equipment and complex methods for result visualization and analysis, limits access to diagnostic tests. To address these barriers, we have developed Capillary-based Quaking Induced Conversion (Cap-QuIC), a cost-effective alternative. By using capillary action of post QuIC amplified material , Cap-QuIC eliminates the need for complex and large plate readers and greatly simplifies the analysis of diagnostic test results. We demonstrate the potential of Cap-QuIC as a versatile detection tool for a wide range of misfolded proteins by successfully distinguishing misfolded from healthy proteins associated with synucleinopathies (α-synuclein) and chronic wasting disease (prions). We also showcase Cap-QuIC's ability to accurately diagnose biological tissue samples from wild white-tailed deer afflicted with Chronic Wasting Disease. By introducing capillaries after amplification, Cap-QuIC enables the visual detection of misfolded proteins, offering a more accessible approach to neurodegenerative disease diagnostics. This innovative method promises to overcome the limitations of current diagnostic tools, providing a valuable tool for easily accessible and accurate detection of protein misfolding disorders.

Disclosures: H. Jeong: None. **P. Christenson:** None. **H. Ahn:** None. **M. Li:** None. **G. Rowden:** None. **R. Shoemaker:** None. **P. Larsen:** None. **H. Park:** None. **S. Oh:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.19/X24

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH Grant 90090487

Title: Novel inhibitors of the brain metallopeptidase glutamate carboxypeptidase II (GCPII) identified through innovative high throughput screening approaches

Authors: *R. WISEMAN[1], N. HOXIE[2], T. TSUKAMOTO[3], B. S. SLUSHER[3];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Div. of Preclinical Innovation, Natl. Ctr. for Advancing Translational Sci. (NCATS), Natl. Inst. of Hlth. (NIH), Gaithersburg, MD; [3]Johns Hopkins Drug Discovery, Baltimore, MD

Abstract: Cognitive deficits are a comorbidity in a range of disorders that continue to impact quality of life even when primary symptoms are controlled. There is an unmet need for therapeutics with a novel mechanism to address cognitive symptoms. One enzyme of potential relevance is glutamate carboxypeptidase II (GCPII), a brain metallopeptidase whose activity is significantly upregulated in activated microglia. The primary role of brain GCPII is cleaving the dipeptide N-acetylaspartyl glutamate (NAAG) into NAA and glutamate. Pathologic upregulation of GCPII is thought to exacerbate glutamatergic dysregulation in several neurological disorders by increasing glutamate and decreasing NAAG, the latter of which serves as an agonist at the pro-cognitive metabotropic glutamate receptor 3. For these reasons, GCPII inhibition represents a promising therapeutic strategy. In support of this, several potent, selective active site GCPII inhibitors have shown preclinical efficacy in rodent and primate models but have not been developed clinically due to poor pharmacokinetics and brain penetration. Our current efforts are focused on identifying new inhibitor scaffolds by employing two innovative high-throughput screening (HTS) approaches. 1.) We used a DNA encoded library (DEL), allowing for billions of compounds to be screened in a single assay. The main goal of the DEL screen was to identify allosteric binders binding away from the enzyme active site, in pockets allowing for lower polarity and charge density. In our screening condition, we utilized saturating concentrations of the active site inhibitor 2-PMPA to block active site binding. Off-DNA synthesis of the top 6 non-active site binders was completed, and the compounds were tested in binding and enzymatic activity assays. The compounds did not inhibit enzymatic activity. Work with an affinity selection mass spectrometry assay is ongoing utilizing bead-immobilized recombinant protein to

confirm the DEL hits are true binders. 2.) We used a newly created dual-stream LC/MS substrate cleavage assay to screen several compound libraries. Hits from this assay were resynthesized and tested in an orthogonal enzymatic assay. We identified multiple hits with low micromolar potency against GCPII including cefsulodin (IC50 = 4 μM) and amaranth (IC50 = 0.3 μM). Significant inhibition was also confirmed in GCPII expressing cells. Mode of inhibition studies are ongoing. As a brain penetrant antibiotic, cefsulodin is of particular interest and mouse target engagement studies are ongoing. Our new screening approaches identified novel inhibitors of GCPII that could serve as molecular templates for further chemical optimization.

Disclosures: R. Wiseman: None. **T. Tsukamoto:** None. **B.S. Slusher:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.20/X25

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: ALS Association Grant 19-SI-471
NIH Grant 1RF1AG055053-01A1

Title: Development of a Seed Amplification Assay for the detection of misfolded SOD1 in biological tissue

Authors: *T. ALLISON, F. WANG, V. BANERJEE, C. SOTO;
The Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease characterized by the selective loss of both upper and lower motor neurons. Cytoplasmic inclusions containing misfolded and aggregated mutant superoxide dismutase 1 (SOD1) are a pathological hallmark for a subset of familial ALS cases. The implications of these inclusions in disease progression, as well as the involvement of misfolded wild-type SOD1, is still up for debate. Using recombinant monomeric wild-type (WT) SOD1 protein as the substrate, we have developed a Seed Amplification Assay (SAA) capable of detecting the aggregated SOD1 seeds in highly diluted (up to 10 million fold) brain homogenates from ALS patients with the A4V-SOD1 missense mutation. Furthermore, we were also able to show seeds produced from three different recombinant SOD1 mutant constructs (AV4, G93A, and G85R) are capable of seeding the WT SOD1 substrate, providing convincing evidence that the WT SOD1 may be used as a universal substrate in this assay to detect seeds in patients with a range of SOD1 mutations. In addition to advancing our understanding of the disease biology underlying ALS, our assay can be applied for drug discovery by screening inhibitory compounds against SOD1 aggregation.

Moreover, the SOD1-SAA has the potential to develop into a biochemical test that can detect SOD1 aggregates in biological fluids, such as blood, and facilitate the diagnosis of ALS (SOD1).

Disclosures: T. Allison: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent application filed for the development of this technology. **F. Wang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent application filed for the development of this technology. **V. Banerjee:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent application filed for the development of this technology. **C. Soto:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent application filed for the development of this technology.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.21/X26

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Minnesota Environment Trust Fund, State of Minnesota

Title: Exploring novel immunodiagnostic tools for prion disease

Authors: *R. L. SHOEMAKER[1], S. GRESCH[1], R. SHIKIYA[2], A. J. BLOCK[3], N. LURNDAHL[1], G. ROWDEN[1], S. STONE[1], S. S. LICHTENBERG[1], J. C. BARTZ[2], P. A. LARSEN[1];
[1]Dept. of Biomed. and Vet. Sci., Univ. of Minnesota, Saint Paul, MN; [2]Dept. of Med. Microbiology and Immunol., Creighton Univ., Omaha, NE; [3]Microbiology, Immunology, and Pathology, Colorado State Univ., Fort Collins, CO

Abstract: Prion Diseases, or Transmissible Spongiform Encephalopathies (TSEs), are rapidly progressive and fatal neurodegenerative diseases of mammals. TSEs of global importance include Creutzfeldt-Jakob Disease (CJD) in humans, Chronic Wasting Disease (CWD) in cervids, and Bovine Spongiform Encephalopathy (BSE) in cattle. These diseases occur when the normal cellular prion protein (PrP^{C}) misfolds, producing the infectious isoform PrP^{Sc},which can readily self propagate with no nucleic acid intermediate. PrP^{Sc} aggregates are insoluble self-molecules, which results in a large number of limitations pertaining to the prevention, detection, and treatment of TSEs; including a lack of accessible isoform-specific antibodies. With annual cases of CWD and CJD on the rise, there is an urgent need to develop new diagnostic tools to facilitate new surveillance and management options. Here, we describe a quantitative PCR

method to explore prion protein epitope variability and availability by leveraging proximity ligation technology (PLA). Utilizing a repertoire of ten known monoclonal anti-PrP antibodies, we establish “functional” and “non-functional” antibody probe pairs to assess differences in prion protein conformations. To date, we have identified four unique probe pairs to multiple prion strains, including: hamster PrP^{C} and PrP^{Sc} (Hyper) as well as deer PrP^{C} and PrP^{Sc} (CWD). For example, antibody probe combination 8H4 and 3F4 is specific to hamster PrP^{C}, but non-specific to other sample and strain types. Sample infectivity was confirmed by real-time quaking induced conversion and western blotting, and validated by a PrP knockout control. In light of our results, we posit that exploring PrP^{Sc} strain diversity with available anti-PrP antibodies will lead to the development of ultrasensitive qPCR-PLA Protein Assays for precise detection and quantification of particular PrP^{Sc} strains. This approach addresses current gaps in prion research by providing a versatile tool for studying prion strain diversity and timely intervention strategies that ultimately enhance surveillance efforts.

Disclosures: R.L. Shoemaker: None. **S. Gresch:** None. **R. Shikiya:** None. **A.J. Block:** None. **N. Lurndahl:** None. **G. Rowden:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Priogen Corp.. **S. Stone:** None. **S.S. Lichtenberg:** None. **J.C. Bartz:** None. **P.A. Larsen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Priogen Corp..

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.22/X27

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH/NIMH
NINDS (BRAIN Initiative/Heal Initiative)
Stanford Wu-Tsai Neurosciences Institute
NASEM Ford Foundation
Focused Ultrasound Foundation
Anonymous Donor (to Stanford Radiology)

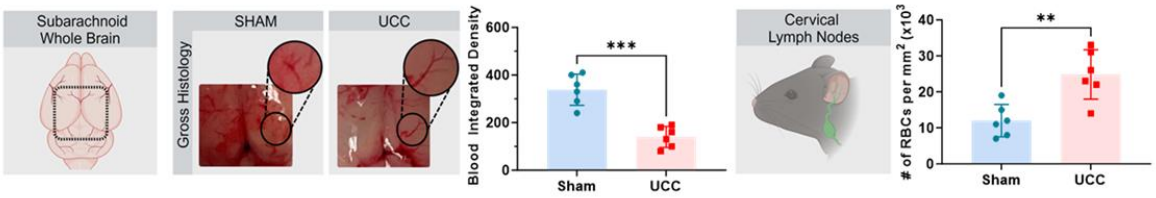
Title: Ultrasonic cerebrospinal fluid clearance of hemorrhage to the cervical lymph nodes improves outcomes in hemorrhagic brain injury

Authors: *M. AZADIAN[1], N. MACEDO[1], E. MARKARIAN[1], B. YU[1], R. M. FAME[2], P. M. GEORGE[3], R. AIRAN[4];
[1]Stanford Univ. Sch. of Med., Stanford, CA; [2]Neurosurg., Stanford Univ. Sch. of Med., Stanford,

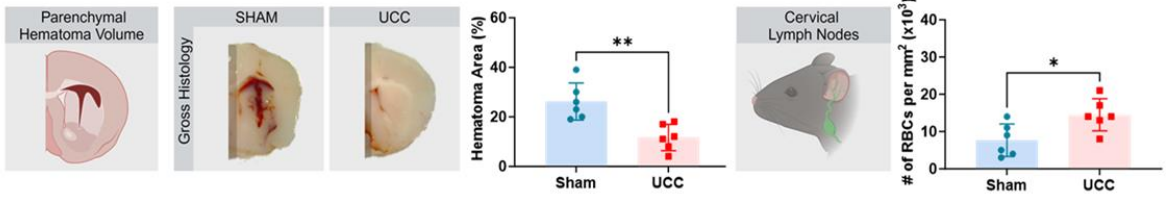
CA; [3]Neurol., Stanford Univ. Sch. of Med., Stanford, CA; [4]Radiology, Stanford Univ. Sch. of Med., Stanford, CA

Abstract: Disease and aging byproduct accumulation in the brain interstitium exacerbates disease progression and severity. The meningeal lymphatic system is responsible for clearing these toxic substances but in pathological conditions this clearance is compromised. We have developed a noninvasive low intensity transcranial focused ultrasound (FUS) protocol, ultrasonic cerebrospinal fluid clearance (UCC), that facilitates the removal of pathogenic substances from the cerebrospinal fluid (CSF) and the brain interstitium. We optimized a FUS protocol (250kHz, 0.45 MPa, 4Hz PRF, 50ms pulses x3 sessions) and validated that it can clear an aging byproduct (neurofilament light chain) in aged mice and also that it could clear blood products from the central nervous system in two models of hemorrhagic brain injury: subarachnoid hemorrhage (SAH) and intracerebral hemorrhage (ICH). With clearance from either the CSF or interstitial compartments, these blood products were detected in higher levels in the cervical lymph nodes, indicating clearance through the meningeal lymphatic system. UCC treated hemorrhagic brain injury mice showed reduced neuroinflammatory and neurocytotoxic profiles, with decreased hematoma volume, neuronal degeneration, microglial activation, and astrocytic response. The protocol also improved post-hemorrhage functional and behavioral outcomes and, importantly, increased survival. This protocol was blocked by mechanosensitive-channel antagonism and is effective when applied in anesthetized subjects, indicating a mechanosensitive channel mediated mechanism that does not depend on sensory stimulation or a specific neural activity pattern induction. Notably, this protocol qualifies for an FDA non-significant risk designation given its low intensity, making it readily clinically translatable. Overall, our results demonstrate that UCC significantly promotes the clearance of hemorrhage and other harmful substances from the brain via the meningeal lymphatic system, potentially offering a new therapeutic tool for varied neurological disorders.

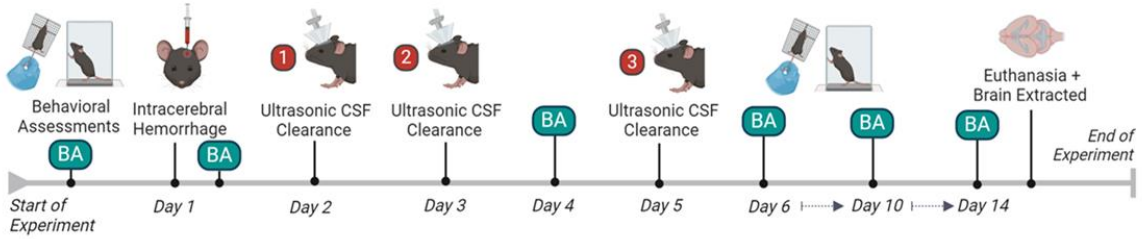
1. Ultrasonic CSF clearance enhances RBC removal from CSF to lymph nodes



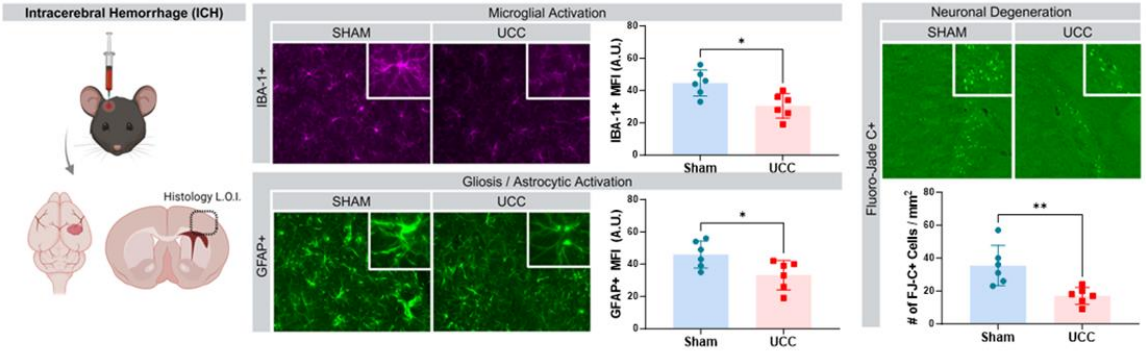
2. Ultrasonic CSF clearance enhances RBC removal from brain interstitium to lymph nodes



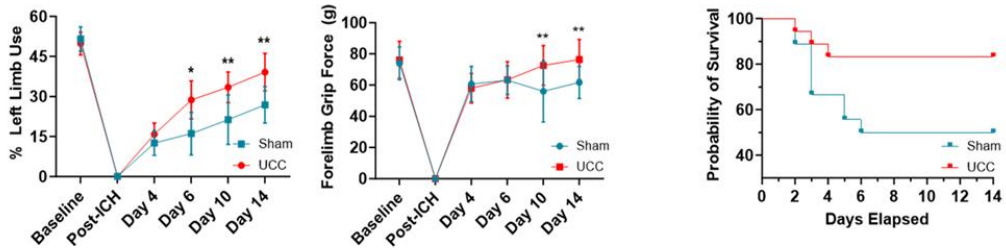
Experimental Timeline for Assessing Outcomes:



3. Ultrasonic CSF clearance reduces neuroinflammatory & neurocytotoxic profiles



4. Ultrasonic CSF clearance of hemorrhage improves functional outcomes & survival rates



Disclosures: M. Azadian: None. **N. Macedo:** None. **E. Markarian:** None. **B. Yu:** None. **R.M. Fame:** None. **P.M. George:** None. **R. Airan:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.23/X28

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH- RO1- DE031832

Title: Saliva-based Electrochemical Biosensor for Stroke Risk Prediction

Authors: *H. GEORGE[1], J. WU[2], Y. SUN[3], X.-J. LI[4], Y. YAN[5], R. PESAVENTO[6], M. MATHEW THOPPIL[7];
[1]Biomed. Engin., Univ. of Illinois Chicago, Rockford, IL; [2]computer Sci., Illinois Inst. of Technol., Illinois, IL; [3]Univ. of Illinois Chicago, Chicago, IL; [4]Dept. of Biomed. Sci., Univ. of Illinois, Rockford, IL; [5]Computer Sci., Illinois Inst. of Technol., Chicago, IL; [6]Oral Biol., Univ. of Illinois Chicago, Chicago, IL; [7]Biomed. Engin., Univ. of Illinois, Rockford, IL

Abstract: Saliva-based Electrochemical Biosensor for Stroke Risk Prediction Haritha George[1], Junyi Wu [2], Yani Sun [3], Yan Yan[2], Russell P. Pesavento[4], Xue-Jun Li[1], Mathew T Mathew[1]
[1]Department of Biomedical Engineering, University of Illinois Chicago, IL, USA, [2]Department of Computer Science, Illinois Institute of Technology, Chicago, [3]Department of Civil, Materials & Environmental Engineering, University of Illinois Chicago, IL, USA, [4]Department of Oral Biology, College of Dentistry, University of Illinois Chicago, IL, USA. Oral health as an indicator of systemic health is becoming increasingly relevant with diseases like periodontitis having links to systemic diseases. According to CDC every 40 seconds, someone in the United States has a stroke. CDC mentions that 80% of strokes can be prevented. This study considers periodontitis biomarker Matrix Metallo Proteinase 9 (MMP9) and oxidative stress biomarker Glutathione (GSH) to be used for early stroke risk prediction. The antioxidant effect of GSH on MMP9 was checked. Electrochemical analysis of artificial Saliva was done with increasing MMP9 and GSH protein concentrations independently. The combination of GSH and MMP9 was also done in different test conditions to check the antioxidant effect. Electrochemical Impedance Spectroscopy (EIS) and Cyclic Voltammetry (CV) were conducted. From EIS data, change in capacitance and charge transfer (CV area) was calculated for each test condition. The electrochemical properties obtained could be used together with the resistance of solution (Rsol), polarization resistance (Rp), and constant phase element (CPE) as machine learning data input for stroke risk prediction. Scanning Electron Microscopy (SEM) with Energy Dispersive Spectroscopy (EDS) was employed to observe the change in the electrode surface. The elemental composition on the sensor surface showed the antigen-antibody presence. ELISA and confocal imaging enabled biological characterization of MMP9 and GSH antibody-antigen interaction. Machine learning models using MMP9 and GSH electrochemical data were applied for the prediction of risk levels of stroke with periodontitis and salivary oxidative stress. The detection of MMP9 and GSH by electrochemical biosensors indicates the potential to use them as

electrochemical biomarkers and the data for ML-driven prediction tool for stroke risk. Also the effect of GSH can be seen as the electrochemical trend followed GSH in lower concentrations of MMP9. To know the alterations of MMP9 and GSH in patients' saliva samples and cellular level changes to MMP9 in the presence of GSH, the expression levels and pathways involved will also be checked in future studies.

Disclosures: H. George: None. **J. Wu:** None. **Y. Sun:** None. **X. Li:** None. **Y. Yan:** None. **R. Pesavento:** None. **M. Mathew Thoppil:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.24/Web Only

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: FPIS2024-INR-6865
SALUD-2018-2-B-S-45803

Title: Artificial intelligence-based model for estimation of biomarkers of upper extremity sensorimotor function derived from electroencephalography and magnetic resonance imaging

Authors: *J. CANTILLO-NEGRETE[1], **R. I. CARINO-ESCOBAR**[2], M. E. RODRIGUEZ-GARCIA[4], C. HERNÁNDEZ[3], A. RAMIREZ[3], M. PACHECO-GALLEGOS[3], J. QUINZAÑOS FRESNEDO[3], P. CARRILLO-MORA[3];
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Abstract: Stroke upper extremity sensorimotor function assessment requires trained clinical personnel. However, artificial intelligence could allow the identification of biomarkers of sensorimotor function derived from the patient's brain and automatize clinical assessments. In this study, the Feature-Ranked Self-Growing Forest algorithm, a novel machine learning method, was used to create a model to predict upper extremity sensorimotor function in stroke patients. The model was generated using Fugl-Meyer Assessment for the Upper Extremity (FMA-UE) scores from 19 stroke patients, measured three times per patient across an experimental intervention protocol (Clinical Trial registry = NCT04724824). Variables used for prediction were comprised by 15 features included demographic and clinical information, and metrics computed from electroencephalography (EEG) and magnetic resonance imaging (MRI). With the computed model, sensorimotor function scores were predicted with a mean absolute error of 4.5 points, with the most relevant feature for this assessment being the asymmetry of white matter

integrity in the corticospinal tract measured via the fractional anisotropy derived from diffusor tensor MRI. Other variables that had a high contribution for assessing upper extremity function were the hemispheric dominance in functional MRI indicated by the laterality index, the stroke location (cortical, subcortical, or cortical + subcortical), the affected hemisphere, the hemispheric dominance in EEG indicated by the laterality coefficient in the beta band, and age. The automated prediction error was lower than the reported minimal clinically important difference of 5.2 points for the FMA-UE, allowing to confirm the feasibility of estimating in clinical terms, upper extremity sensorimotor function in stroke using a machine learning algorithm. Furthermore, the level of spared neurological tissue within the corticospinal tract, as well as the stroke location, patient's age, and the lateralization of brain function during motor tasks, seem to be suitable biomarkers of upper extremity sensorimotor function when combined into a single nonlinear model. Although the explored automated assessment needs the acquisition of electroencephalography and magnetic resonance images, it is not susceptible to subjectivity and to the level of experience of personnel that applies the clinical assessment. Potential applications for this method are expert systems for aiding physicians to diagnose upper extremity sensorimotor function, and a tool that clinical personnel can use as reference when training for performing stroke patients' upper extremity assessments.

Disclosures: J. Cantillo-Negrete: None. **R.I. Carino-Escobar:** None. **M.E. Rodriguez-Garcia:** None. **C. Hernández:** None. **A. Ramirez:** None. **M. Pacheco-Gallegos:** None. **J. Quinzaños Fresnedo:** None. **P. Carrillo-Mora:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.25/X29

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH Grant 5R01NS112940-05
NIH Grant F31NS135993-01

Title: Post-stroke injection of a porous hydrogel scaffold with nanoparticle-bound vascular endothelial growth factor encourages axonal sprouting and glial migration into the infarct

Authors: *E. M. RATHBUN[1], S. DODDIPALLI[1], K. ERNING[2], N. V. PHAN[2], T. SEGURA[2], S. T. CARMICHAEL[1];
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Abstract: Ischemic stroke accounts for 87% of all stroke cases and is a leading cause of disability in adults in the U.S. Angiogenesis and axonal sprouting are two key components in post-stroke neural repair associated with functional recovery in humans and animal models, with

much to be discovered regarding signaling and association between blood vessels, axons, and glial cells. Manipulation of the tissue could induce structural and chemical changes to increase pro-repair processes and functional recovery. After stroke, the infarct remains devoid of axons; however, this characteristic can be overcome by replacing the fibrotic core with biomaterials to encourage pro-repair mechanisms. In order to examine innate cellular responses to a potential stroke therapeutic, we induced ischemia in the mouse primary motor cortex with photothrombosis and injected a biocompatible hydrogel scaffold with nanoparticle-immobilized vascular endothelial growth factor (VEGF) into the stroke site, yielding the identification of cell types and processes within the hydrogel that are indicative of neural repair. Primarily, axons sprout into the hydrogel and the remaining infarct tissue over time from the peri-infarct cortex and the corpus callosum. Preliminary studies suggest this axonal growth is enhanced by VEGF signaling from the injected hydrogel and that the cell bodies of the sprouting neurons are located in the peri-infarct and the contralesional cortex. Additionally, angiogenesis occurs within the porous hydrogel, which we hypothesize to have downstream effects on migration of other cell types. To study the longitudinal aspects of angiogenesis and perfusion in the peri-infarct, infarct, and hydrogel, we employed two-photon volumetric microscopy of blood flow and endothelial cells in live head-fixed mice performing a rotating grid walk test. These studies elucidate the timeline of vascular changes and functional recovery after stroke. Finally, we have characterized the presence of astrocytes, oligodendrocyte progenitor cells, and doublecortin-positive progenitors within the hydrogel scaffold, thereby exposing further potential targets for understanding post-stroke neural repair in the context of novel biomaterials.

Disclosures: E.M. Rathbun: None. **K. Erning:** None. **N.V. Phan:** None. **T. Segura:** None. **S.T. Carmichael:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.26/X30

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: ANID Exploracion 13220082
ANID Fondecyt Regular 1200880

Title: Protective Mechanisms in Retinal Aging: The Role of XBP1s in Alzheimer's Disease Mouse Models

Authors: *L. MEDINA[1], M. ORELLANA[1], C. ROJAS[2], M. CHACÓN[1], C. DURAN-ANIOTZ[3], C. HETZ[4], D. PONCE[5], D. NEIRA[5], J. ARAYA[6], A. G. PALACIOS[5];
[1]Univ. of Santiago de Chile, Santiago, Chile; [2]Pontificia Univ. Católica de Chile, Santiago,

Chile; [3]Univ. Adolfo Ibáñez, Santiago, Chile; [4]Inst. of Biomed. Sci., Santiago, Chile; [5]Univ. de Valparaiso, Valparaiso, Chile; [6]Univ. Santo Tomás, Santiago, Chile

Abstract: The retina has long been considered a window into the brain, generating significant interest in identifying potential ocular biomarkers for brain disorders. Functional changes in the retina can be detected using electroretinography (ERG). In fact, recent research has revealed alterations in the characteristics of the ERG in patients with Alzheimer's disease (AD). However, the effects of aging and neurodegeneration on retinal electrophysiology remain unclear, as do the most effective tools to detect these changes. Furthermore, recently proposed protective mechanisms against brain cell senescence, such as the overexpression of the transcription factor XBP1s, may be reflected in retinal electrophysiology. In this study we used entropy tools to explore the complexity of retinal dynamics in two age stages: young (2-5 months) and adult (5-8 months) in four mouse models: wild-type (WT) (n=8 young, 9 adult), AD 5xFAD transgenic model (n = 9, 10), Tg ^{XBP1s}mice (n = 7, 6), and a crossbreed Tg ^{XBP1s} / 5xFAD (n = 6, 7). We applied a chirp light stimulus—a flashlight followed by sinusoidal light of increasing frequency and then increasing amplitude light—to retinas ex vivo while recording µERG responses from a multielectrode recording array (MEA). We calculated the multiscale entropy (MSE) of the µERG signals and estimated a complexity index as the slope of the linear regression of the MSE curves. The median complexity index (upper: lower quartile) of all young groups was positive: WT young 4.6 (6:1.4), 5xFAD young 2.4 (3.5: -1.7), Tg ^{XBP1s} young 1.7 (2.4: 0.18) and Tg ^{XBP1s} / 5xFAD young 0.94 (1.7: -1.4), which was higher than those of the respective older groups: WT adult -2.2 (0.98:-11.4), 5xFAD adult -5.3 (-3:-8.9), Tg ^{XBP1s} adult 0.09 (3.4:-2.1) and Tg ^{XBP1s} / 5xFAD adult 0.89 (1.7:-1.4). This reduction in complexity among older groups supports the theory of loss of complexity with age. Furthermore, the 5xFAD groups exhibited lower complexity compared to their respective WT groups, consistent with a theory of loss of complexity in the disease. Tg ^{XBP1s} overexpression appeared to confer protection against aging, a benefit that extended to Tg ^{XBP1s} / 5xFAD animals, suggesting that Tg ^{XBP1s} may counteract aging and neurodegenerative mechanisms in the retina. These findings not only advance our understanding of retinal electrophysiology with aging but also have significant implications for the identification of new AD biomarkers and the development of novel therapeutic strategies.

Disclosures: L. Medina: None. **M. Orellana:** None. **C. Rojas:** None. **M. Chacón:** None. **C. Duran-Aniotz:** None. **C. Hetz:** None. **D. Ponce:** None. **D. Neira:** None. **J. Araya:** None. **A.G. Palacios:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.27/X31

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Interplay of calcium signaling and Homer protein function in neurodegeneration during AMD: Modulatory effects of smoking and systemic diseases

Authors: *M. GAJENDRAN, D. MORCOS, **P. KOULEN**;
Ophthalmology, Univ. of Missouri-Kansas City, Kansas City, MO

Abstract: The purpose of the present study was to explore the role of intracellular calcium signaling pathways in the context of the progression of age-related macular degeneration (AMD). Specifically, Homer protein-regulated calcium signaling and its effects on neuroprotection, neuroplasticity, and neurodegeneration was analyzed with a focus on how smoking and systemic diseases modulate these processes across various stages of AMD, as classified by the Minnesota Grading System (MGS). We analyzed retinal transcriptional profiles from 453 postmortem samples of the Eye Genotype Expression database, with a specific emphasis on the interaction between Homer protein signaling and calcium dynamics. These pathways are essential for maintaining the function of retinal neurons and are closely associated with neuroprotective responses and synaptic plasticity. Our study assessed the impact of extrinsic factors such as cigarette smoking and systemic diseases such as hypertension and cardiovascular conditions on these signaling pathways during AMD progression. Our findings indicate distinct, stage-specific changes in gene expression related to calcium signaling pathways, modulated by Homer proteins. These molecular alterations are linked to differential effects on neuroprotection, neuroplasticity, and the extent of neurodegeneration across MGS stages. Smoking and systemic diseases were found to have varying levels of impact on these pathways, suggesting that their modulatory effects are potentially implicated in the progression and pathology of AMD. Our study highlights the critical role of Homer protein signaling in regulating pathways pivotal for neuroprotection and neuroplasticity in the context of AMD. The detailed examination of how smoking and systemic diseases influence these pathways identifies novel potential molecular mechanisms of AMD progression. These mechanisms pave the way for developing new targeted interventions that specifically address neurodegenerative aspects of AMD, and have the potential for developing personalized therapeutic strategies geared towards individual disease stages and risk factors.

Disclosures: M. Gajendran: None. **D. Morcos:** None. **P. Koulen:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.28/X32

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: P50 HD103536-7954
R21 EB033122

Title: Age related and sex specific progression of auditory neurophysiological deficits in the Cln3 -/- mice model

Authors: *Y. DING[1], K. H. WANG[2], J. J. FOXE[3], E. G. FREEDMAN[4];
[1]Neurosci., Univ. of Rochester Med. Ctr., Rochester, NY; [2]Dept. of Neurosci., Univ. of Rochester, Rochester, NY; [3]Neurosci., Univ. of Rochester, Bronx, NY; [4]Del Monte Inst. for Neurosci., Univ. of Rochester, Rochester, NY

Abstract: CLN3 disease is a prevalent form of Neuronal Ceroid Lipofuscinosis (NCL) due to mutations in the *CLN3* gene and characterized pathologically by intracellular accumulation of ceroid lipofuscin. Common CLN3 disease symptoms include early vision loss followed by cognitive decline. However, objective neurophysiological markers of disease progression and the underlying neural circuit mechanisms in the brain are not well established, in part because the early appearance of peripheral visual deficits precludes vision-based neurophysiological tests. Building on our pilot electroencephalography (EEG) measurements of auditory processing changes in CLN3 human subjects, we utilized a mouse knockout model (Cln3 -/-) to investigate neurophysiological deficits in auditory processing and the underlying cellular pathology in auditory pathways. With implanted high-density EEG electrode arrays, we repeatedly conducted auditory duration mismatch negativity (MMN) tests to probe auditory change detection and sound discrimination across ages. Our results show that wild-type (WT) mice for both sex displayed a robust MMN response from 3 to 9 month of age. In contrast, Cln3 -/- male mice started with a normal MMN response at 3-month, then proceeded to a nearly complete absence of MMN response by 5-month. Interestingly, MMN responses reappeared in 7 to 9-month-old Cln3 -/- male mice, while other waveform features of auditory-evoked potentials (AEPs) remained different between WT male and Cln3 -/- male mice. On the other hand, Cln3 -/- female mice had consistent MMN deficits from 3 to 9 month of age, suggesting a more severe central auditory processing deficit. Additional recordings of auditory brainstem responses (ABRs) confirmed that the robust reduction of MMN in Cln3-/- mice is not caused by peripheral hearing loss but of central origin. To further examine age-dependent progression of pathological changes, we used immunostaining and confocal microscopy to image the accumulation of Subunit C of Mitochondrial ATP Synthase (SCMAS), a common marker for ceroid lipofuscin, in auditory pathways. We found an early appearance of SCMAS+ ceroid lipofuscin in the auditory thalamus, particularly the reticular nucleus (TRN), followed by the auditory and frontal cortices. On the other hand, brainstem auditory areas were much less affected. Taken together, our studies reveal robust age and sex-related deficits in the central auditory processing pathways of Cln3-/- mice, which supports the use of auditory MMN as a clinically relevant neurophysiological biomarker for the progression of CLN3 disease and provides a foundation to uncover the underlying thalamocortical mechanisms.

Disclosures: Y. Ding: None. **K.H. Wang:** None. **J.J. Foxe:** None. **E.G. Freedman:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.29/X33

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Neuronal connectivity in MS: the relationship between MRI graph parameter and transcranial magnetic stimulation connectivity measures

Authors: *A. M. AURIAT[1], V. J. LI[4], G. MELKUS[1], A. SY[5], L. WALKER[6], S. CHAKRABORTY[6], S. THEBAULT[6], C. TORRES[2], M. FREEDMAN[6], H. ATKINS[6], R. AVIV[3];
[1]Radiology, Radiation Oncology and Med. Physics, [2]Radiology, [3]Univ. of Ottawa, Ottawa, ON, Canada; [4]The Ottawa Hosp. Res. Institute, Ottawa, ON, Canada, Ottawa, ON, Canada; [5]Fac. of Medicine, Univ. of Ottawa, Ottawa, ON, Canada; [6]The Ottawa Hosp., Ottawa, ON, Canada

Abstract: Background: Clinical network neuroscience has been striving to enhance our understanding of network disruptions that impact functional performance. The application of graph theory modelling to structural T1-weighted MRI, is a promising approach to assess neuronal network disruption that may underpin clinical presentation in individuals with Multiple Sclerosis. We hypothesized that these markers would correlate with markers of functional connectivity ascertained by transcranial magnetic stimulation (TMS).Methods: 18 patients with active multiple sclerosis underwent standardized MRI scans and transcranial magnetic stimulation testing. Network extraction of MRI graph perimeters were derived from realigned and segmented cortical grey matter T1-weighted images. Volumetric quantification of total thalamic, white matter, and gray matter was completed with a semiautomated segmentation pipeline (FSL), volumes were corrected for differences in total intracranial volume. We studied correlations between graph parameters and motor evoked potential latency (MEP) and cortico-motor conduction time (CMCT). Serum neurofilament light chain (sNfL) levels, a neuron specific intermediate protein, was quantified as a biomarker of neuro-axonal damage. Correlations with a $p<0.05$ were reported.Results: Clinical outcome as assessed with expanded disability status scale (EDSS) correlated with both MEP latency ($r=0.86$, $p<0.0001$), and CMCT ($r=0.72$, $p=0.002$). MEP latency was significantly correlated with network size (0.658, $p = 0.003$), Betweenness (0.609; $p=0.007$), Lambda (-0.503, $p = 0.03$), and Sigma $r=0.4474$, $p=0.047$). CMCT significantly correlated to size ($r=0.58$, $p=0.018$), and Betweenness ($r=0.57$, $p=0.02$). Several measures of network connectivity (Degree, Density, Clustering Coefficient, Path Length, Lambda, and Sigma significantly correlated to total gray matter. MEP latency and CMCT did not relate to brain volumes. sNFL correlated to both MEP latency (0.514, $p=0.035$), and CMCT ($r=0.503$, $p=0.034$), as well as Degree ($r=-0.416$, $p=0.043$). Conclusion: MRI network parameters may represent convenient biomarkers of motor disability in MS. These measures can be derived from standard MRI sequences which are already collected as part of the standard of MS patient care.

Disclosures: A.M. Auriat: None. **V.J. Li:** None. **G. Melkus:** None. **A. Sy:** None. **L. Walker:** None. **S. Chakraborty:** None. **S. Thebault:** None. **C. Torres:** None. **M. Freedman:** None. **H. Atkins:** None. **R. Aviv:** None.

Poster

PSTR429

Biomarker, Drug Discovery, and Experimental Therapeutics in Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR429.30/X34

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Discovery of a novel NLRP3 inhibitor with good CNS penetration

Authors: *L. WU, L. ZHAO, D. CHEN, N. LIU, J. SHEN;
NeuShen Therapeut., Shanghai, China

Abstract: Discovery of a novel NLRP3 inhibitor with good CNS penetrationThe current treatments for neurodegenerative diseases, including Alzheimer's and Parkinson's, are largely for controlling symptoms and have a limited effect on disease progression. The latest advancement in Alzheimer’s disease research suggests that neuroinflammatory mechanisms closely related to toxic proteins may have the potential to modify the disease in Alzheimer's disease.
NLRP3 is a key regulator of the innate immune response. Dysregulation of NLRP3 function has been associated with numerous immune diseases. Inhibition of NLRP3 has promising application prospects in the field of autoimmune therapy. A number of NLRP3 inhibitors are currently being investigated in clinical trials for the treatment of various indications. However, the structures of most inhibitors contain a sulfonylurea moiety, which restricts their applicability in the treatment of central nervous system disorders. Recently, a number of novel scaffolds without sulfonylurea have been reported, including pyridazine analogues. The removal of the CNS-unfriendly sulfonylurea moiety from these molecules has resulted in a significant improvement in CNS permeability, while maintaining good in vitro and in vivo activity.
A novel small molecule NLRP3 inhibitor, devoid of a sulfonylurea moiety, has been identified. Its THP1 and PBMC IL-1β inhibition IC_{50} values are all below 10 nM. The rat primary microglial IL-1β inhibitory IC_{50} value was also below 10 nM. This compound is not a Pgp substrate and exhibits favorable ADME/PK properties, including brain penetration (B/P > 1). In the LPS-induced inflammation PD model, this molecule was found to dose dependently reduce LPS-induced IL-1β. Furthermore, the hERG activity was significantly reduced to over 10 μM, indicating that the cardiovascular risks commonly associated with many NLRP3 inhibitors are also significantly mitigated. Further details will be presented.

Disclosures: L. Wu: None. **L. Zhao:** None. **D. Chen:** None. **N. Liu:** None. **J. Shen:** None.

Poster

PSTR430

Optogenetic Tools

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR430.01/X35

Topic: I.08. Methods to Modulate Neural Activity

Support: KAKENHI 20J13905

Title: A neural circuit targeting technique for investigating functional input-output organization in the nervous system

Authors: *Y. KASUGA[1,2], X. GU[1], T. OHNUKI[1], A. UEMATSU[3], J. P. JOHANSEN[1,2]; [1]Lab. for the Neural Circuitry of Learning and Memory, RIKEN Ctr. for Brain Sci., Wako-Shi, Japan; [2]Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Meguro-Ku, Japan; [3]Human Informatics and Interaction Res. Inst., Natl. Inst. of Advanced Industrial Sci. and Technol., Tsukuba-Shi, Japan

Abstract: Neurons communicate information across circuits and the function of cells in these circuits is based on both the afferent inputs they receive and the efferent outputs they make to other brain regions. To study the activity and function of specific neuronal populations, anterograde and retrograde viral approaches have been employed to define neural circuit elements by inputs or outputs, respectively. However, what is missing is a way to study the function of neurons based on both their inputs and outputs. Applying a combination of multiple recombinases and anterograde/retrograde viruses, we developed a technique called input/output Projection-based INtersectional Circuit-tagging Enabled by Recombinases (PINCER) to target specific neuronal cell types and investigate functional input/output organization in neural circuits. We show the logic and application of this technique with *in vivo* calcium imaging and optogenetics approaches to investigate the activity and function of an input/output defined neuronal populations in the lateral and basal nuclei of the amygdala (LA/B) defined by input from the cuneiform nucleus (CnF) and output to the central amygdala (CeA) during aversive associative learning in rodents. We compare the function and neural activity of input/output defined cells to CnF-input or CeA-output alone defined LA/B populations. The comparison reveals that LA/B cells classified by inputs or outputs alone serve more general aversive learning, memory and salience functions, while cells defined by both inputs and outputs function specifically for aversive memory formation. This technique allows neuroscientists to identify novel subclasses of cells based on their combinatorial input/output anatomical connectivity, providing a tool for finer dissection of the functional properties of specific neural circuits.

Disclosures: Y. Kasuga: None. **X. Gu:** None. **T. Ohnuki:** None. **A. Uematsu:** None. **J.P. Johansen:** None.

Poster

PSTR430

Optogenetic Tools

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR430.02/Y1

Topic: I.08. Methods to Modulate Neural Activity

Support: Innovate UK
ADInstruments

Title: The development and testing of a novel, wirelessly powered telemeter for simultaneous optogenetic stimulation and electroencephalogram recording in adult wistar rats

Authors: *B. REES[1], S. D. GREENHILL[2], P. GRIFFITHS[3];
[1]Aston Univ., Birmingham, United Kingdom; [2]Neurosci., Aston Univ., Birmingham, United Kingdom; [3]ADInstruments, Oxford, United Kingdom

Abstract: Many neuroscience studies in rodents are limited spatially and temporally by the use of tethered devices, head-fixed recordings or battery powered telemetric implants. When subtle behavioural readouts are needed, protruding implants may affect neurophysiological and psychobehavioural parameters. We have successfully deployed and continue to develop ADInstruments' KAHA Wireless Optogenetics Biopotential Telemeter which allows simultaneous user-defined optogenetic stimulation (460nm) and continuous electroencephalogram recording at 2kHz. Powered by inductive charging to remove the limitations of battery-life and allowing for higher sampling rates, this device enables long-term, uninterrupted data collection and intervention with more data gathered from fewer animals. ChR2 (H134R) was expressed in the somatosensory cortex of male Wistar rats via AAV8. After two weeks with rats reaching 175g, a KAHA telemeter was implanted intraperitoneally with the optic fibre positioned over the left somatosensory cortex and 2 skull screws positioned at AP:-3, ML:-2.5/+2.5 and AP:-3 (from bregma) to record cortical EEG. Across a range of stimulus parameters, cortical rhythms at 2, 5, 10 and 20 Hz were entrained over multiple recording sessions in awake rats (7) and anaesthetised rats (5). We also present a telemeter implantation method with telemeter and head-gear entirely enclosed subcutaneously, with explant and optic fibre-connectorisation protocols to increase reusability. Overall, this study provides the first in-animal validation of this device for entraining cortical rhythms using the onboard optogenetics stimulation capability, showing it to be a potentially powerful tool in chronic, longitudinal optogenetic experiments in rats, affording significant ethical benefits and removing behavioural confounds to the experiment.

Disclosures: B. Rees: A. Employment/Salary (full or part-time):; Aston 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.; Innovate UK, ADInstruments. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); ADInstruments. **S.D. Greenhill:** A. Employment/Salary (full or part-time):; Aston 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.; Innovate UK, ADInstruments. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); ADInstruments. **P. Griffiths:** A. Employment/Salary (full or part-time):; ADInstruments. 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.; Innovate UK, ADInstruments. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); ADInstruments.

Poster

PSTR430

Optogenetic Tools

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR430.03/Y2

Topic: I.08. Methods to Modulate Neural Activity

Support: Max Planck Society

Title: Dual-color nanophotonic neural probes with on-shank directional coupler demultiplexers for optogenetic stimulation and electrophysiological recording

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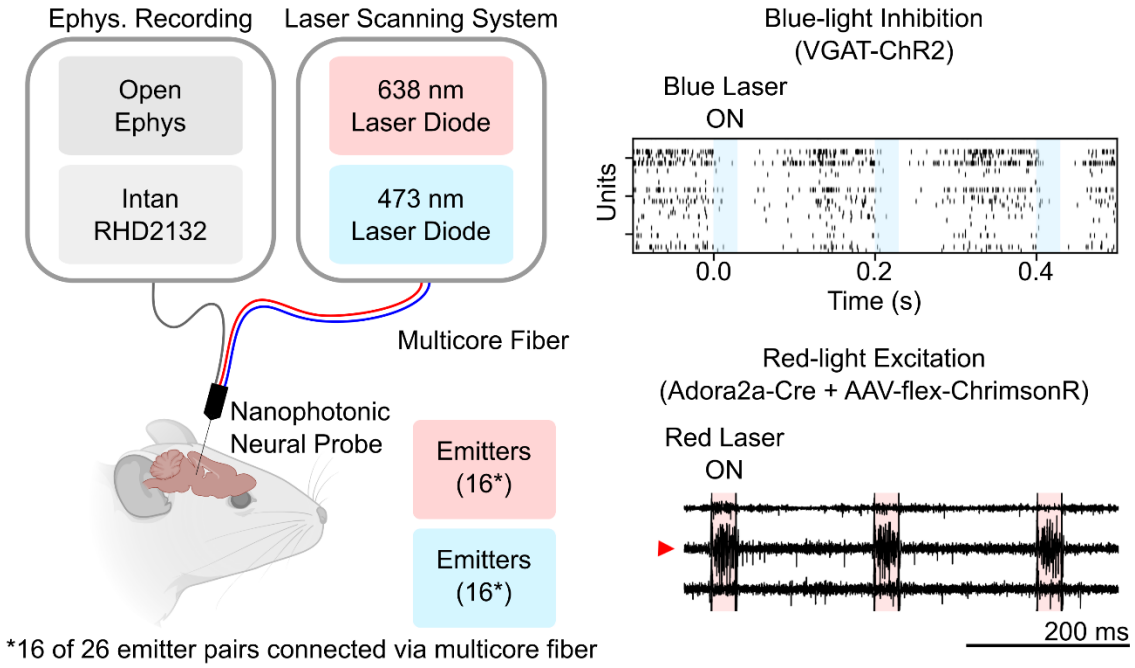
Abstract: Optogenetics enables researchers to manipulate neural activity through wavelength-selective, cell-type specific excitation, inhibition, and modulation. Tools for delivering multicolor photostimulation to deep tissue targets with sufficient spatiotemporal resolution and optical power while maintaining a small form factor remain challenging to realize. Here, we demonstrate compact nanophotonic neural probes for blue and red photostimulation and electrophysiological recording. Neural probes with 6 mm long shanks, 26 TiN recording electrodes, and 26 pairs of SiN grating coupler emitters (emitter/electrode pitch: 188 µm; span: 4.80 mm) were designed and fabricated in a wafer-scale integrated photonic platform at Advanced Micro Foundry (AMF), Singapore. Devices were polished to achieve a shank thickness of 37 ± 18 µm (mean ± SD) (n=4 probes). Electrode impedances were reduced using laser surface roughening from a nominal impedance of 3.47 ± 0.15 MΩ (n=75 electrodes) to a final impedance of 0.32 ± 0.12 MΩ (n=90 electrodes). On-shank directional coupler filters

enable compact wavelength demultiplexing of blue- (473 nm) and red- (638 nm) light to specially designed grating coupler emitters. Insertion loss for neural probes after packaging for blue- and red-light grating coupler emitters were measured as 27.1 ± 4.7 dB (n=64 emitters) and 29.5 ± 4.3 dB (n=64 emitters), respectively. Neural probes were connected to a custom dual-color laser scanning system (473 nm, 300mW; 638 nm, 180 mW) via a 16-core multicore fiber for photostimulation through 16 of 26 emitter pairs, with average emitter output powers up to 214 µW and 88 µW for blue- and red-light, respectively. We validated the neural probe functionalities by achieving selective blue-light induced inhibition in a VGAT-ChR2 mouse and selective red-light induced excitation in an Adora2a-Cre + AAV-flex-ChrimsonR mouse. Given its two emission wavelengths and long site span, this probe will facilitate experiments involving bidirectional circuit manipulations across both shallow and deep structures simultaneously.



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Poster

PSTR430

Optogenetic Tools

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR430.04/Y3

Topic: I.08. Methods to Modulate Neural Activity

Support: HOLOVIS-AdG" ERC2019-ADG-885090

Title: A flexible two-photon endoscope for in depth fast activity imaging and cell-precise optogenetic photo-stimulation of hippocampal pyramidal neurons in freely moving animals

Authors: *R. CASTILLO NEGRETE, D. DECOMBE, C. TOURAIN, N. ACANTO, V. EMILIANI;
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Abstract: Main Text
A key question in neuroscience is to unravel causal relations between neuronal circuits and behavior. The precise study of neuronal circuits requires to measure and manipulate neuronal activity with high spatial (single cell) and temporal resolution within large ensembles. All-optical experiments based on two-photon (2P) calcium imaging and 2P optogenetic photostimulation are currently a very promising solution to investigate neuronal circuits *in vivo* in mice [1-6]. However, they have so far focused on experiments in head restrained mice. We recently developed a flexible two-photon microendoscope (2P-FENDO) capable of all-optical brain investigation at near cellular resolution in freely moving mice. The system performs fast two-photon (2P) functional imaging and 2P holographic photostimulation of single and multiple cells using axially confined extended spots[7]. In its first demonstration, we used the system to manipulate neuronal circuits in the L2/3 of the barrel and visual cortex. Here, we exploited the characteristics and advantages of the 2P-FENDO in the deeper hippocampus, a crucial brain structure being involved in spatial navigation and memory formation. The hippocampus plays a central role in the formation and retrieval of spatial memories, allowing individuals to navigate and remember their surroundings [11]. Proof-of-principle experiments were performed in head restrained and freely moving mice co-expressing GCaMP8s and the opsin ChRoME in the CA1 region of the hippocampus. In a field of view of 250 µm in diameter, we demonstrate functional imaging at a frame rate of up to 100 Hz and precise photostimulation of single and multiple cells in the hippocampus. With the ability to image and control neuronal activity at single cell resolution in the hippocampus of freely moving animals, 2P-FENDO will help deciphering the neuronal networks responsible for creating a cognitive map of an environment and contributing to our ability to navigate, form memories, and understand spatial relationships, which are essential for adaptive behavior andsurvival.
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Poster

PSTR430

Optogenetic Tools

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR430.05/Y4

Topic: I.08. Methods to Modulate Neural Activity

Support: ANR-17-CE16-0021
ANR-19-CE16-0026
ANR-10-LABX-65
ANR-18-IAHU-01
HOLOVIS-AdG" ERC2019-ADG-885090

Title: Scanless two-photon voltage imaging for all-optical neurophysiology

Authors: *I. BENDIFALLAH[1], R. R. SIMS[1], C. GRIMM[1], A. MOHAMED LAFIRDEEN[1], S. DOMINGUEZ[1], C. CHAN[1], X. LU[2], B. FORGET[1], F. ST-PIERRE[3,4,5,6], D. TANESE[1], E. PAPAGIAKOUMOU[1], V. EMILIANI[1];
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Abstract: Parallel light-sculpting methods have been used to perform scanless two-photon photostimulation of multiple neurons simultaneously during all-optical neurophysiology experiments [1-3]. In this work, we demonstrate that scanless two-photon excitation also enables high-resolution, high-contrast, voltage imaging by efficiently exciting fluorescence in a large fraction of the cellular soma during high-duty-cycle recordings [4]. We present a thorough characterization of scanless two-photon voltage imaging using existing parallel approaches and lasers with different repetition rates. We demonstrate voltage recordings of high-frequency spike trains and sub-threshold depolarizations in intact brain tissue from neurons expressing the soma-targeted genetically encoded voltage indicator JEDI-2P-kv [5]. Using a low repetition-rate laser, we perform recordings from multiple neurons simultaneously in vivo. We further demonstrate that scanless light-sculpting illumination methods enable two-photon voltage imaging of rhodopsin-based voltage indicators. These indicators are typically much brighter than those based on voltage sensing domains but have not previously been demonstrated to function under two-photon excitation [6,7]. Finally, by co-expressing JEDI-2P-kv and the channelrhodopsin ChroME-ST in neurons of hippocampal organotypic slices, we perform single-beam, simultaneous, two-photon voltage imaging and photostimulation. This enables in-situ validation of the precise number and timing of light-evoked action potentials and will pave the way for rapid and scalable identification of functional brain connections in intact neural circuits.

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Disclosures: I. Bendifallah: None. **R.R. Sims:** None. **C. Grimm:** None. **A. Mohamed lafirdeen:** None. **S. Dominguez:** None. **C. Chan:** None. **X. Lu:** None. **B. Forget:** None. **F. St-Pierre:** None. **D. Tanese:** None. **E. Papagiakoumou:** None. **V. Emiliani:** None.

Poster

PSTR430

Optogenetic Tools

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR430.06/Y5

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH EY030998
NIH T32EY007125
NIH P50HD103536

Title: Inhibitory Neuron and PV-Subtype Specific AAV Targeting in the Marmoset Monkey

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Abstract: Inhibitory neurons are critical in regulating the brain's activity to shape neural tuning/ stimulus selectivity (Katzner et al 2011), increase temporal precision (Wehr and Zador 2003), and pace oscillations (Atallah and Scanziani 2009; Poo and Isaacso 2009). However, tools for modulating inhibitory neurons in non-human primates have been limited. Previously the DLX5/6 enhancer has been shown to achieve fast and reversible neuronal inactivation in the macaque monkey by targeting inhibitory neurons (De, El-Shamayleh, Horwitz 2020). Here we sought to first characterize AAV-DLX-ChR2 expression and optogenetic neuronal inactivation with the marmoset monkey, and then further test recently developed AAV constructs that selectively target the PV-subtype of inhibitory neurons (Mich et al 2021; Vormstein-Schneider et al 2020). We injected AAV-DLX5/6-ChR2 into the prefrontal cortex of the marmoset brain, and after waiting over 6 months for expression, used blue light stimulation delivered in brief pulses (30-

50ms pulses at 5-10Hz) through a thin layer of clear Kwik-sil at the cortical surface. We find in this preparation that a small fraction of neurons responded with fast excitatory responses, reflecting they are putative inhibitory cells, while the majority of the population (>80%) undergoes a fast and reversible suppression of activity. Using laminar recording electrodes, we could also demonstrate that suppression fell off with cortical depth but still remained effective even in deep layers. We have also begun testing two AAV constructs, AAV(PhP.eB) - **eHGT_140h**-ChR2-YFP and AAV(PhP.eB)-**S5E2**-HBB-ChR2-YFP, which have been reported to target PV interneurons in mice (Mich et al 2021; Vormstein-Schneider et al 2020). Both viruses were injected at different sites within area V1 of marmoset monkey cortex. We found that both viruses were expressed in the visual cortex but appeared to avoid the input layer. Using PV-immunostaining to co-label neurons, we found in preliminary data that the cortical PV enhancer S5E2 is more specific than eHGT_140h. The application of these tools in non-human primates will facilitate the identification of specific cell types in electrophysiology recordings and clarify the role of inhibitory neurons in primate brain function.

Disclosures: A. Bucklaew: None. **L. Shaw:** None. **K.H. Wang:** None. **J.F. Mitchell:** None.

Poster

PSTR430

Optogenetic Tools

Location: MCP Hall A

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Program #/Poster #: PSTR430.07/Y6

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH NINDS R01NS118188

Title: Drumbeat Optogenetics: Improving the firing frequency of channelrhodopsin-2 in the peripheral nervous system

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Abstract: Vagus Nerve Stimulation for the treatment of refractory epilepsy typically occurs at frequencies of 20 - 30 Hz; however, there is evidence to suggest that high frequency stimulation (> 80 Hz) might inhibit seizures more effectively [1]. Optogenetics is being considered as an alternative for electrical stimulation in the peripheral nervous system (PNS) because of the potential to genetically target opsins to specific fibers and eliminate off-target side effects [2]. Channelrhodopsin-2 (ChR2) is widely used in neuroscience research and is expressed well in the PNS of transgenic mice. However, it is rapidly desensitized to light and has relatively slow kinetics which makes it difficult to excite action potentials at frequencies ≥ 40 Hz [3, 4]. Here,

we investigate an optical stimulation scheme called “Drumbeat Optogenetics.” During drumbeat stimulation, two fiber optic cannulas are placed at separate locations along the length of a peripheral nerve, and the two regions are alternately stimulated using pulsed light from a 473 nm laser. Theoretically, drumbeat stimulation allows the ChR2 at one location to recover for a slightly longer inter-pulse duration while stimulation occurs at the alternate location. To test this hypothesis, we use e*x-vivo,* whole nerve electrophysiology to record compound action potentials (CAPs) from the sciatic nerves of a pilot cohort of ChAT-ChR2(H134R)-YFP mice. We perform a preliminary assessment of the experimental parameter space to determine the optimal power, pulse width, and inter-sweep interval for optogenetic experiments. We also assess the performance of drumbeat stimulation over a range of optical stimulation frequencies (10 - 60 Hz) and compare the results to optogenetic stimulation of individual nerve regions and electrical stimulation. Overall, this work seeks to provide a better understanding of how to improve the efficacy of optogenetic stimulation in the PNS.

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Disclosures: T. Welton: None. **A. Fontaine:** None. **R. Weir:** None. **J.H. Caldwell:** None. **D. Restrepo:** None. **E. Gibson:** None.

Poster

PSTR430

Optogenetic Tools

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Program #/Poster #: PSTR430.08/Y7

Topic: I.08. Methods to Modulate Neural Activity

Support: NSERC

Title: Optical interrogation of cAMP signaling in behaving animals reveals cAMP-dependent neural circuit activity underlying hippocampus-dependent learning and memory

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Abstract: The spatiotemporal organization of hippocampal circuit activity is critical for hippocampus-dependent learning and memory. However, the molecular mechanisms underlying the coordination of hippocampal neural activity and their task-related dynamics remain unclear. Here, we investigate the role of cAMP (adenosine 3′,5′-cyclic monophosphate) signaling to regulate hippocampal CA1 neural activity in object recognition memory by *in vivo* calcium imaging with cellular cAMP manipulation in freely-behaving animals. cAMP is a ubiquitous second messenger that serves for certain types of synaptic plasticity as well as learning and memory. We previously reported the function of cAMP to rapidly & spatiotemporally enhance synaptic strength and neuronal depolarization in murine hippocampal slices, suggesting that cAMP may not only serve synaptic potentiation and neuronal activity but may also affect patterns of neural circuit activity for short-term memory (STM). To test this, we first determined the spatiotemporal effect of cAMP in short-term object recognition memory using mice that virally express a constitutively active cAMP-specific phosphodiesterase or light-sensitive cAMP metabolic enzymes in murine CA1 neurons with photoactivation by an implanted wireless fiber optic LED. During object recognition memory tests, we found a deficit in object memory following cAMP suppression specifically during training, while cAMP enhancement during training promotes STM, indicating a cAMP function that is critical for formation of short-term object memory. To directly observe the role of cAMP in hippocampal CA1 neural circuit activity during STM formation, we virally coexpressed a calcium indicator (GCaMP8m) with cAMP metabolic enzymes, then implanted a GRIN lens coupled with the wireless fiber optic LED by our custom headmount system. We optically recorded activity of CA1 neurons with or without cAMP manipulation by micro-endoscopic calcium imaging in freely-behaving mice during the object recognition STM tests, then fit a latent space dynamical system model to estimate population dynamics underlying memory task-specific behaviors. We found that cAMP perturbation disrupts the task-related structure and dynamics in the latent embedding of population activity during STM, which correlates with the observed deficit in memory behavior by cAMP manipulation. Thus, our results reveal that the spatiotemporal cAMP signal is critical for CA1 neural activity that facilitates hippocampus-dependent memory. We will further discuss our approach to characterize the cAMP mechanism of action to regulate population dynamics during hippocampus-dependent STM and LTM.

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Poster

PSTR430

Optogenetic Tools

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Topic: I.08. Methods to Modulate Neural Activity

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R01 NS125307 (MPI: Rosene/Rushmore)
5UG3MH120095 (Levi/Ting)
U01-NS094362 (Zemelman)
U01-NS094330 (Zemelman)

Title: Multimodal characterization of optogenetic approaches for assessing inhibitory neuron function in macaque monkeys

Authors: M. MEDALLA[1], C. A. MOJICA[1], ***P. BOUCHER**[3], B. SNYDER[1], A. MORE[1], H. BHATT[1], M. YAHAYA[1], T. WANG[4], K. LEE[5], S. CARRILL[2], D. L. ROSENE[1], R. J. RUSHMORE, III[1], T. L. MOORE[1], B. ZEMELMAN[6], J. T. TING[7], B. P. LEVI[7], J. I. LUEBKE[1], C. CHANDRASEKARAN[1];
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Abstract: How inhibitory neurons orchestrate sensation, cognition, and motor control is an open question. In mice, transgenic approaches have helped address this question, whereas a corresponding understanding in macaque monkeys has been elusive. Recent studies identified regulatory elements and red-shifted opsins that enable viral constructs to selectively target inhibitory neurons. However, the capabilities of these viral constructs to selectively express opsins in inhibitory neurons and performance in altering and "optotagging" inhibitory neurons of the macaque monkey is not fully resolved.
We combined in-vivo electrophysiology, ex-vivo slice electrophysiology, and immunohistochemistry to assess the efficacy of inhibitory neuron-specific viral constructs in 4 macaque monkeys (W, M, T, B), complemented with parallel experiments in 6 mice. We focused on three promising constructs: AAV1-h56D-bReaCHeS-tdTomato, CN4858: AAV/PHP.eB-DLX2.0-ChR2(H134R)-EYFP, CN2068: AAV/PHP.eB-DLX2.0-ChrimsonR-tdTomato.
We found that these viral constructs selectively target inhibitory neurons in multiple brain areas of macaques (and mice). First, the h56D construct selectively expressed bReaCHeS-tdTomato in inhibitory neurons of macaque premotor cortex, and in-vivo photostimulation (561 nm laser) suppressed putative excitatory neurons (case W), and increased multiunit activity of putative inhibitory neurons (case M). Second, the CN4858 construct selectively expressed ChR2(H134R)-EYFP in inhibitory neurons of the macaque somatosensory cortex (S1, case M). Photostimulation (473 nm) of ChR2(H134R)+ inhibitory terminals in ex-vivo slices evoked reliable IPSCs in excitatory neurons recorded with whole-cell patch-clamp, and these IPSCs were blocked by the $GABA_AR$ antagonist, bicuculline. Finally, the CN2068 construct expressed ChrimsonR-tdTomato in inhibitory neurons of mouse V1 and S1 and monkey S1 (case T). In mice, in-vivo photostimulation (561 nm laser) led to robust low-latency spikes in putative inhibitory neurons. Subsequent ex-vivo slice experiments in the same animal confirmed functional light-evoked IPSCs. Post-hoc immunohistochemistry and confocal imaging of fluorescent reporters together with inhibitory neuron markers confirmed the selective expression of these opsins in parvalbumin (PV) and non-PV type inhibitory neurons in both species. Collectively, these results suggest that viral vectors with cell-specific regulatory elements can alter the activity of inhibitory neurons and thus enable study of neural circuit dynamics in the macaque monkey.

Disclosures: M. Medalla: None. **C.A. Mojica:** None. **P. Boucher:** None. **B. Snyder:** None. **A. More:** None. **H. Bhatt:** None. **M. Yahaya:** None. **T. Wang:** None. **K. Lee:** None. **S. Carrill:** None. **D.L. Rosene:** None. **R.J. Rushmore:** None. **T.L. Moore:** None. **B. Zemelman:** None. **J.T. Ting:** None. **B.P. Levi:** None. **J.I. Luebke:** None. **C. Chandrasekaran:** None.

Poster

PSTR430

Optogenetic Tools

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR430.10/Y9

Topic: I.08. Methods to Modulate Neural Activity

Title: Neuronal and behavioral manipulations by retrograde pathway-selective optogenetic activation in non-human primates

Authors: *X. YU[1], A. GOPAL P A[2], K.-I. INOUE[3], M. TAKADA[3], O. HIKOSAKA[4]; [1]NIH, Bethesda, MD; [2]Lab. Of Sensorimotor Res., NIH, Bethesda, MD; [3]Ctr. for the Evolutionary Origins of Human Behavior, Kyoto Univ., Inuyama, Aichi, Japan; [4]Lab. Sensorimotor Res., Natl. Eye Inst., Bethesda, MD

Abstract: Pathway-selective optogenetics provides powerful approaches to studying the central mechanisms underlying cognitive functions by achieving precise manipulation of specific neurons within a given neural circuit. For example, retrograde viral vectors with optogenetic constructs can selectively tag neurons that send inputs to a target area, allowing the control of these tagged neurons to investigate their functional contributions to a particular neural circuit. Although retrograde optogenetics has widely been utilized in rodents, it has not been extensively explored in awake nonhuman primates (NHPs).Here, we injected a retrograde viral vector expressing channelrhodopsin-2 (RetroAAV2-hSyn-ChR2-GFP) into the superior colliculus (SC) of macaques to identify and manipulate neuronal populations that project directly to SC, and then assessed their roles in oculomotor behavior. We first targeted neurons projecting from the frontal eye field (FEF) to SC. When optically stimulating in FEF, we found that around 30% of the recorded neurons were activated within 5ms, indicating successful retrograde labeling. Notably, optical stimulation of these labeled FEF neurons reliably triggered contralateral saccadic eye movements within 40 ms of stimulation onset, comparable to electrical stimulation at the same site. Next, we characterized the functional identity of the labeled FEF neurons using a variety of behavioral tasks and obtained the data suggesting that a diverse range of functional subtypes—visual-, visual-motor-, and motor-related neurons—project to SC. To further characterize the SC projection patterns of these FEF neurons, we simultaneously recorded neurons across the different layers of SC while optically stimulating FEF. Our results showed that most of the activated SC neurons belonged to the class of visual-motor-related neurons and were localized within the intermediate layers of SC. Outside of FEF, we also found optically activated neurons in the substantial nigra pars reticulata (SNr), indicating successful labeling of GABAergic

projection neurons, albeit a lower proportion (~10%) compared with the FEF neurons. In summary, the present study demonstrated, for the first time, the feasibility of using retrograde pathway-selective optogenetics to label upstream neurons, explore neural circuit interactions, and manipulate behaviors in NHP. This technique opens a novel possibility of investigating neural circuits and their roles for complex behaviors in NHP models.

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Poster

PSTR430

Optogenetic Tools

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Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant R01NS119395
NIH P51OD010425
NIH U42OD011123
NSF Graduate Research Fellowships Program (GRFP)

Title: Large-scale connectivity changes induced by targeted optogenetic inhibition in non-human primate cortex

Authors: *J. ZHOU[1], K. KHATEEB[2], J. BLOCH[1], T. BELLOIR[1], A. YAZDAN-SHAHMORAD[3];
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Abstract: Numerous neurological disorders originate from aberrant neural dynamics and connectivity within the brain. By leveraging the brain's inherent plasticity, targeted neuromodulation techniques can be developed to reorganize specific connections, serving as therapies for various brain disorders. Among these techniques, optogenetic tools enable spatiotemporally precise modulation by allowing rapid excitation or inhibition of selected neuronal groups with light. Although both single-site and paired optogenetic stimulation have been shown to enhance functional connectivity within the brain, it remains unclear whether these tools can also disrupt connections and dissociate specific locations from the broader network. To address this gap, we examined network-level changes following optogenetic inhibition in the posterior parietal cortex (PPC) of two non-human primates using a multimodal neural interface. After virally infusing the PPC with a red-shifted inhibitory opsin (Jaws), we delivered 5 blocks of 10-minute, single-site, 5 Hz laser illumination (638nm) at selected locations over the PPC. To monitor the neural dynamics in response to light modulation, we recorded electrocorticography signals before, during, and after each inhibition session. Pairwise coherence between electrodes

were computed to estimate the changes in functional connectivity. We found that when targeting locations with strong Jaws expression and significant light evoked neural response, gamma coherence between the illuminated site and the whole network gradually decreased after each inhibition block. Conversely, no significant changes in gamma coherence were observed during no-laser control sessions or when targeting regions without distinct opsin expression. These results suggest that targeted neuronal inhibition can effectively decouple specific locations from the brain network.

Disclosures: J. Zhou: None. **K. Khateeb:** None. **J. Bloch:** None. **T. Belloir:** None. **A. Yazdan-Shahmorad:** None.

Poster

PSTR430

Optogenetic Tools

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR430.12/Y11

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF NCS-FO #2024364
NSF #2142911

Title: Simultaneous Widefield Calcium Imaging and Optogenetic Control for Linking Brain Activity and Behavior

Authors: *N. MATVEEVA[1], A. LI[4], Z. YE[2], N. A. STEINMETZ[3];
[2]department of biological structure, [3]Biol. Structure, [1]Univ. of Washington, Seattle, WA; [4]Biol. Struture, Univ. of Washington Grad. Program In Neurosci., Seattle, WA

Abstract: Coordination of dynamic activity across the cerebral cortex is essential to perception and cognition, supporting computations like sensorimotor integration (Zatka-Haas et al., 2020) and maintaining goal-directed task engagement (Jacobs et al., 2020). However, understanding direct causal links between population dynamics and behavior requires the ability to measure and manipulate activity across multiple cortical regions simultaneously. Here, we develop a novel system for simultaneously recording and manipulating activity in any dorsal cortical area in awake mice. Transgenic GCaMP mice (CaMKII-tTA.tetO-G6s or -G8s) are injected systemically with an AAV-PHP DLX2.0-Chrimson virus. The red-shifted Chrimson opsin expresses in all inhibitory neurons and GCaMP expresses in excitatory neurons. This preparation is stable over many months and thus well-suited for long-term behavioral experiments. We performed widefield single-photon calcium imaging over the entire dorsal cortical surface (as described in Ye et al. 2023) and galvo-targeted stimulation with a 638 nm laser. There was no interference between the light channels of the imaging and light responsivity of the opsin. Indeed, we can elicit inhibitory activity via opsin activation with high spatial and temporal precision: the calcium indicator responds to the laser within 10 ms, and the activity returns to baseline in ~100

ms. The spatial extent can be as small as .8 mm or as large as 2.5 mm in diameter depending on laser power. This technique allows for studying the effect of cortical perturbations on cortex-wide activity and on behavior. The ability to simultaneously measure and manipulate further affords the option to design control algorithms that guide desired activity patterns in closed-loop. In the future, we will use the combination of techniques to understand the brain-wide mechanisms underlying goal-directed behaviors.

Disclosures: N. Matveeva: None. **A. Li:** None. **Z. Ye:** None. **N.A. Steinmetz:** None.

Poster

PSTR430

Optogenetic Tools

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR430.13/Y12

Topic: I.08. Methods to Modulate Neural Activity

Support: JST SPRING JPMJSP2109

Title: Neuronal Connectivity Impairment Due to Chronic Hypoperfusion Measured by Two-Photon Optogenetics and Simultaneous Calcium Imaging

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Abstract: Two-photon optogenetics and simultaneous calcium imaging can be used to assess neuronal connectivity in the brain. This method enables visualization of the response with respect to the activity of the optically stimulated neuron, providing a direct assessment of the connectivity between neurons. Previous studies have employed this method to evaluate connectivity strength ex vivo. However, the application of two-photon optogenetics-based assessments of neuronal connectivity in in vivo pathophysiological research remains to be determined. In this study, we developed a method for evaluating neuronal connectivity in vivo and applied it to investigate connectivity impairments in a hypoperfusion mouse model. In these experiments, GCaMP6s-expressing transgenic mice underwent cranial window surgery and were introduced with C1V1-mScarlet, a type of red-shifted channelrhodopsin, with an adeno-associated virus. Additionally, the mice underwent unilateral common carotid artery occlusion (CCAO) surgery to prepare a hypoperfusion model. Two-photon optical stimulation (1064 nm) of C1V1-positive neurons and simultaneous calcium imaging (920 nm) of the target and surrounding cells were performed in awake mice. Neuronal connectivity was analyzed based on the correlation coefficients between the normalized percent changes in GCaMP6s fluorescence intensity ($\Delta F/F$) of the target and surrounding cells. The correlation coefficients were high for neurons that were synchronized with the activity of the target neuron in healthy mice, indicating that neuronal connectivity in the live brain was visualized using the two-photon optogenetics-based technique. Neuronal connectivity was evaluated using a CCAO model. The mean

correlation coefficient in each neuron significantly decreased at 1, 2, and 4 weeks after CCAO surgery, whereas it remained unchanged in the control group (n=5). Furthermore, there was no significant decrease in the resting-state neuronal synchrony in the CCAO group. Our results suggest that the two-photon optogenetics-based method is a sensitive detector of neuronal connectivity impairment in the hypoperfusion model. The two-photon optogenetics-based technique is expected to be useful for in vivo neural connectivity assessments in brain diseases like stroke, dementia, and psychiatric disorders.

Disclosures: M. Yoshioka: None. **H. Takuwa:** None.

Poster

PSTR430

Optogenetic Tools

Location: MCP Hall A

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Program #/Poster #: PSTR430.14/Y13

Topic: I.08. Methods to Modulate Neural Activity

Support: NRF-2022R1I1A4063209
2022R1A2C2005062

Title: Optogenetic sleep modulation in rat medial prefrontal cortex

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Abstract: Numerous studies have explored methods to enhance sleep quality using brain stimulation. The quality of sleep is influenced by sleep depth, duration, onset latency, and the balance between non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. Specifically, sleep spindles and slow waves during NREM sleep are regarded as key factors for cognitive functions. Sleep spindles are implicated in long-term memory consolidation, while slow waves play a crucial role in memory reinforcement. Most sleep studies target the cortical-thalamic circuit known as the corticothalamic reticular nucleus (TRN), the origin of sleep spindles. However, this study focuses on the medial prefrontal cortex (mPFC), a brain region oscillating neural signals with TRN and thalamus as part of a major neural circuit involved in sleep-wake control as a target brain area for optogenetic stimulation. In this study, three different frequencies (2 Hz, 10 Hz, and spindle-like stimulation) of light stimulation were applied to Sprague-Dawley rats expressing channelrhopsin-2 (ChR2) in the mPFC region. Sleep patterns were assessed before and after optogenetic stimulation over six hours for each experimental day to assess long-term changes in brain stimulation. Data were recorded at a sampling rate of 20 kHz, and EEG and EMG signals were analyzed to track changes in sleep phases. The analysis comprehensively investigated the temporal dynamics of non-rapid eye movement (NREM) and

rapid eye movement (REM) sleep phases, transitions between these stages, and the manifestation of sleep spindles. In this study, we confirmed that optogenetic stimulation of the mPFC effectively modulates sleep patterns, improving sleep quality and cognitive function.

Disclosures: Y. Lee: None. **Y. Lee:** None. **S. Hwang:** None. **S. Jun:** None.

Poster

PSTR430

Optogenetic Tools

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR430.15/Y14

Topic: I.08. Methods to Modulate Neural Activity

Title: Targeting high throughput optogenetics and constrained imaging in all optical electrophysiology

Authors: *M. KÖNIG[1], C. MOON[1], N. PATHAK[1], J. BUTSCHER[2], L. KURIAN[3], M. SCHUBERT[1], M. GATHER[1];
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Abstract: Despite taking promising steps into interdisciplinary domains beyond neuroscience, current optogenetics also continues to face challenges, both on the biological and the technological side. For example, high throughput optogenetics in drug screenings is hindered by the availability of optogenetic samples, and assessing optogenetically evoked activity *in vivo* is constrained by the lack of transparent light sources. Working to address these issues, we utilized the piggyBac transposase to engineer an optogenetic human induced pluripotent stem cell (hiPSC) line with constitutive CheRiff expression under the ubiquitously expressed PGK promoter. Using 3D, cardiac like structures (Cardioids) differentiated in a 96 well plate within seven days, we showcase high throughput optogenetics for toxicity assays. Cardioids derived from hiPSCs exhibit robust light-induced, stimulus locked contractions which follow different stimulation frequencies. Given its capability to generate various types of excitable tissue, this hiPSC based approach can serve as a versatile platform for a wide range of optogenetic applications. Furthermore, we developed a semitransparent light source based on an electrochemiluminescent device (ECLD) whose emission meets the excitation spectrum and fulfills the light demands of the highly sensitive opsin ChRmine [1]. High transparency across the visible spectrum is ensured by two thin indium tin oxide electrodes with a thin fluid-state electroactive layer in between. We demonstrate calcium imaging through the active area of the device, and resolve spiking in primary hippocampal neurons that is evoked by optical stimulation from the ECLD [2]. The high transparency and small footprint of the ECLD (2.4 x 2.4 cm) facilitates simple integration on existing microscopes, and might ultimately allow integration in

cranial windows or implants, as well as on on-chip microscopes.

References

[1] J. H. Marshel *et al.*, "Cortical layer-specific critical dynamics triggering perception," *Science* (2019), **365**, doi: 10.1126/science.aaw5202.
[2] C. K. Moon, J. F. Butscher, and M. C. Gather, "An Exciplex-Based Light-Emission Pathway for Solution-State Electrochemiluminescent Devices," *Adv. Mater.* (2023), **35**, doi: 10.1002/adma.202302544.

Disclosures: M. König: None. **C. Moon:** None. **N. Pathak:** None. **J. Butscher:** None. **L. Kurian:** None. **M. Schubert:** None. **M. Gather:** None.

Poster

PSTR430

Optogenetic Tools

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Program #/Poster #: PSTR430.16/Y15

Topic: I.08. Methods to Modulate Neural Activity

Support: IHU-FOReSIGHT
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ERC2019-ADG-885090
ANR-HOLOVIS
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Marie Skłodowska-Curie grant #813457

Title: High-throughput in vivo synaptic connectivity mapping of neuronal micro-circuits using two-photon holographic optogenetics and compressive sensing

Authors: *D. TANESE[1], I.-W. CHEN[1], C. CHAN[1], P. NAVARRO[3], V. DE SARS[2], E. RONZITTI[1], K. G. OWEISS[3], V. EMILIANI[1];
[1]Photonics Dept., [2]Vision Inst., Paris, France; [3]Electrical & Computer Engin., Univ. of Florida, Gainesville, FL

Abstract: Understanding the intricate synaptic connectivity in living neural circuits is crucial for unraveling the relationship between network structure and function, as well as its evolution during development, learning, and recovery from injury. However, current methodologies for identifying connected neurons *in vivo* suffer from limitations, particularly with regards to their throughput. In this study, we introduce a novel framework for *in vivo* connectivity mapping that combines two-photon holographic optogenetics for activating single or multiple potential presynaptic neurons, whole-cell recording of postsynaptic responses, and a compressive sensing strategy for efficiently retrieving individual postsynaptic neurons' responses when multiple potential presynaptic neurons are simultaneously activated. The approach was validated in the

layer 2/3 of the visual cortex in anesthetized mice, enabling rapid probing of up to 100 cells in approximately 5 minutes. By identifying tens of synaptic pairs, including their connection strength, kinetics, and spatial distribution, this method showcases its potential to significantly advance circuit reconstruction in large neuronal networks with lower invasiveness compared to multi-patch approaches. Moreover, through simultaneous multi-cell stimulation and compressive sensing, we demonstrate up to a three-fold reduction in the number of required measurements to infer connectivity with limited loss in accuracy, thereby enabling high-throughput connectivity mapping *in vivo*. These results pave the way for a more efficient and rapid investigation of neuronal circuits, leading to deeper insights into brain function and plasticity.

Disclosures: D. Tanese: None. **I. Chen:** None. **C. Chan:** None. **P. Navarro:** None. **V. de Sars:** None. **E. Ronzitti:** None. **K.G. Oweiss:** None. **V. Emiliani:** None.

Poster

PSTR430

Optogenetic Tools

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR430.17/Y16

Topic: I.08. Methods to Modulate Neural Activity

Support: U19NS107464
U19NS112953

Title: A multiscale all-optical approach to interrogate neural connectivity

Authors: *M. KARADAS[1,2], J. V. GILL[3], D. RINBERG[4,2], S. SHOHAM[3,5];
[1]NYU Langone Hlth., New York, NY; [2]Neuroscience Institute, NYU Langone Health, New York, NY; [3]Neurosci. Inst., NYU Langone Hlth., New York, NY; [4]Neurosci. Inst., NYU Neurosci. Inst., New York, NY; [5]Tech4Health, NYU Langone Health, New York, NY

Abstract: Neural circuits achieve their computational power through their connectivity. Behaviors linking sensation to action are governed by the precise interplay of neurons communicating over multiple spatial scales, such as between brain areas and layers of processing, as well as within local populations of neurons. Yet, circuit computations are usually only inferred by observing the activity of neurons, not the connections between them. Here, we describe a system based on a custom 2-photon microscope that permits multiscale optogenetic interrogation of the effective connectivity between neurons. This system combines a pathway for large-scale 1-photon patterned illumination using a digital micromirror device (DMD), along with a pathway for precise holographic 2-photon stimulation using a spatial light modulator (SLM) at a fine spatial scale. The system permits imaging of many neurons (100s-1,000s) over a 2-mm field of view with subcellular resolution and recording stability over months. Using this combined approach, we demonstrate our ability to identify neurons by both their tuning to sensory stimuli and the effective input they receive from other circuits. We demonstrate this

using two model systems, the mouse olfactory bulb and the somatosensory cortex. In the olfactory bulb, we used DMD pattern stimulation to activate individual glomerular channels expressing ChR2 and demonstrate that we can identify groups of mitral and tufted cells receiving direct input from their parent glomerulus. We investigated the functional interaction between glomerulus channels by stimulating pairs of glomeruli and measuring the impact on their connected neurons. Then, we interrogated the effective connections between individual mitral and tufted cells using holographic stimulation. Surprisingly, we observed a mixture of excitatory and inhibitory influence, even though only inhibitory interneurons mediate the anatomical connections between mitral cells. We related the sign and strength of coupling between mitral cells to distances in odor tuning and differences in glomerular input to determine how local connectivity reshapes odor representations, as well as extend this technique to study the connections between and within whisker barrels in the somatosensory cortex.

Disclosures: M. Karadas: None. **J.V. Gill:** None. **D. Rinberg:** None. **S. Shoham:** None.

Poster

PSTR430

Optogenetic Tools

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Program #/Poster #: PSTR430.18/Y17

Topic: I.08. Methods to Modulate Neural Activity

Support: DIRP, NIMH, USA, ZIAMH002797, ZIAMH002971
BRAIN initiative Grant U19 NS107464-01

Title: Evaluating pattern efficacy in holographic stimulation of single neurons in awake mice using all-optical interrogation

Authors: *B. GIFFORD, T. L. RIBEIRO, A. VAKILI, V. SINFUEGO, D. PLENZ;
Section on Critical Brain Dynamics, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: All-optical interrogations, in which lasers are used to simultaneously monitor and stimulate neuronal populations, have revolutionized neuroscience. They allow for unprecedented precision in studying and controlling brain activity when combined with genetic targeting of neuronal subtypes and precisely stimulated, individual neurons. This approach, though, requires precise alignment of the holographic stimulation plane with the imaging plane which can be technically challenging. Here, we introduce a method to readily check for proper alignment and achieve performance with cellular and sub-cellular precision.
Our holographic pathway consists of a low-repetition, high-powered laser beam (LightConversion) shaped by the high resolution Spatial Light Modulator (MeadowLark) routed through a Galvo-Galvo mirror set. Our imaging pathway is composed of a high-repetition, low-powered laser (Discovery NX; Coherent) routed through a Resonant-Galvo mirror set. Alignment of the two planes was achieved by shifting the xy-position and rotation of the galvo-

galvo mirrors in the holographic pathway, quickly redirecting the misaligned beam with little to no observed distortion. We aligned our system using fluorescent microbeads (polystyrene, 542/612 nm, 3.2 µm diameter, density = 1.05 g/cm^3) suspended in agarose gel and tested the calibration efficacy by reconstructing the Point Spread Function (PSF) of the beam. We evaluated the alignment and spatial precision of our holographic stimulation by examining on-off target effects shown by the PSFs of three separate holographic beam profiles - 1) top-hat pattern for larger 10 µm spots (LS); 2) 2 µm diffraction-limited spot (DL); and 3) 4 diffraction-limited spots in a diamond pattern (4DL). The 4DL pattern exhibited the highest precision, illustrating that our stimulation system can finely control the combination of DL beams, or beams at their highest possible radiance for a given power, to create a beam large enough to cover the somatic cross-sectional area for neuronal stimulation. We tested our stimulation efficacy in awake mice, simultaneously imaging and exciting individual pyramidal neurons in layer II/III of the primary visual cortex (V1). We obtained laser power vs. neuronal stimulation efficacy for various experimental conditions (ChrimsonR/GCaMP7s AAV vs. bicistronic ChrimsonR/GCaMP8s and stimulation in visual cortex vs. frontal cortex through a microprism). We demonstrate sub-cellular spatial precision in our all-optical approach, down to 2 µm. Even increasing to the 10 µm LS and 4DL spots allows us to robustly stimulate cells at low power (<10 mW) with negligible off-target stimulation.

Disclosures: B. Gifford: None. **T.L. Ribeiro:** None. **A. Vakili:** None. **V. Sinfuego:** None. **D. Plenz:** None.

Poster

PSTR430

Optogenetic Tools

Location: MCP Hall A

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Program #/Poster #: PSTR430.19/Y18

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant 5R03DA050962-02

Title: Enhanced food consumption through optogenetic stimulation of hypothalamic AgRP neurons in transgenic zebrafish larvae

Authors: *H. MEHRABI[1], P. BANSAL[1], J. JUTOY[1], E. JUNG[2];
[1]Mechanical and Industrial Engin., Univ. of Illinois at Chicago, Chicago, IL; [2]MIE, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Agouti Related Protein (AgRP) neurons, situated in the hypothalamus, regulate hunger and associated behaviors such as food-seeking and compulsive eating upon activation. These neurons are typically activated by ghrelin during starvation, binding to ghrelin receptors on their surface to elicit these behaviors. In this study, we employed channelrhodopsin-Kaede (chR2-Kaede), a photoactivatable protein expressed in AgRP neurons, to induce a feeding

response. We used 6dpf transgenic zebrafish larvae Tg(agrp1:ChR2-Kaede) with expressed chR2-Kaede and wild-type (ABWT) larvae to compare food intake. Food consumption was assessed by recording the food suction behavior of partially immobilized larvae, allowing free mouth movement in water containing suspended food particles. The speed of food particle movement induced by suction was analyzed and quantified using Particle Image Velocimetry (PIV). Results indicated that acute photoactivation of AgRP-chR2-Kaede neurons significantly enhanced food-suction behavior in transgenic zebrafish larvae compared to wild-type. Furthermore, increased food-suction behavior was observed in previously fed AgRP larvae. These findings provide insights into hunger-related behaviors and their neural circuits in a novel transgenic zebrafish model, potentially advancing understanding of the neural mechanisms responsive to various chemical stimuli, including drugs of abuse.

Disclosures: H. Mehrabi: None. **P. Bansal:** None. **J. Jutoy:** None. **E. Jung:** None.

Poster

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Optogenetic Tools

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Program #/Poster #: PSTR430.20/Y19

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH DIRP ZIAMH002797
BRAIN initiative U19 NS107464-01

Title: Neuronal avalanches and non-linear responses to holographic perturbation in the visual cortex of awake mice

Authors: *T. L. RIBEIRO, B. GIFFORD, V. SINFUEGO, D. PLENZ;
Sect Crit Brain Dynamics, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Neuronal synchronization in cortex has recently been shown at the cellular level to organize as scale-invariant parabolic avalanches, both during ongoing as well as sensory-stimulus evoked activity (Ribeiro, Capek et al., 2023). To further understand the contribution of local groups of neurons in the formation of parabolic avalanches, we combined a low-repetition, high-power laser (Carbide/Orpheus; LightConversion) and a high-resolution spatial light modulator (MeadowLark) to achieve successful 2-photon (2P) excitation of individual neurons expressing the opsin ChrimsonR in the awake mouse. Simultaneously, we imaged layer II/III pyramidal neurons of widefield-identified primary visual cortex (V1) expressing the GECI GCaMP8s using a Resonant-Galvo pathway and a high-repetition, low-power laser (Discovery NX; Coherent). The activity of ~150 - 300 neurons was recorded using 2P imaging in a ~450 μm x 450 μm area (100 - 200 μm depth) at ~45 Hz framerate while holographically stimulating (100 ms; <10 mW per target) 32 pyramidal neurons co-expressing the opsin (~100 trials per group). The chosen 32 targets were grouped together for stimulation in subsequent recordings, going

from group size of 1 neuron to 2, 4, 8, 16 and 32. The groups were formed hierarchically, by consecutively merging groups that are spatially close. Images were denoised using a machine-learning-based algorithm and denoised calcium traces were then deconvolved for spike extraction. During stimulation of the target cells, a subset of non-stimulated cells responded significantly to the perturbation (> 92% baseline spike count). Population responses to the perturbation increased non-linearly with the size of perturbation (measure either by number of stimulated targets or total number of induced spikes). Correlation between targets paired together increased as the targets continued to be stimulated simultaneously, suggesting a Hebbian strengthening of connections between them. Avalanches were power-law distributed in size and duration, and scaled parabolically, even as correlations and drive increased, suggesting a mechanism to balance those changes and maintain critical dynamics in the system, optimizing numerous aspects of information process for the brain.

Disclosures: T.L. Ribeiro: None. **B. Gifford:** None. **V. Sinfuego:** None. **D. Plenz:** None.

Poster

PSTR430

Optogenetic Tools

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR430.21/Y20

Topic: I.08. Methods to Modulate Neural Activity

Title: Imaging and stimulating neurons in freely behaving rodents using a multi-color multi-region miniscope

Authors: A. PAPANICOLAOU, J. KANEM, **T. MAHMOUDI**, Y. LEE, R. CHRISTO, C. SANTOS, *Y. SOUDAGAR;
Bruker, Toronto, ON, Canada

Abstract: Advances in technology are unveiling new perspectives on the structural connectivity and functional dynamics of brain circuits. Optogenetic modulation combined with imaging offers insight into the roles and dynamic interactions of neuronal populations driving specific behaviors. Here we report a pioneering multi-color multi-region miniscope capable of simultaneously and longitudinally imaging and modulating neuronal activity in up to four areas of the animal brain. Generally, transgenic mice or viral expression are utilized to express cell-type-specific multicolor fluorescence tags, such as GCaMP or RGECO for imaging, and Channel Rhodopsin and ChrimsonR for optogenetics stimulation. Up to four surgically implantable GRIN Lens-Connectors (GLCs) enable imaging/stimulation of hard-to-reach brain regions in various animal species. During imaging/stimulation with behavior sessions, imaging fiber bundles (IFBs) transmit three different illumination colors, from multiple LED sources, to the implanted GLCs: Blue (470nm +/-12nm, up to 5mW) for GFP imaging or Channel Rhodopsin stimulation, green (550nm +/-10nm, up to 0.9mW) for RFP imaging and red (630nm +/-10nm, up to 2.5mW) for

CrimsonR optical stimulation. Each GLC forms the image of neurons above its distal end, which is then transmitted to the camera via the IFB. This design maintains optical components away from the animal's head, enabling lightweight implants (<1.5g) with a small footprint. We report successful simultaneous imaging of neurons in a combination of deep and shallow brain areas, the hippocampus, amygdala, and medial prefrontal cortex, during open-field behavior paradigm in mice. Further more, we report a time management system that enables the synchronization of the brain and behavior movies with time-tagging of eight behavioral event channels. We demonstrate how this time management system and our developed control electronics enable the capability of closed-loop optogenetics stimulation, with diverse optogenetics protocols.This novel multi-color multi-region miniscope facilitates accurate multiregional longitudinal assessments of neuronal subpopulations in freely behaving subjects, providing a circuit-level understanding of brain function. The combined optogenetics capability with imaging, allows one to not just study correlations between neuronal activity and particular behavior, but establish a causal connection. Such insights accelerate our comprehension of brain mechanisms and optimize therapeutics for brain disorders.

Disclosures: A. Papanicolaou: A. Employment/Salary (full or part-time):; Bruker (Full Time). **J. Kanem:** A. Employment/Salary (full or part-time):; Bruker (Full Time). **T. Mahmoudi:** A. Employment/Salary (full or part-time):; Bruker (Full Time). **Y. Lee:** A. Employment/Salary (full or part-time):; Bruker (Full Time). **R. Christo:** A. Employment/Salary (full or part-time):; Bruker (Full Time). **C. Santos:** A. Employment/Salary (full or part-time):; Bruker (Full Time). **Y. Soudagar:** A. Employment/Salary (full or part-time):; Bruker (Full Time).

Poster

PSTR430

Optogenetic Tools

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR430.22/Y21

Topic: I.08. Methods to Modulate Neural Activity

Title: A test bed for evaluating fast excitatory opsins

Authors: Z. MOHAMMADREZAEE, *J. R. VOISARD, A. ARVANITOGIANNIS, P. SHIZGAL;
Concordia Univ., Montréal, QC, Canada

Abstract: Excitatory opsins are critical for determining the causal roles of neural populations. Their effectiveness depends on photocurrent magnitude and on inducing firing rates that match those of the target population. The most commonly used opsin, channelrhodopsin-2, produces modest photocurrents with a photocycle much longer than the minimum inter-spike intervals seen in large, myelinated axons. While the common target of the optogenetic stimulation are cell bodies and axon terminals, the effectiveness of optogenetic stimulation in large myelinated axons

remains relatively unexplored. The pyramidal tract contains a substantial population of such axons. Their high maximal firing rate and the observability of the induced response make them a promising test bed for evaluating the effectiveness of excitatory opsins in activating large myelinated axons. Here, we describe a procedure for determining the relative effectiveness of fast, potent opsins in stimulating the pyramidal tract axons. As a first step, we used electrical stimulation to fire pyramidal-tract axons and developed a rating scale for stimulation-induced movement. Reliable estimates of frequency-following were obtained. These provide a benchmark against which the ability of various opsins to induce high-frequency firing in myelinated axons can be assessed.

Disclosures: Z. Mohammadrezaee: None. **J.R. Voisard:** None. **A. Arvanitogiannis:** None. **P. Shizgal:** None.

Poster

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Optogenetic Tools

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Program #/Poster #: PSTR430.23/Y22

Topic: I.08. Methods to Modulate Neural Activity

Support: 1K99NS128250-01

Title: A real-time closed-loop system for modeling and manipulating neural population dynamics for action using targeted 2-photon stimulation

Authors: *V. ATHALYE[1], I. RODRIGUES-VAZ[2], D. S. PETERKA[2], R. M. COSTA[3]; [1]Zuckerman Inst., Columbia Univ., New York City, NY; [2]Zuckerman Inst., Columbia Univ., New York, NY; [3]Zuckerman Mind Brain Behavior Inst., The Allen Inst., Seattle, WA

Abstract: A fundamental goal of neuroscience is to reveal the dynamics and effect of specific neural populations for generating behaviors. In the past decade, there have been major advances in the development of all-optical interfaces to interrogate and control neural activity. However, one major challenge has been implementing these tools in real time for fast control of ongoing neural activity linked to motor action. Another challenge has been to monitor and stimulate specific cells deep in the brain. Here we developed a system for modeling population dynamics that encode specific features of motor action, and for translating this model into a closed-loop stimulation protocol to test the model. The system automatically defines which pattern to stimulate and when, and we used this system to stimulate deep in the brain through a GRIN lens. With this flexible control, this system promises to reveal principles of dynamics for how a specific neural population drives future neural activity and motor action. We have implemented this in a behavioral task in which head-fixed mice performed two forelimb actions in a self-paced manner, consisting of a push or pull force on an immobile joystick without overt movement. We used fast 2-photon microscopy to image the calcium activity (via GCaMP6f) of ~100 neurons in

dorsolateral striatum through a GRIN lens, and we optimized a protocol to track the same neurons over many days (30-60 days) of behavioral training. We found that dynamics that were shared across neurons stably encoded what action the animal would perform across days. We applied Factor Analysis to extract a low-dimensional latent state that captures information of how neurons are co-activated, and a linear Support Vector Machine to reliably decode this latent state into an overt behavior - whether the animal would perform a push versus pull - that worked across days. We then designed a system that stimulated a series of individual neurons that participated most in the latent states that predicted action. The system used custom hardware to trigger stimulation in closed-loop with 1ms latency based on a threshold crossing of force amplitude. Our preliminary results (10 animal-sessions across 4 animals) showed that activating an ensemble specific to one action increased the force amplitude only of that action. Altogether, we have developed a system to model and manipulate neural dynamics driving action, which allows us to design and trigger stimulation patterns deep in the brain and with low-latency, not only based on behavioral events but also on ongoing neural activity. Our preliminary results reveal that the dorsolateral striatum has more granular control of action than previously thought.

Disclosures: V. Athalye: None. **I. Rodrigues-Vaz:** None. **D.S. Peterka:** None. **R.M. Costa:** None.

Poster

PSTR430

Optogenetic Tools

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR430.24/Y23

Topic: I.08. Methods to Modulate Neural Activity

Support: HHMI Gilliam Fellowship

Title: Parapinopsin: a photoswitchable GPCR for two-photon optogenetics

Authors: *B. BROWN[1], B. A. COPITS[2], R. W. GEREAU IV[3];
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Abstract: The development of optogenetic tools has greatly advanced the capability of researchers to understand functions of the nervous system by allowing for the precise regulation of activity within neurons. Optogenetics uses light-activated ion channels, ion pumps, or G-Protein coupled receptor (GPCR-based) proteins called opsins to excite or inhibit neurons. Paired with advances in microscopy and genetics, optogenetics can be used to control specific populations of cells in a time-locked, spatially specific manner. The experiments possible with optogenetics depend largely on the capabilities and limitations of the opsin used; thus, there have been continuous efforts to design and employ new opsins with a variety of characteristics to allow for more diverse optogenetic manipulations. An example of this has been the more recent

developments of two-photon (2P) activated optogenetic tools and GPCR- based opsins, the focus of this study.
Despite the key role optogenetics has played within neuroscience, the number of tools available for neuronal excitation greatly outnumber those for neuronal inhibition. Most inhibitory opsins available are ionotropic and are restricted by off-target effects, poor performance at synaptic terminals, and require constant light to maintain their effect. Inhibitory GPCR-based systems are effective at synaptic inhibition, but few of these optogenetic tools are available; to our knowledge, none of these have been implemented as tools for multi-photon optogenetics. This spectral confinement excludes inhibitory optogenetics from many of the advantages of 2P microscopy such as greater depth penetration and more precise z-axis targeting.
In 2021, we (Copits et. al. 2021) and Mahn et. al. (2021) published studies addressing some limitations of current inhibitory optogenetic tools. Our publication identified parapinopsin (PPO) as a bistable GPCR-coupled opsin that rapidly and reversibly inhibits synaptic terminals. Our publication included preliminary data suggesting that PPO could be utilized in studies where 2P activation is favorable over single-photon (1P) activation; we now have new data supporting PPO as a 2P-activated inhibitory opsin. Building on our last publication, we recently discovered that 2P-acticated PPO can couple to G-protein Inwardly Rectifying K+ channels (GIRK channels), which can allow for GPCR-based inhibition in neurons via. We will present: 1) a characterization of GIRK current responses to 2P activation of PPO and 2) an exploration of the capabilities of 2P-activated PPO (2P-PPO) in neurons.

Disclosures: B. Brown: None. **B.A. Copits:** None. **R.W. Gereau:** Other; Neurolux.

Poster

PSTR430

Optogenetic Tools

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Program #/Poster #: PSTR430.25/Y24

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF Frontiers BCS 1926676
NSF Frontiers BCS 1926668
NIH 1UF1NS116241
NIH R01NS118188

Title: A MEMS miniature two-photon microscope, incorporating patterned optogenetic stimulation for comprehensive all-optical neural recording and modulation - Opto2P-FCM

Authors: *M. ZOHRABI[1], G. FUTIA[3], C. M. MCCULLOUGH[4], A. TEEL[5], F. M. SIMOES DE SOUZA[6], R. OROKE[2], V. BRIGHT[2], D. RESTREPO[7], E. GIBSON[8], J. T. GOPINATH[2]; [1]Electrical, Computer & Energy Engin., [2]Univ. of Colorado Boulder, Boulder, CO; [3]Dept. of Bioengineering, Univ. of Colorado Anschutz Med. Campus, Denver, CO; [4]Bioengineering,

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Abstract: In vivo miniature microscopes are invaluable tools in various fields of research, including neuroscience, biology, and medicine. Particularly in neuroscience, these devices are used to study neuronal dynamics, synaptic transmission, and network connectivity in awake, behaving animals, providing insights into brain function and its behavioral context. Multiphoton miniature microscopes provide enhanced depth access and improved cellular localization compared to their one-photon microscope counterparts. However, these microscopes necessitate a fiber tether to deliver ultrafast excitation light. Previously, two-photon (2P) miniature microscopes have been engineered by using either a coherent-imaging fiber bundle (CFB) and distal scanning element or an on-board MEMS scanner. Here, we demonstrate the first of a kind 2P photostimulation to the MEMS based 2P miniature microscopes, Opto2P-FCM, enabling simultaneous imaging and photostimulation by precisely activating or inhibiting specific subsets of cells in an overlapping field of view of 250 × 250 microns. 2P photostimulation using 1030 nm light is holographically patterned with a spatial light modulator (SLM) relayed through a CFB to the miniature microscope. The SLM provides rapid switching of illumination patterns to stimulate select regions (or cells) of interest. In addition to 2P photostimulation, the Opto2P-FCM has several novel features including the delivery of the 920 nm imaging laser through a standard, commercially available polarization maintaining (PM) fiber, fabrication of the device housing using a consumer-grade resin 3D printer and using off-the-shelf optical components. In-vivo 2P imaging and photostimulation in the somatosensory cortex of a head-fixed mouse expressing jGCaMP7s and ChRmine is demonstrated using the Opto2P-FCM.

Disclosures: M. Zohrabi: None. **G. Futia:** None. **C.M. McCullough:** None. **A. Teel:** None. **F.M. Simoes de Souza:** None. **R. Oroke:** None. **V. Bright:** None. **D. Restrepo:** None. **E. Gibson:** None. **J.T. Gopinath:** None.

Poster

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Optogenetic Tools

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Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant MH122987
NIH Grant GM140130

Title: Functional labeling of individualized postsynaptic neurons using optogenetics and trans-tango in Drosophila (FLIPSOT)

Authors: *L. NI;
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Abstract: A population of neurons interconnected by synapses constitutes a neural circuit, which performs specific functions upon activation. It is essential to identify both anatomical and functional entities of neural circuits to comprehend the components and processes necessary for healthy brain function and the changes that characterize brain disorders. To date, few methods are available to study these two aspects of a neural circuit simultaneously. In this study, we developed FLIPSOT, or functional labeling of individualized postsynaptic neurons using optogenetics and *trans*-Tango. FLIPSOT uses (1) *trans*-Tango to access postsynaptic neurons genetically, (2) optogenetic approaches to activate (FLIPSOTa) or inhibit (FLIPSOTi) postsynaptic neurons in a random and sparse manner, and (3) fluorescence markers tagged with optogenetic genes to visualize these neurons. Therefore, FLIPSOT allows using a presynaptic driver to identify the behavioral function of individual postsynaptic neurons. It is readily applied to identify functions of individual postsynaptic neurons and has the potential to be adapted for use in mammalian circuits.

Disclosures: L. Ni: None.

Poster

PSTR430

Optogenetic Tools

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR430.27/Y26

Topic: I.08. Methods to Modulate Neural Activity

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Simons Foundation grant 872599SPI
Alfred P. Sloan Foundation grant SP-2022-19027
The Christina Enroth-Cugell and David Cugell fellowship

Title: Synthesizing artificial perception with wireless transcranial optogenetic arrays

Authors: *M. WU[1], Y. YANG[2], J. ZHANG[1], C. H. GOOD[3], A. BANKS[3], L. PINTO[4], J. M. COX[4], Y. KOZOROVITSKIY[5], J. ROGERS[1];
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Abstract: Perception is a fundamental cognitive process by which organisms interpret environmental signals, forming conscious representations of the external world. This process is crucial for understanding and interacting with our surroundings. The development of artificial perception, which bypasses traditional sensory pathways, opens new possibilities for both healthy individuals and those with sensory impairments to experience extended realities. This advancement is key in the ongoing evolution of modern brain-machine interfaces (BMIs), although challenges persist in conveying encoded information without direct sensory input or physical connections. In our research, we made a platform for the wireless transmission of digital data directly to a widespread network of cortical neurons. We employed a minimally invasive array of individually addressable optogenetic stimulators, capable of delivering precise activation patterns to specific brain areas through the skull. The subdermal implantable design of our device facilitates the exploration of artificial perception under naturalistic conditions. Furthermore, our detailed simulations of light penetration and thermal management through the skull yield valuable insights for future designs of optical BMIs. With this innovative technology, we developed a framework to analyze artificially induced cortical activation patterns in relation to perception. Our results demonstrate that the ability to distinguish artificial perceptions correlates with the similarity of spatial features and is influenced by the sequence of cortical activations. These findings reveal a core principle regarding how synthetic cortical activity is interpreted, validating the effectiveness of our engineering approach in addressing new biological questions. Our techniques allow for the precise, spatiotemporal manipulation of neural representations across extensive brain networks. The versatility and scalability of our platform, combined with open-source tools, enable in-depth exploration of fundamental perceptual mechanisms. This research holds significant implications for the advancement of BMIs, expanding the range of studies into artificial neural syntaxes comprehensible to the brain.

Disclosures: M. Wu: None. **Y. Yang:** None. **J. zhang:** None. **C.H. Good:** None. **A. Banks:** None. **L. Pinto:** None. **J.M. Cox:** None. **Y. Kozorovitskiy:** None. **J. Rogers:** None.

Poster

PSTR430

Optogenetic Tools

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Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Award 5R43NS127710
NSF Award 2212906
OEDIT Advance Industries Grant CTGG1 2024-2090

Title: Next-generation ultrathin endomicroscopy for high-resolution, minimally invasive imaging of deep brain structures

Authors: *L. LAMBOT[1], S. E. MONTGOMERY[1], O. TZANG[2,3], R. PIESTUN[2], A. CARAVACA AGUIRRE[1];
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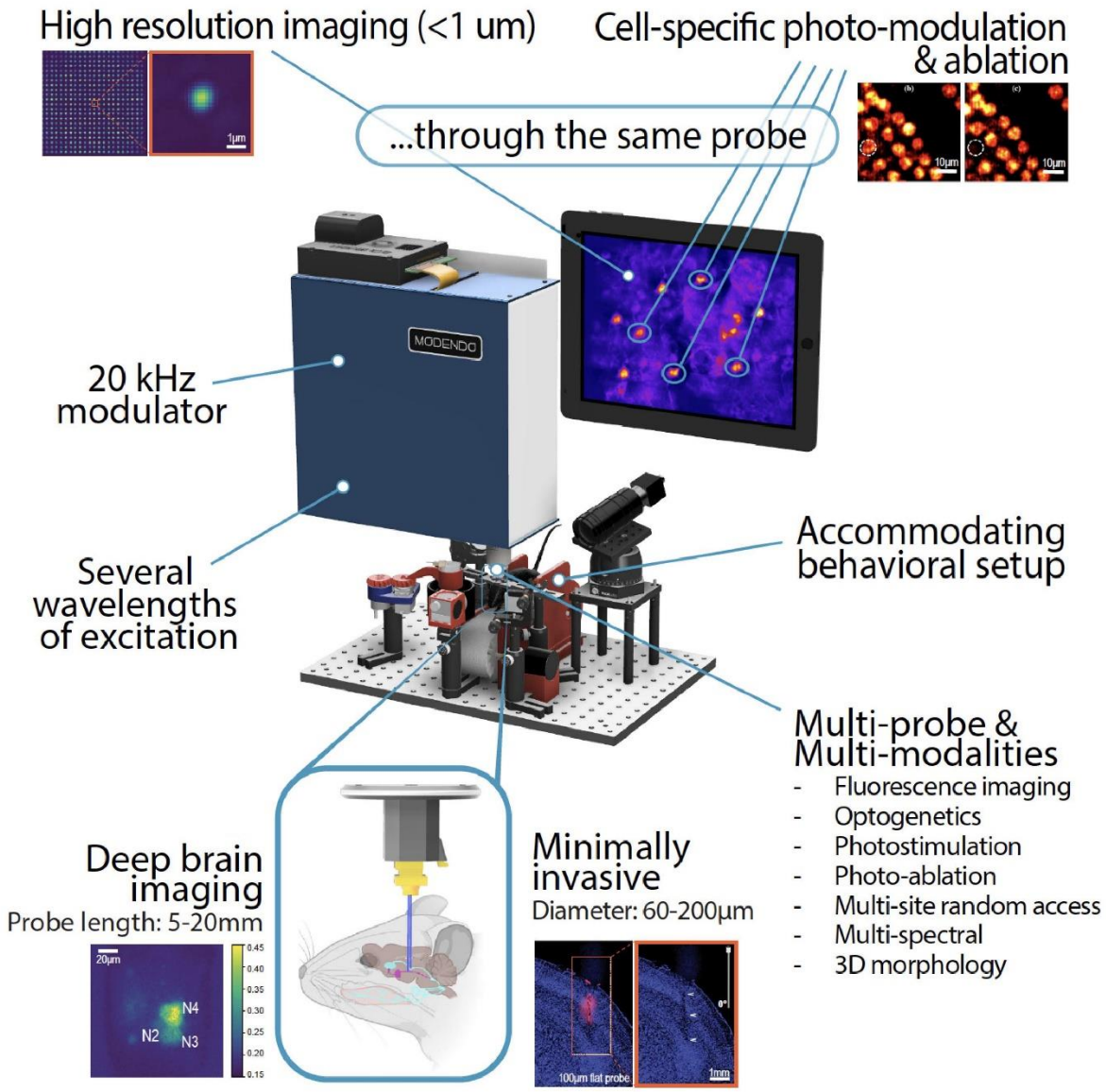
Abstract: Over the past decade, the notion that isolated neuron populations support cognition and behavior has shifted towards an understanding that complex behaviors emerge from interactions within anatomically connected, specialized networks. Advances in brain imaging techniques have expanded the tools available to explore the functional organization of these networks. However, traditional methods face significant limitations, damaging the tissue, disrupting synaptic connections, and destroying the brain's integral circuit architecture. Here, we introduce a commercial prototype that employs wavefront shaping through a hair-thin probe to resolve cellular and sub-cellular structures with spatial, sub-micron precision. Utilizing computational optics, this system generates arbitrary, digitally programmed light patterns at the distal tip of the probe at a speed of 23 kHz. Real-time high-resolution 2D and 3D images are acquired via a user-friendly interface. Our ultrathin endomicroscope is compact, mobile, and well-suited for head-fixed recordings in rodents while supporting multiple laser inputs. Endoscopic imaging experiments conducted *in vitro* with phantom brains containing fluorescent beads, *ex vivo* brain slices, as well as *in vivo* in mice demonstrate single-cell precision across multiple imaging planes, including the capability to visualize somatic and dendritic compartments with minimal tissue impact. Multi-color imaging through the same fiber probe facilitates the visualization of fluorescent reporters and the acquisition of neuronal activity of distinct ensembles using genetically encoded calcium indicators. Current efforts in developing this technology focus on single-cell optogenetic modulation and multi-site recordings during both anesthetized and awake, behaving animal experiments. This ultrathin endomicroscopy opens new avenues for both structural and functional imaging, providing unprecedented access to deep brain structures in vivo and the capability to map and manipulate cells across intact neural networks with minimal damage.



Disclosures: L. Lambot: None. **S.E. Montgomery:** None. **O. Tzang:** None. **R. Piestun:** None. **A. Caravaca Aguirre:** None.

Poster

PSTR430

Optogenetic Tools

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Program #/Poster #: PSTR430.29/Y28

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant U01NS118288
NIH Grant RF1NS133657

Title: Efficiency and limitation of K^+-conducting channelrhodopsins as optogenetic inhibitors

Authors: *Y. GOU[1], Y. WANG[3], Z.-L. CAI[2], H. CHEN[2], M. XUE[2];
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Abstract: Optogenetically manipulating neuronal activities, either by inhibition or activation, helps understand the necessity or sufficiency of neurons in brain functions. However, there are no optimal optical silencers, as the existing molecular tools produce small photocurrents, have low temporal accuracy, or create undesired excitatory effects. The discovery of K^+-conducting channelrhodopsins (KCRs) HcKCR1 and WiChR, generates new possibilities for neural inhibition, as potassium channels are naturally utilized to repolarize membrane potentials in neurons. Therefore, we sought to measure the efficacy of KCRs in silencing neural activities. We firstly reduced the cellular toxicity and enhanced the membrane trafficking of KCRs by the addition of trafficking motifs. We then expressed KCRs in either the lateral geniculate nucleus (LGN) or primary visual cortex (V1) and determined how light activation of KCRs in V1 modulated visual activities. We found that KCR activation can inhibit visual activities in V1 in both preparations. However, we also observed that KCR activation can excite neurons and cause seizures besides the inhibitory effects. Therefore, the use of KCRs to inhibit neural activities must be carefully examined for different experimental conditions.

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Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.01/Y29

Topic: I.04. Physiological Methods

Title: Local and Inter-areal Circuit Recruitment Patterns of Precisely-Defined Cortical Neuronal Ensembles

Authors: *A. DRINNENBERG[1], A. ATTINGER[2], A. RAVENTOS[3], C. RAMAKRISHNAN[3], T. L. DAIGLE[5], L. SIVERTS[7], S. QUIRIN[4], S. GANGULI[3], B. TASIC[6], H. ZENG[8], L. M. GIOCOMO[2], K. DEISSEROTH[9];
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Abstract: The diverse and numerous cell types of the brain can be considered as the elementary units of brain function. Genetic access to molecularly defined cell types for specific observation and perturbation has revealed important insights into brain function. Yet, in regions heavily shaped by experience– like the mammalian neocortex– neurons with the same molecular identity can exhibit unrelated or even oppositional function. In these cases, distinct targeting strategies are needed to manipulate the circuit's functional units, such as two-photon holographic all-optical approaches, which have opened new possibilities for causal perturbation of functionally defined ensembles. Here we probe aspects of the functional logic of neocortex by testing how large ensembles with distinct functional properties influence each other in local and inter-areal circuits. We first substantially improved the 'read-write' accessibility of cortical circuits by developing expression-enhanced, ribosome-tethered probes and transgenic lines to deliver the excitatory channelrhodopsin ChRmine stably and reliably over far greater spatial and temporal scales than previously achieved. Our approach enables all-optical experiments with decreased neuropil contamination and 'read-write' access to >1,000 individually-specified neurons, and 'read' access to >10,000 individually-specified neurons, in a single field-of-view. We next stimulated large functional ensembles in visual cortex and found that, consistent with known connectivity rules, neurons with similar orientation tuning or highly-correlated responses to natural scenes preferentially recruit each other. A classifier trained on synaptically-evoked activity of the non-stimulated population was able to discriminate different ensembles, even if the stimulation was restricted to a neighboring cortical area. While some synaptically-recruited cells ('followers') were unique for a given ensemble, surprisingly, we also found cells that became recruited by any large ensemble written into the circuit. These general-purpose followers were found to be enriched in deeper layers and highly synchronized during spontaneous activity, and were observed to exhibit specific visual properties, suggesting functional contributions to specific brain states and cognitions.

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Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.02/Web Only

Topic: I.04. Physiological Methods

Title: Personalized percutaneous network mapping enables acute amelioration of refractory chronic pain

Authors: *Y. HUANG[1], T. LIU[2], B. REID[1], N. KABOODVAND[2], A. CHIBUKHCHYAN[2], K. PATRON[2], L. YAMADA[3], E. CHOI[4], A. FOGARTY[2], P. D. MCGEOCH[1], A. G. RAMAYYA[2], P. NUYUJUKIAN[5], K. DEISSEROTH[2], V. BUCH[1];
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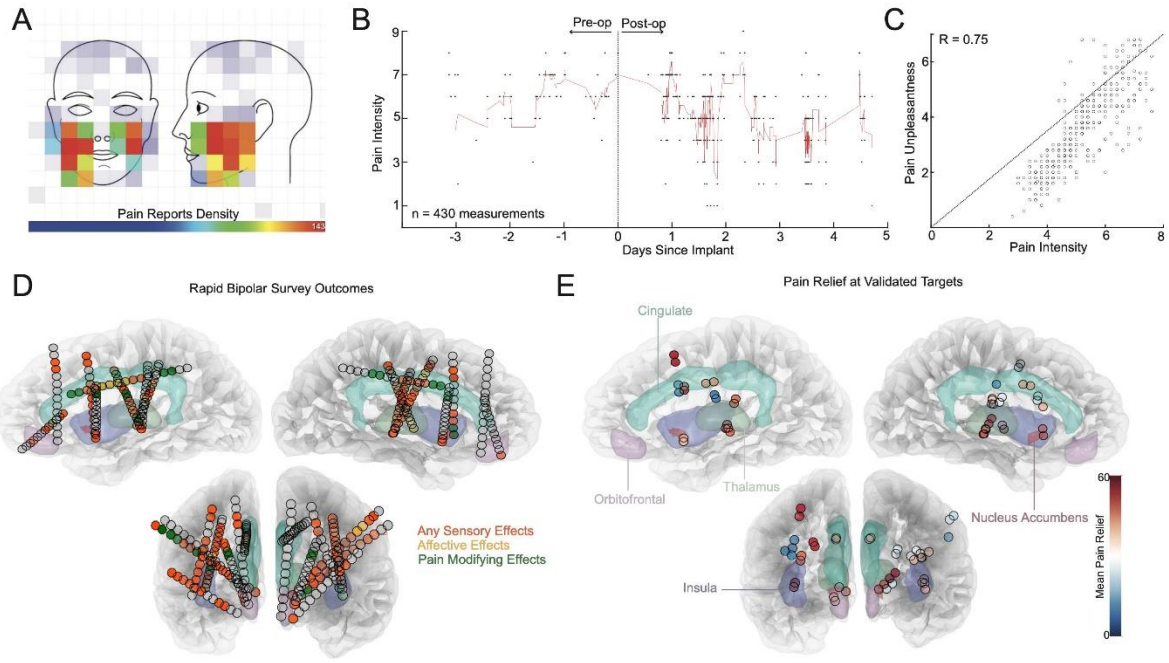
Abstract: Chronic pain poses a significant burden, with limited effective treatments for refractory cases. Deep brain stimulation (DBS) has shown promise, but response rates are heterogeneous, and long-term pain relief is often not achieved. We considered that personalized network mapping using intracranial stimulation may improve outcomes. As part of an institutional innovative care trial, we present the results of our first patient with refractory right-sided atypical facial pain and left-sided anesthesia dolorosa who underwent multisite intracranial monitoring and stimulation. Using a high fidelity custom-built human neural recording and stimulating infrastructure, we performed 430 naturalistic (provocation induced using naturalistic stimuli) and temporally-precise assessments of pain unpleasantness and intensity over 5 inpatient days. Rapid bipolar stimulations were surveyed across 124 non-overlapping sites, identifying sensory changes in 55%, affective changes in 6%, and pain modifying changes in 15% of sites. Validation of candidate sites resulted in 26 distinct anatomical targets, including known (paraventricular gray matter, anterior cingulate, face motor cortex, sensory thalamus) and novel (anterior and posterior insula, caudate, inferior fronto-occipital fasciculus) sites providing pain relief. Right-sided stimulation achieved a mean pain relief of 34% for left anesthesia dolorosa from baseline ($n=21$, $p<0.001$, paired t-test) and 54% from provoked maximal pain ($n=8$, $p<0.001$). Left-sided stimulation achieved a mean pain relief of 39% for right atypical facial pain from baseline ($n=23$, $p<0.001$) and 39% from provoked maximal pain ($n=9$, $p=0.002$). Sham stimulation failed to provide significant relief (7% reduction, $n=7$, $p=0.34$). Pain alleviation was rapid in onset and reproducible. These findings demonstrate the feasibility of rapid, percutaneous, multisite brain mapping to identify therapeutic targets and potentially guide personalized DBS for chronic pain. *first 3 authors contributed equally, last 3 authors are co-senior status.



Disclosures: Y. Huang: None. **T. Liu:** None. **B. Reid:** None. **N. kaboodvand:** None. **A. Chibukhchyan:** None. **K. Patron:** None. **L. Yamada:** None. **A. Fogarty:** None. **P.D. McGeoch:** None. **A.G. Ramayya:** None. **P. Nuyujukian:** None. **K. Deisseroth:** None. **V. Buch:** None.

Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.03/Y30

Topic: I.04. Physiological Methods

Support: R01MH075957

Title: Structure-guided generation of diverse inhibitory functionality using protist-derived microbial channels

Authors: *Y. KIM[1], P. Y. WANG[2], Y. JO[3], A. DRINNENBERG[4], S. TAJIMA[5], C. RAMAKRISHNAN[2], K. DEISSEROTH[6];
[1]Stanford Univ., Palo Alto, CA; [2]Stanford Univ., Stanford, CA; [3]Applied Physics, Stanford Univ., Stanford, CA; [4]Stanford Univ. CNC, Stanford Univ., Palo Alto, CA; [5]Univ. of Tokyo, Tokyo, Japan; [6]Stanford, Stanford, CA

Abstract: The KCR channelrhodopsins represent a class of light-gated ion channels that allow the passage of K^+ ions with a certain amount of selectivity of Na^+ ions. Previously, we elucidated the first high-resolution structures of KCRs, uncovering a novel mechanism for achieving K^+ selectivity, along with structure-guided variants featuring new functionalities. In this study, we demonstrate the potential applications of these novel KCR variants across various experimental settings in neuroscience, spanning from single-cell stimulation to population dynamics. The C110T variant displays prolonged kinetics (up to 1000-fold) and improved sensitivity. When also coupled with the Y222A mutation, which switches channel functionality from excitatory to inhibitory by allowing both Na^+ and K^+ flux (Erev shift to ~0 mV), C110T mutant-expressing cells display extraordinarily high currents and light sensitivity which enables precise and high-speed modulation of behavior in mice with light delivery from outside the skull (even for deep midbrain targets). Furthermore, the KALI (K^+-selectivity-Augmented Light-gated Ion-channel, H225F) variants exhibit significantly hyperpolarized reversal potentials (shifted by more than 10 mV) for both KCR1 and KCR2 variants (denoted as KALI-1 and KALI-2), and demonstrate markedly improved performance for single-cell- and population-level optogenetics. Finally, we have resolved the high-resolution structures of all three of these enhanced variants (C110T, Y222A, H225F) and conducted computational, electrophysiological, and biochemical analyses to further elucidate structural and mechanistic details of designed kinetics, selectivity, and functionality in biological systems. Together, our findings not only provide a framework for the development of next-generation inhibitory optogenetic tools but also define and elucidate a novel non-tetrameric mechanism for robust K^+ selectivity in ion channels.

Disclosures: Y. Kim: None. **P.Y. Wang:** None. **Y. Jo:** None. **A. Drinnenberg:** None. **S. Tajima:** None. **C. Ramakrishnan:** None. **K. Deisseroth:** None.

Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.04/Y31

Topic: I.04. Physiological Methods

Support: HHMI

Title: Identification of Control Hubs Governing Large-Scale Cortical Networks

Authors: *J. KOCHALKA[1], A. MITRA[2], K. SHENG[3], S. DRUCKMANN[3], K. DEISSEROTH[3];
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Abstract: Mammalian cognition may involve coordination of neural activity across distinct and distributed sets of cortical areas, many of which have been assigned to large-scale functional

networks based on activity in sensorimotor and cognitive tasks. While these networks have been presumed to be important for behavior, their governing principles (for example, whether particular nodes causally recruit these network activity patterns)— remain completely unknown. To address this question, we began with imaging cortex-wide spontaneous activity in head-fixed, awake mice (N=3), assessing Ca2+ signals via GCaMP6f within excitatory cells located predominantly in layer II/III. Utilizing convolutional non-negative matrix factorization to capture spatiotemporal regularities in these data, we discovered six factors, or "basis motifs"— closely corresponding to previously reported functional networks— representing consistently repeated temporal patterns of spontaneous cortical activation. To examine the potential contributions of specific cell types to these network-wide events, we extended our imaging assay to new cohorts of mice expressing GCaMP in specific neuronal subpopulations, defined by vasoactive intestinal polypeptide (N=3), somatostatin (N=3), or CaMKIIα (N=6) genetic regulatory elements; we observed remarkably similar motif structure in all cases. Based on this reliable structure of the basis motifs, we hypothesized that activity within regions contributing to early features of a specific spatiotemporal pattern would elicit consistent later (downstream) patterns in the corresponding network. To test this hypothesis, we developed an optical system that enabled simultaneous one-photon patterned optogenetic control combined with wide-field Ca2+ imaging. Consistent with our prediction, we found that single, brief optical pulses in "source" areas of cortical networks were sufficient to elicit downstream "sink" neural responses. To assess the behavioral impact of this basis-motif activity, we developed a visual detection task wherein water-deprived mice (N=6) were trained to lick for a water reward in response to a small, low-contrast stimulus appearing on an otherwise gray screen. Bringing our all-optical assay to this behavioral paradigm revealed that causal and specific recruitment of Vibrissal, Motor, and Default Mode network motifs (but not other observed network motifs) prior to stimulus onset significantly improved performance (increasing hit rate and reducing reaction time). Together, these results highlight the critical contribution of large-scale network dynamics to even relatively simple sensorimotor behaviors.

Disclosures: J. Kochalka: None. **A. Mitra:** None. **K. Sheng:** None. **S. Druckmann:** None. **K. Deisseroth:** None.

Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.05/Y32

Topic: I.04. Physiological Methods

Support: The Shurl and Kay Curci Foundation
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Title: Circuitry of the preparatory period: a role for thalamocortical circuit dynamics in optimizing spontaneous attentional control prior to cognitive engagement

Authors: *M. HEDLUND[1], S. SADEGHZADEH[1], J. BERNABEI[3], G. NG[4], T. HO[1], C. SHILYANSKY[5], A. G. RICHARDSON[6], K. DEISSEROTH[1], V. BUCH[2];
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Abstract: Human cognition acts through the complex coordination of interconnected networks. Spontaneous network states prior to cognitive engagement may prime the brain for differential cognitive performance. Thalamocortical (TC) communication is known to be a crucial element of attentional processes in humans and mice. In this multi-species study, we find that spontaneous TC communication dynamics predict performance on a trial-by-trial basis. We employed a temporal expectancy task in 24 humans and 6 wild type mice, consisting of an initial cue followed by a go cue separated by a variable time interval, and a response to the go cue. Human subjects were implanted with iEEG, and mice were injected with a fluorescent Ca2+ indicator (GCAMP6m)-carrying AAV with expression targeted to CaMKIIa+ neurons of the mediodorsal thalamus (MD), and with neural activity subsequently recorded using fiber photometry in MD and prefrontal cortex (PFC). In humans, spectral power and graph communicability (Qexp) (a measure of network-wide communication) were calculated for each of four canonical frequency bands within the 500 ms preparatory (spontaneous, before initial cue) or anticipatory (task-activated, before go cue) periods. The feature space was reduced by performing a univariate linear regression bootstrapped 1000x with a random 80% of trials for each iteration. Features that had a statistically significant relationship with reaction time (RT) on >35% of iterations were selected for further analysis because this threshold performed best in an SVM to classify fast vs slow trials. To see if any anatomical region had metastable effects for predicting RT across subjects, we performed a rank-sum test of selected vs non-selected features from each region. Low frequency white matter Qexp, particularly in TC circuitry, predicts upcoming RT in the preparatory period while predictive features switch to high frequency spectral activity in the anticipatory period. We further explored TC circuit dynamics in mice and observed a robust correlation between RT and activity in MD-PFC TC projections. For trials with the fastest RTs (0-20ms), TC activity surged approximately 4s prior to initial cue onset (preparatory) as well prior to the go cue (anticipatory), compared to other trials (20-500ms) ($p<0.001$). When comparing the fastest vs. the slowest third of trials with RT<100ms, activity significantly increased for the fastest trials in the preparatory period but not the anticipatory period ($p<0.001$). In concordance with our human results, these findings indicate that enhanced TC communication primes mammalian neural circuitry during the preparatory period for robust and swift performance.

Disclosures: M. Hedlund: None. **S. Sadeghzadeh:** None. **J. Bernabei:** None. **G. Ng:** None. **T. Ho:** None. **C. Shilyansky:** None. **A.G. Richardson:** None. **K. Deisseroth:** None. **V. Buch:** None.

Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.06/Z1

Topic: I.04. Physiological Methods

Title: Simultaneous single-cell imaging across cortex, thalamus, and striatum reveals features underlying the circuit dysfunction of tardive dyskinesia

Authors: *L. ENCARNACION-RIVERA[1], J. H. JENNINGS[2], S. QUIRIN[2], A. CORDERO[2], K. DEISSEROTH[2,3];
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Abstract: A primary function of the brain is to select and implement adaptive behaviors. This role requires robust and specific control over activation and inhibition of motor programs, in a balance that is dysregulated in movement disorders such as tardive dyskinesia (TD). TD is characterized by involuntary movement of the orofacial region and extremities, and arises iatrogenically from prescription use of antipsychotic drugs. Although the circuit architecture for canonical 'direct' and 'indirect' pathways thought to govern action selection and initiation has been well-characterized, we know little about how alterations to this circuit engender involuntary movement. To address this, we developed a novel imaging modality we term Multi-Grin Imaging (MGI), whereby we couple gradient index lenses to high-density fiber bundles to image from multiple deep brain regions simultaneously at single-cell resolution. First, we deployed MGI to record from primary motor cortex (M1) (n=1709 neurons), dorsomedial thalamus (MDT) (n=719 neurons), and dorsomedial striatum (DMS) (n=760 neurons) during spontaneous orofacial behavior (n=7 animals). We discovered differential encoding of orofacial movement (M1<MDT, $p<0.001$ | DMS<MDT, $p<0.001$) and interregion correlation among M1, MDT, and DMS populations (DMS & M1 > MDT & M1, $p<0.001$ | DMS & M1 > MDT & DMS, $p<0.001$). We next validated a mouse model for TD by chronically administering the D2 receptor antagonist haloperidol, giving rise to a state characterized by abnormal TD-like movements. In this state, we identifed potentiated activity across M1 (n=1761 neurons $p<0.001$), MDT (n=889 neurons $p<0.001$) and DMS (n=664 neurons $p<0.001$), as well as inter-region decoupling (DMS & M1, MDT & M1, MDT & DMS $p<0.001$), all as key dysregulatory features of the TD state. Consistent with a role for these dynamics in generating involuntary movement, the VMAT2 inhibitor tetrabenazine (n=4 animals) rescued aspects of both the normal neural activity dynamics (increased activity DMS, $p<0.001$ | increased DMS & M1, MDT & DMS $p<0.001$) and the normal behavior (decreased movement, $p<0.05$). To probe circuit dynamics specifically generating involuntary movements, we compared neural activity across M1, MDT, and DMS during involuntary movements clinically analogous to TD presentation in humans (tongue protrusion or TP) vs. a qualitatively similar natural movement (voluntary licking or VL) (n=7 animals). We observed that TP is readily distinguishable from VL specifically through abnormally early striatal activation ($p<0.001$). Overall, our results provide an integrated neural circuit-dynamics and behavioral framework for the involuntary movements of TD.

Disclosures: L. Encarnacion-Rivera: None. **J.H. Jennings:** None. **S. Quirin:** None. **A. Cordero:** None. **K. Deisseroth:** None.

Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.07/Z2

Topic: I.04. Physiological Methods

Support: Fonds de recherche du Québec - Santé (chercheurs-boursiers en intelligence artificielle)
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NSF FOUNDATIONS award (NSF1926800)
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Title: Identifying and modeling brain-wide neural activity structure in larval zebrafish

Authors: *Y. DUAN[1], M. G. PERICH[2], M. BEIRAN[3], T. BENSTER[4], H. CHAUDHRY[6], A. S. ANDALMAN[5], E. S. CARTER[7], K. RAJAN[7], K. DEISSEROTH[5];
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Abstract: Behaving animals continually reconcile the internal states of brain-wide neural circuits with incoming sensory and environmental evidence to evaluate when and how to act. The brains of animals including humans exploit many evolutionary innovations, chiefly modularity—observable at the level of anatomically-defined brain regions, cortical layers, and cell types among others—that can be repurposed in a compositional manner to endow the animal with a highly flexible behavioral repertoire. Accordingly, behavioral output shows its own modularity, yet these behavioral modules seldom correspond directly to traditional notions of modularity in the brain. It remains unclear how to link neural and behavioral modularity in a compositional manner. Here, we propose a comprehensive framework—compositional modes—to identify overarching compositional structure spanning specialized submodules such as brain regions. Our framework directly links the behavioral repertoire with distributed patterns of population activity brain-wide at multiple concurrent spatial and temporal scales. We applied our

method to longitudinal, whole-brain, cellular-resolution neural recordings from larval zebrafish exhibiting spontaneous behavior. We built and analyzed large-scale recurrent neural network models that reproduced the long time-scale dynamics of over 10,000 simultaneously-recorded neurons. We combined this model's outputs-connectivity and inter-region currents-with tensor decomposition to infer, in an unsupervised manner, the compositional modes that describe the brain-wide flow of source and target currents. We reveal highly conserved compositional modes across individuals despite the spontaneous nature of the behavior, and demonstrate experimentally that compositional modes can be manipulated in a consistent manner through behavioral and pharmacological manipulation. Our results show that even spontaneous behavior in different individuals can be decomposed and understood using a relatively small number of neurobehavioral modules—the compositional modes—and elucidate a compositional neural basis of behavior. The striking consistency of these modes across individuals points towards a preserved, compositional basis across individuals. Inspired by such consistency across individuals, we use state-of-the-art state space models from machine learning to build unifying generative models of brain-wide neural activities of different individuals. These models allow us to gain deeper insights into the shared neural dynamics across individual vertebrate animals, as well as identify unique differences underlying diversity in behavior.

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Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.08/Z3

Topic: I.04. Physiological Methods

Support: NIH Grant K08 MH131888
HHMI

Title: Cortex-wide optical screen reveals top-down pathway for adaptive modulation of visual perception

Authors: *A. MITRA[1], J. KOCHALKA[1], S. QUIRIN[2], S. DRUCKMANN[3], S. GANGULI[3], K. DEISSEROTH[4];
[1]Stanford Univ., San Francisco, CA; [2]Stanford Univ., Palo Alto, CA; [3]Stanford Univ., Stanford, CA; [4]Stanford, Stanford, CA

Abstract: Title: Cortex-Wide Optical Screen Reveals Top-Down Pathway for Adaptive Modulation of Visual Perception Anish Mitra*, John Kochalka*, Chandan Kadur, Sean Quirin, Charu Ramakrishnan, Shaul Druckman, Surya Ganguli, Karl Deisseroth[†]* Equal contribution[†]

Corresponding author: deissero@stanford.edu**Abstract**Spontaneous neural activity drives significant variability in visual sensory-evoked responses and perceptual decisions. However, whether this spontaneous activity represents random noise that interferes with perception, or instead a cognitive process for modeling and predicting environmental features, remains uncertain. To investigate this relationship, we developed a two-alternative forced choice visual detection task in mice and examined spontaneous activity at both the cortex-wide and single-neuron scales. A large-scale optical screen using wide-field cortical imaging, in a task requiring specific discrimination of noise vs. formed geometrical visual stimuli, revealed that the spontaneous state of the retrosplenial (RSP) and visual cortices before stimulus onset predicted subsequent perceptual reports. Specific spontaneous activity patterns (that is, neural activity preceding visual stimuli), assessed in the form of single-cell resolution neuronal population dynamics in both RSP and visual cortex, were implicated in these specific perceptual decisions. Furthermore, suggesting an adaptive cognitive process acting through specific neural circuit pathways, the content of this spontaneous activity was observed to adaptively adjust based on environmental statistics and prior errors, and was modulated in a top-down manner from RSP to visual cortex. Consistent with this interpretation, optogenetic inhibition of activity in projection neurons from RSP to visual cortex gave rise to corresponding specific biases in perceptual reports. Finally, a pharmacological agent known to contribute to formed illusory percepts in humans (LSD) favored perceptual reports of formed visual stimuli (even when not present) rather than noise, in a manner consistent with disruption of this top-down influence. Taken together, our findings underscore a crucial role for spontaneous neural activity in shaping visual perception by predicting sensory information and highlight the importance of the retrosplenial cortex in this process as identified through a large-scale optical screen followed by causal investigation and single-cell resolution measurement.

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Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

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Topic: I.04. Physiological Methods

Support: Youth Innovation Promotion Association, CAS Grant 2021266
STI2030-Major Projects (2021ZD0204503)
National Natural Science Foundation of China (32125020)

Title: Parallelized Multiple-beams Two-photon Microscopy for Whole Brain Imaging and Reconstruction of Individual Neurons

Authors: *Z. SHI, Y. ZHAO, L. CONG, L. BAI, L. YE, K. WANG;
Inst. of Neurosci., Chinese Acad. of Sci., Shanghai, China

Abstract: Recent advancements in microscopy and tissue processing techniques have significantly broadened our ability to investigate neuronal structures across extensive spatial scales, from nanometers to whole-brain dimensions. However, traditional high-resolution imaging methods often require invasive sectioning and complex tiling, which can distort tissue architecture and introduce optical aberrations. Addressing these challenges, we have developed a dual-color, parallelized-multiple beams two-photon microscopy system capable of imaging an entire, intact, cleared brain at sub-micrometer resolution with minimal distortions and defocusing, maintaining long-term stability essential for comprehensive neuronal reconstruction. Featuring a numerical aperture of approximately 1.014, it achieves a lateral resolution of about 360 nm and an axial resolution of about 2 μm. By dividing the excitation light source into eight parallel beams for simultaneous scanning and signal collection, it takes approximately 100 hours to automatically image an intact mouse brain. We evaluated the performance of this system by conducting dual-color imaging on an intact cleared mouse brain with sparsely labeled neurons in the visual cortex, achieving high-resolution, brain-wide neuronal reconstruction. Compared to existing imaging methods for cleared samples, our approach eliminates the need for physical sectioning and allows for the preservation of the intact brain for multiple rounds of imaging, without any loss of information.

Disclosures: Z. Shi: None. **Y. Zhao:** None. **L. cong:** None. **L. Bai:** None. **L. Ye:** None. **K. Wang:** None.

Poster

PSTR431

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Topic: I.04. Physiological Methods

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Research
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Title: Minimally invasive optical clearing media for fluorescence imaging of live tissues ex vivo and in vivo

Authors: *S. INAGAKI[1], N. NAKAGAWA[2], Y. KAMBE[3], S. FUJIMOTO[4], Y. TAGAWA[5], T. K. SATO[6], T. IMAI[7];
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Abstract: Tissue-clearing agents have been utilized for three-dimensional fluorescence imaging of fixed tissues, but not for live tissues due to their toxicity. Here we demonstrate a strategy for minimally invasive optical clearing of live mammalian tissues for fluorescence microscopy. We found that the refractive index (RI) mismatch between the cytosol and the extracellular medium is the major cause of light scattering in live cells, suggesting that minimizing the RI mismatch could facilitate the optical clearing of live cells and tissues. We then screened for minimally-invasive tissue clearing agents, focusing on high molecular weight and low osmolarity chemicals. We found that one chemical allows for normal calcium responses and proliferation of cultured cells when dissolved in cultured media to achieve the optimal RI. With additional optimization of the ionic compositions, we established a tissue clearing medium for live tissues, named SeeDB-Live. SeeDB-Live cleared live spheroids and organoids without affecting their proliferation. SeeDB-Live minimally affected the electrophysiological properties of neurons and allowed calcium imaging of acute brain slices up to ~2-fold depth. For example, we were able to image the spontaneous activity of mitral cells in the olfactory bulb at a depth of ~100 μm using conventional confocal microscopy. When applied *in vivo*, SeeDB-Live improved the maximum imaging depth of two-photon imaging in the mouse brain. For example, the brightness of layer 5 (L5) neurons was ~4-fold brighter after the SeeDB-Live treatment, allowing for imaging basal dendrites. SeeDB-Live did not affect the sensory responses in the primary visual cortex and olfactory bulb. SeeDB-Live is particularly powerful for one-photon voltage imaging of neurons with genetically encoded voltage indicators (GEVIs). Using a GEVI and a high-speed CMOS camera, we performed 2 kHz widefield imaging and observed backpropagating action potentials along dendrites in acute brain slices in single-trial experiments, without averaging. Moreover, we were able to reliably detect action potentials in cortical layer 2/3 pyramidal neurons *in vivo* using the simple widefield imaging setup. Thus, SeeDB-Live expands the imaging scale as well as the modalities for studying the dynamics of neuronal circuits, paving the way for deeper insights into brain functions.

Disclosures: S. Inagaki: None. **N. Nakagawa:** None. **Y. Kambe:** None. **S. Fujimoto:** None. **Y. Tagawa:** None. **T.K. Sato:** None. **T. Imai:** None.

Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.11/Z6

Topic: I.04. Physiological Methods

Support: NIH/NIDCD R01 DC014701
NIH/NIDCD R01 DC019124

Title: Integrated workflow for counting fluorescently labeled neurons in atlas-segmented whole mouse brains

Authors: *Y. CHEN, M. EINHORN, C. YANG, T. A. CLELAND;
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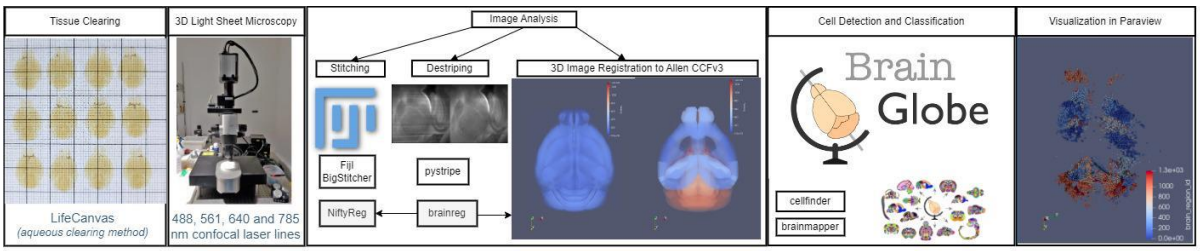
Abstract: Our laboratory is interested in the patterns of activity across brain regions that are recruited into the neural representations of odors as they acquire meaning in diverse ways. To do this, we developed an integrated workflow incorporating behavioral methods, immediate-early gene expression labeling, brain clearing, light-sheet imaging, and computational analysis using open source tools. In a pilot study, FosTRAP genetically modified mice were presented with one of three test odorants (the fox odor component 2,4,5-trimethyl thiazoline (TMT) or the nominally aversive odorants 2-methylbutyric acid or isoamylamine), or a blank stimulus, in a test arena. Behavioral responses were recorded to video and analyzed for freezing/immobility and place preference using Annolid software. Neurons expressing the immediate-early gene c-Fos following odorant exposure were fluorescently labeled via induction of the TRAP method during behavioral performance and/or by postmortem immunohistochemistry. Brains were perfused, extracted, and optically cleared using Lifecanvas aqueous methods, positioned using custom 3D printed mounts, and imaged with a Lavision Ultramicroscope II light sheet microscope. Tiles were stitched using the Fiji BiggerStitcher plugin; images then were destriped when necessary and registered to the Allen Common Coordinate Framework v3 (CCFv3) using the BrainGlobe brainreg tool. Cell detection and classification were performed using the BrainGlobe cellfinder tool with some custom modifications. Labeled cells then were assigned to segmented brain regions according to two CCFv3-registered atlases (Franklin and Paxinos, Allen Mouse Brain Atlas). Results then were visualized using Paraview, enabling interactive exploration and further analysis of the 3D brain volume, including the overlay of labeled cells onto atlas-delineated brain regions or cross-sections.



Disclosures: Y. Chen: None. **M. Einhorn:** None. **C. Yang:** None. **T.A. Cleland:** None.

Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

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Program #/Poster #: PSTR431.12/Z7

Topic: I.04. Physiological Methods

Support: NIH grant 1R01MH11704201
NIH grant 1RF1NS126043
Howard Hughes Medical Institute

Title: Wide area all-optical electrophysiology reveals connectivity and dynamics in cortical Layer 1

Authors: *Y. QI[1], V. J. PAROT[3], D. WONG-CAMPOS[2], P. PARK[2], A. E. COHEN[1]; [1]Chem. and Chem. Biol., [2]Harvard Univ., Cambridge, MA; [3]Inst. for Biol. and Med. Engin., Catholic Univ. of Chile, Macul, Chile

Abstract: Cortical Layer 1 (L1) comprises a sparse population of inhibitory interneurons. These cells modulate sensory processing in the underlying cortex and have powerful reciprocal inhibition among each other. The collective dynamics of the L1 network remain unknown. We combined advances in voltage indicators, channelrhodopsins, and microscopes, to perform simultaneous high-speed voltage imaging and targeted optogenetic perturbation of ~300 L1 interneurons in the barrel cortex of awake mice. We first recorded the spontaneous voltage dynamics over 6 mm^2 of L1 and observed that 1) during quiet wakefulness, global subthreshold oscillations manifest as propagating wavefronts rather than synchronous oscillations, 2) whisking induces global depolarization followed by a depression of population activity, 3) distinct sub-ensembles of neurons showed activity which correlated with distinct aspects of behavior, 4) the covariance structure of the population activity is cell-type dependent. Next, we measured the electrophysiological properties of these neurons by stimulating individual neurons under increasing strengths of optogenetic drive. We demonstrate that 1) the in vivo electrophysiological properties (firing rate adaptation, membrane constant, after-hyperpolarization etc.) cluster around cell types, 2) these properties correlate with spontaneous firing patterns during locomotion and free whisking. Finally, we mapped the gap junctional and synaptic connections among these neurons by repeatedly stimulating each cell and probing the subthreshold responses of all others. We find that 1) both synaptic and gap junctional strength decay with distance, 2) synaptic and gap junctional strengths co-vary, 3) synaptic connectivity is cell type dependent, 4) mutual inhibition explains whisking induced activity depression.

Disclosures: Y. Qi: None. **A.E. Cohen:** None.

Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

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Program #/Poster #: PSTR431.13/Z8

Topic: I.04. Physiological Methods

Support: NIH Grant NS122123
NIH Grant NS103518

Title: System and method for calcium imaging and electrophysiology in non-human primates

Authors: *B. PESARAN[1], I. C. GARWOOD[1], K. WINGEL[1], J. HAGGERTY[1], A. CHARLES[2], J. CHOI[3], A. DUBEY[1];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Johns Hopkins Univ. - Homewood Campus, Lutherville-Timonium, MD; [3]New York Univ., New York, NY

Abstract: Analyzing multiregional networks requires simultaneous access to large expanses of the brain such that we can interrogate the activity of the neurons, their locations, and their biological properties such as projections targets, cell types, and functional roles. Recently work has enabled such experiments in NHP by developing a “brain observatory”: a large window for optical access across many brain areas combined with a robotically mounted microscope, a hexascope, that can flexibly reposition to image across the window. Here, we report methods to perform micro-electrocorticography from the cortical surface simultaneously with activity dependent imaging of GCaMP8m in neurons. We first prepared a non-human primate (NHP, male rhesus macaque) for imaging by surgically implanting a cortical window over a craniotomy and durotomy, and injecting AAV1-hSyn-jGCaMP8m-WPRE (Addgene). The hexascope stage performed translational movements (x,y,z) and rotational movements around the x,y,z axes (rotx,roty,rotz). Widefield fluorescence imaging expression was performed using a 2x magnification objective to provide a ~4.6 x 2.6 mm field-of-view. Two-photon imaging was performed using a 16x magnification objective to provide a ~600 um x 600 um field-of-view. uECoG was performed using a 200 um contact size, 750 um spacing, 61 electrode array in a liquid-crystal polymer substrate. The monkey performed a center-our reaching task on a touchscreen with the contralateral arm. Imaging, electrophysiology, task event, and kinematic joint state time-stamps were synchronized. We show that we can perform accurate, 10-20 um, localization of neuron recordings by computing a pose difference estimate from stitching and merging images obtained across reference and sample imaging sessions performed on different days. We will discuss how cellular localization accuracy has the potential to support the ability to construct multimodal maps of distributed neuronal populations using imaging and electrophysiology. Hexascope imaging offers a new solution to the problem of repeatedly targeting micron scale recordings in centimeter-sized expanses of tissue and allows individualized brain maps of a specific subject to be constructed at cellular scale.

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Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

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Program #/Poster #: PSTR431.14/Z9

Topic: I.08. Methods to Modulate Neural Activity

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NSF GRFP Grant DGE-2036197

Title: Locomotor manipulation through optical neuronal silencing with photocaged saxitoxin

Authors: *N. MILICIC[1], C. D. MAKINSON[2], D. E. EHRLICH[1], D. J. WILLIAMS[3]; [1]Dept. of Integrative Biol., Univ. of Wisconsin-Madison, Madison, WI; [2]Neurol., Columbia Univ., New York, NY; [3]Columbia Univ., New York, NY

Abstract: The ability to stimulate and inhibit neurons with light holds therapeutic promise and has revolutionized neuroscience research. However, current methods for optically inhibiting neurons require genetic manipulation and have unpredictable and sometimes paradoxically stimulatory effects due to their indirect mechanisms of action, competing with neural excitation rather than outright blocking it. Here we present a direct, potent, user-friendly chemical approach for optically silencing neurons. We rendered the naturally occurring paralytic, saxitoxin, transiently inert through chemical protection with a novel nitrobenzyl-derived photocleavable group. Light-induced uncaging of this photocaged toxin, STX-bpc, effects rapid release of active toxin and transient, spatially precise sodium channel blockade. We demonstrate the efficacy of this reagent for parametrically manipulating action potentials in mammalian neurons and brain slice. We show its potency for silencing neural activity in vivo by blocking and dissecting the startle reflex in larval zebrafish. Photo-uncaging of STX-bpc is a straightforward method for non-invasive, reversible, spatiotemporally precise neural silencing without the need for genetic access, removing barriers for comparative research and potential translation to humans.

Disclosures: N. Milicic: None. **C.D. Makinson:** None. **D.E. Ehrlich:** None. **D.J. Williams:** None.

Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.15/Z10

Topic: I.04. Physiological Methods

Title: Advanced Surgical Techniques for Longitudinal Cellular-level Visualization in Mouse Brain

Authors: *S. HONG[1], H. KWON[2], K. AKYILDIZ[3], H. KIM[4];
[1]KAIST, Daejeon, Korea, Republic of; [2]IVIM Technol., Inc., Korea, Korea, Republic of; [3]IVIM Technol., Inc., Daejeon, Korea, Republic of; [4]IVIM Technol., Inc., Seoul, Korea, Republic of

Abstract: In societies with an aging population, degenerative brain diseases such as Alzheimer's, Parkinson's, and Huntington's Diseases have become as widespread as cancer. There is a growing focus on developing treatments that target protein plaques, like beta-amyloid and alpha-synuclein, and on understanding the initial mechanisms of these diseases. Given the complex and variable nature of these conditions and their progression rates among individuals, it's essential to achieve significant results in the preclinical stages. Traditional methods typically involve ex vivo imaging, where medication is administered, followed by the harvesting of brain tissue for analysis at predetermined intervals.
To address these challenges, our goal was to refine surgical techniques for long-term, cellular-level brain imaging in living organisms. This would allow for the continuous observation and analysis of the effects of pharmaceuticals over time. We have advanced methods for installing cranial imaging windows that permit the monitoring of various brain cells, including neurons, blood vessels, astrocytes, and microglial cells, over several months. However, the hippocampus, located deeper than the cortex, was not adequately observable with traditional cranial windows. To solve this, we enhanced a technique that involves inserting a cannula into the cortex to bring the optical lens closer to the hippocampus, thus enabling effective longitudinal in vivo imaging. This approach has allowed for detailed observation of hippocampal neurons.
Our research effectively demonstrates the viability of these surgical techniques for brain imaging, underscoring their critical role in deciphering disease mechanisms and aiding drug development. The use of longitudinal in vivo brain imaging through intravital microscopy is set to become an indispensable tool in future neurological research.

Disclosures: S. Hong: None. **H. Kwon:** None. **K. Akyildiz:** None. **H. Kim:** None.

Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.16/Z11

Topic: I.04. Physiological Methods

Support: NIH grant NS128947
Alzheimer's Association grant 22-AAIIA-718504

Title: Label-free tracking of myelin degeneration in the subcortical white matter of live mice using third harmonic generation microscopy with adaptive optics

Authors: *N. HONG[1,2], N. CHERNAVSKY[2], L. J. TRIGIANI[2], M. LAMONT[2], N. NISHIMURA[2], C. B. SCHAFFER[2];
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Abstract: Understanding the structural integrity and pathological changes in myelin is integral for studying diseases of the central nervous system (CNS), such as multiple sclerosis (MS) and dementia. Third-harmonic generation (THG) microscopy is a label-free imaging technique with high contrast at "bold" optical interfaces, such as the lipid wrapping of myelin around axons in the CNS. Here, we demonstrate longitudinal, non-invasive imaging of dynamic demyelination and remyelination processes within the white matter of live mice using THG. Using 1320-nm, 50-fs excitation pulses to drive THG, we imaged the three-dimensional structure of subcortical white matter (WM) in adult mice. Using an implanted cranial window, we achieved consistent visualization of myelinated axons, ranging in diameter from 0.6 to 2 μm. Next, we drove MS-like demyelination through a repeat cuprizone diet and collecting data from the same WM region on a weekly basis. By the fourth week of cuprizone administration, we detected bleb formations, consistent with histologically evaluated myelin blebbing and blistering in otherwise normal-appearing WM in patients with MS. Our longitudinal imaging capability allowed us to visualize blister formation and quantify changes in blister size over eight weeks of cuprizone treatment and then observe decreases in blister count and remyelination events over a subsequent four weeks of recovery. Additionally, we detected more subtle myelin changes during the initial weeks of cuprizone administration, as well as in a mouse model of dementia risk factors. They were characterized by regions of focal hyperintensity, indicating early myelin damage and thickening which results in a brighter THG signal. In conjunction with THG, we also employed three-photon excited fluorescence microscopy to image cellular events impacting or being influenced by changes in myelination. To further enhance image quality and resolution during *in vivo* imaging, we incorporated a deformable mirror to correct for aberrations and enhance THG signal, thereby improving the identification of structural features, including the blister-like formations. In conclusion, we demonstrated that label-free THG microscopy has the capability for longitudinal assessment of myelin integrity within the subcortical WM of live mice, enabling tracking of disease progression over time that could improve our understanding of neurodegenerative pathophysiology. This technique provides both a clearer picture of disease mechanisms at the microscopic level, and holds promise for facilitating the development of novel therapeutic strategies.

Disclosures: N. Hong: None. **N. Chernavsky:** None. **L.J. Trigiani:** None. **M. Lamont:** None. **N. Nishimura:** None. **C.B. Schaffer:** None.

Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.17/Z12

Topic: I.04. Physiological Methods

Support: Italian National Recovery and Resilience Plan (NRRP), M4C2, funded by the European Union NextGenerationEU (CUP B83C22003920001, “THE - Tuscany Health Ecosystems"

Title: Monitoring psychological stress with multispectral optical sensors

Authors: *V. BARYGINA[1], E. BARIA[1], F. GORETTI[2], E. CRAVERO[3], F. S. PAVONE[1,4,5]; [1]Physics and Astronomy, Univ. of Florence, Firenze, Italy; [2]European Lab. for Non-Linear Spectroscopy, Sesto Fiorentino (FI), Italy; [3]Campus Bio Medico Univ. of Rome, Rome, Italy; [4]European Laboratory for Non-Linear Spectroscopy, Sesto Fiorentino (FI), Italy; [5]National Institute of Optics, National Research Council, Firenze (FI), Italy

Abstract: The human skin is a complex organ responsive to physiological and psychological states. The usefulness of skin metabolites as a source of biomarkers was demonstrated for dermatologic, systemic diseases, and psychiatric conditions (major depressive disorder, schizophrenia). It was shown that psychological stress-induced skin reactions can include changes in cytokine secretion acute/chronic secretion of corticosteroids and changes in volatile components in the skin. In the current study, we aim to individuate stress-related skin response with a high-throughput multimodal combination of optical techniques. Raman, reflectance, and fluorescence spectra were recorded on the palm skin of healthy volunteers using a multimodal fiber-probe device developed by our group and previously described. The cognitive-emotional stress was induced with the Montreal Imaging Stress Task. Electrodermal activity, heart rate, and heart rate variability were monitored throughout the experiment. A multimodal approach achieved 100% accuracy in classifying “no-stress” and “stress” conditions. The Raman bands associated with several stress biomarkers such as cortisol, lactic acid, and ceramides correlated with the spectral shifts observed in our experimental conditions. These preliminary results confirm the potential in application of our multispectral optical sensor for the non-invasive detection of psychological stress and psychiatric disease conditions.

Disclosures: V. Barygina: None. **E. Baria:** None. **F. Goretti:** None. **E. Cravero:** None. **F.S. Pavone:** None.

Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

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Topic: I.04. Physiological Methods

Support: NIH/NIA Grant K01AG072046
NIH/NIBIB Grant U24EB028941
NIH/NIA Grant NIA R01AG054598

Title: Shallow-angle delivery cannula for in vivo longitudinal brain imaging

Authors: *S. HOU[1], J. YANG[2], Y. KWON[2], Q. PIAN[3], C. DAUPHINAIS[2], M. CALVO-RODRIGUEZ[4], M. EL KHATIB[5], S. VINOGRADOV[5], S. SAKADZIC[3], B. J. BACSKAI[2]; [2]Neurol., [3]Radiology, [1]Massachusetts Gen. Hosp., Boston, MA; [4]Discovery Neurosci., Foundational Neurosci. Center, AbbVie, Cambridge, MA; [5]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Multiphoton microscopy serves as an essential tool for high-resolution imaging of the living mouse brain. To facilitate optical access to the brain during imaging, the cranial window surgery is commonly used. However, this procedure restricts physical access above the imaging area and hinders the direct delivery of imaging agents and chemical compounds. To overcome this limitation, we have developed a cannula delivery system that enables the implantation of a low-profile cannula nearly parallel to the brain surface at angles as shallow as 8 degrees. The shallow angle of the implanted cannula allows its tip to be centered over a 4 mm cranial window while still being located within the superficial layers of the mouse cortex. Importantly, the cannula does not interfere with the cranial window or with imaging using multiphoton microscopy. We validated the ability of our approach to label and image various cellular targets *in vivo* by infusing a wide assortment of fluorescent cell markers and imaging with multiphoton microscopy. To demonstrate longitudinal imaging with our approach, we successfully tracked degenerating neurons over time in Alzheimer's disease mice after repeated infusion of Fluoro-Jade C (FJC), a fluorophore traditionally employed for staining *ex vivo* tissue sections. When following FJC-labeled cells in the same mouse, we found a dynamic process where cells labeled during one weekly imaging session become unlabeled during subsequent weeks. Furthermore, we demonstrated longitudinal functional imaging of tissue partial pressure of oxygen (pO_2) in awake mice through direct infusion of the phosphorescent oxygen sensor, Oxyphor 2P and *in vivo* phosphorescence lifetime imaging. As expected, we found elevated pO_2 in regions close to the diving arteries and its branches and more uniform reduced pO_2 values in surrounding regions. We also found that the measured pO_2 spatial maps were consistent on a week-to-week basis and the spatial resolution was not compromised by repeated infusion. To determine the dependence of pO_2 on depth in awake mice, we obtained the histogram for each depth and found that there is a shift of the pO_2 distribution towards higher pO_2 values at larger depths, which is consistent with a recent study measuring pO_2 in awake mice at a single time point (Mächler et al., 2022). Our developed intracranial delivery technique should enable a wide range of new longitudinal imaging studies in the mouse brain.

Disclosures: S. Hou: None. **J. Yang:** None. **Y. Kwon:** None. **Q. Pian:** None. **C. Dauphinais:** None. **M. Calvo-Rodriguez:** None. **M. El Khatib:** None. **S. Vinogradov:** None. **S. Sakadzic:** None. **B.J. Bacskai:** None.

Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.19/Z14

Topic: I.04. Physiological Methods

Title: Functional analysis of spontaneous calcium oscillations of iPSC-derived 3D neural organoids and evaluation of responses to neuroactive compounds

Authors: *O. SIRENKO[1], L. CHEW[2];
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Abstract: Neural organoids, derived from human induced pluripotent stem cells (iPSC), are a rapidly developing technology with great potential for understanding brain development, neuronal diseases, and the impact of diverse genetic backgrounds. In the present study we focused on functional characterization of spontaneous activity of neural organoids measured by calcium oscillations. The neural organoids were formed using standardized reagents and protocols from STEMCELL technologies based on the work from Sergiu Pasca's lab (Stanford). In this study, we obtained neural organoids from STEMCELL technologies and cultured them for additional 1-3 weeks with neuronal media.
Morphological characterization of 3D organoids was done by imaging. Organoids diameters ranged from 1800-2000 µm, and the expression of neural markers including TUJ1 and GAFP was detected after fixing and staining organoids with appropriate fluorescently labeled antibodies. Functional characterization of neural activity was done via calcium oscillation assay and was recorded on a FLIPR instrument that measured fast kinetic changes in calcium signal. Calcium oscillations were visualized and analyzed by ScreenWorks PeakPro2 software. In addition, kinetic imaging was recorded via high content imaging instrument IXM-C. Importantly, the calcium-sensitive dye used contains a background fluorescence masking technology that enables sensitive detection of calcium oscillation without need to wash calcium dye. The calcium oscillation patterns were analyzed for multiple parameters including peak count, amplitude, and peak width. Majority of organoids demonstrated spontaneous calcium oscillation activity, with consistent rate of oscillations. Some organoids did not show synchronous activity, but such activity was induced by stimulation with 4-aminopyridine or AMPA. For pharmacological characterization, several compounds were used to show the appropriate functional responses. AMPA and 4-AP addition resulted in dose-dependent increase of frequency of calcium oscillations, while GABA and baclofen caused decrease in oscillation frequencies.
Taken together, this biological system of iPSC-derived 3D neural organoids paired with high-

content imaging and detailed analysis of calcium oscillations demonstrates a promising tool for compound testing.

Disclosures: O. Sirenko: A. Employment/Salary (full or part-time):; Employment full time, Molecular Devices. **L. Chew:** A. Employment/Salary (full or part-time):; Employment full time, STEMCELL Technologies.

Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.20/Z15

Topic: I.04. Physiological Methods

Support: NIH grant R01DK136013
NIH grant R01NS126541

Title: Interdisciplinary imaging platform (IDIP) for biomedical research

Authors: *L. XU[1], A. NIAZ[2], T. WANG[3], T.-D. LI[4], Y. HE[5];
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Abstract: The rapid development of imaging technologies has played a key role in our understanding of neuroscience and biomedicine. Mass spectrometry imaging (MSI) such as matrix-assisted laser desorption ionization (MALDI) MSI has been greatly demanded in the biomedical research field due to their unique label free ability to profile individual molecules in complex biological samples in situ. However, each imaging techniques has its own advantage and limitation. Therefore, it turns promising to integrate MALDI with multiple interdisciplinary imaging to propel biomedical research. Advanced Science Research Center (ASRC) at CUNY has been an imaging hub with its unique capacity to house 6 multi-disciplinary imaging facilities and a broad spectrum of imaging capacities within one building. In the past five years, we have been dedicated to developing the Interdisciplinary imaging platform (IDIP) integrating optical imaging, EM, AFM and furtherly Raman and MRI, with a central focus on MALDI mass spectrometry imaging. The IDIP has greatly enabled us to addressing challenging metabolic and mechanical regulations during development such as neuron-glia interaction and brain-gut axis, and in various human diseases including multiple sclerosis (MS), spinal cord injury, and brain tumor.

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Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

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Program #/Poster #: PSTR431.21/Z16

Topic: I.04. Physiological Methods

Support: KAKENHI 23K06344
KAKENHI 22H00432
Grant-in-Aid for Transformative Research Areas 22H05160
SICORP (AMED)

Title: Developing multiplex imaging platforms for deciphering Ca^{2+}-dependent biochemical signaling in the brain health and disease

Authors: *H. FUJII[1], Y. KONDO[1], K. OTA[1], G. CAI[2], R. SONG[3], H. SONG[1], H. KONDO[1], M. INOUE[4], S.-I. HORIGANE[5], S. TAKEMOTO-KIMURA[5,6], H. BITO[1];
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Abstract: Ca^{2+} transients are triggered by various neuronal events and their precise measurements are essential to investigate synaptic transmission, local dendritic spikes and action potential firing. Among downstream Ca^{2+}-dependent effectors, CaMKIIalpha and calcineurin stand out as they are critical for regulating neuronal plasticity, learning and memory. Thus, better understanding of how Ca^{2+} and the downstream kinase and phosphatase signals are activated during cognitive processes, and deciphering the dynamics of their spatial and temporal codes, are fundamental, yet unanswered, questions in neuroscience. To begin to address this issue, we previously developed a multiprobe platform dFOMA (dual FRET imaging with Optical Manipulation), an multiplexable imaging method for simultaneous measurements of two distinct colocalized biochemical signals. We also created a four-color, fast, sensitive and linear genetically encoded Ca^{2+} indicator (GECI) suite, XCaMPs, to measure neuronal activities of different cell types or different intracellular domains. dFOMA imaging demonstrated that CaMKIIalpha and calcineurin activations operated as distinct chemical decoding readouts of different parameters contained in the patterned neuronal input. To further explore this, we generated an updated dFOMA2.0 platform, by re-engineering brighter and more selective donor/acceptor FRET pairs, while also designing new/improved fluorescent probes for Ca^{2+}, and two Ca^{2+}/calmodulin-dependent plasticity-related enzymes, CaMKIIalpha and calcineurin. The new dual FRET imaging of CaMKII and calcineurin achieved single spine-resolved demonstration of spatio-temporal differences between the kinase- and phosphatase-representations of neuronal activities. In order to augment the versatility of multiplexed neuronal activity imaging, we expanded the color pallet of XCaMPs. Introduction of a new GECI

permitted 6-color Ca^{2+} imaging as well as more a quantitative readout of intracellular Ca^{2+} baselines and transients in multicompartmentalized neurons. Finally, by combining a linearly performing red GECI, a FRET reporter measuring a signaling downstream of Ca^{2+}, and a pharmacological knockout approach, we developed a disease-phenotyping assay system to gain functional and quantitative insights into disease-causing rare gene mutations of Ca^{2+} signaling. Our approach paves the way towards advancing our understanding of key molecular mechanisms underlying neuronal plasticity in brain health and in many neurodevelopmental and neuropathological diseases.

Disclosures: H. Fujii: None. **Y. Kondo:** None. **K. Ota:** None. **G. Cai:** None. **R. Song:** None. **H. Song:** None. **H. Kondo:** None. **M. Inoue:** None. **S. Horigane:** None. **S. Takemoto-Kimura:** None. **H. Bito:** None.

Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.22/Z17

Topic: I.04. Physiological Methods

Title: Mesoscopic and Neuron Level Two-Photon Mouse Imaging

Authors: *M. ABIJAOUDE;
Imaging Sci., Washington Univ. in St Louis, st louis, MO

Abstract: Mesoscopic and Neuron Level Two-Photon Mouse Imaging Melena Abijaoude [a], Shengxuan Chen [b], Arthur Li [b], Annie Bice [c], Joseph Culver [a,b,ca] Imaging Science Ph.D. Program, Washington University in St. Louis, 1 Brookings Drive, St. Louis, Missouri, United States of America, [b] Department of Biomedical Engineering, Washington University in St. Louis, 1 Brookings Drive, St. Louis, Missouri, United States of America, [c] Department. of Radiology Washington University in St. Louis, 1 Brookings Drive, St. Louis, Missouri, United States of America **ABSTRACT**Functional connectivity (FC) analysis has gained prominence in human neuroimaging studies utilizing functional magnetic resonance imaging (fMRI) as a tool in mapping spontaneous brain activity. This method investigates the functional relationships between neural activity and has been used extensively in clinical populations as well as in animal models to probe healthy function and disease-related dysfunction. As FC methods advance; a gap continues to grow between human fcMRI and the detailed genetic and molecular techniques common in mouse models. In this study, we aim to bridge this gap by leveraging recent advancements in large field-of-view two-photon microscopy and genetically encoded calcium indicators (GECIs). Together these tools enable examining FC at both the neuronal level, and mesoscopic level in mouse models. Specifically, we utilize GCaMP6 mice with cranial window and crystal skull placement for imaging layer 2/3. Using a custom large FOV TPM, and a 4x

objective (NA 0.28) we first obtain a structural image of the mouse brain. A smaller ROI is imaged with a 10x objective (NA 0.5). Suite2p is used to process the data and detect neuron. The images in the time series are registered using a phase correlation. Registration is done in two parts, first over the whole image and then over blocks of the image. Cell detection is computed by PCA and then spatially smoothing the principal components and finding the peaks. Then the neurons are detected by extending ROIs spatially around these peaks. This is done iteratively. This provides neuron level analysis as expected. From the same microscope we can also map mesoscopic functional connectivity(1g). Using the 4x objective, we smooth the data using a 30-micron gaussian (FWHM). FC is computed for all pixels with respect to a somatosensory. seed and a retrosplenial seed. neuron. Bilateral connections typically indicate strong connectivity between homotopic contralateral regions that share functionality. While the process takes two objectives, this represents a useful step towards integrating neuron-level analysis with mesoscopic systems-level approach of FC.

Disclosures: M. Abijaoude: None.

Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.23/Z18

Topic: I.04. Physiological Methods

Support: JP21H02801
JP22K21353
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JP23K14294
JP21K19346
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JPMJAX2228
19dm0207078
19dm0207087
19dm0207086
JPMJCR20E4

Title: Large-scale, long-term in vivo two-photon imaging of mouse neuronal structure and function through cranial windows utilizing fluoropolymer nanosheet and light curable resin

Authors: *T. TAKAHASHI[1], M. AGETSUMA[2], J. NABEKURA[3], K. OTOMO[4], Y. OKAMURA[5], T. NEMOTO[6];
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Abstract: Coordinated neural activity across various brain regions including the cerebral cortex and the cerebellum, underpins higher-order brain functions. Large-scale *in vivo* two-photon imaging of living animal brains serves as an important technique for unveiling brain function, permitting the visualization of living brain tissues at sub-cellular resolution. To achieve high-resolution two-photon imaging of living mouse brains, the open skull technique has been employed, involving the replacement of a portion of the cranial bone with a glass coverslip to create a cranial window. This approach allows for long-term imaging at high resolution; however, the diameter of cranial windows is usually limited to around 5 mm to prevent high pressure on brain tissue from a flat glass coverslip. Recently, a method employing polyethylene-oxide-coated CYTOP (PEO-CYTOP) nanosheets as a flexible sealing material for cranial windows has been proposed for *in vivo* two-photon imaging [Takahashi *et al. iScience*, 2020]. These nanosheets, approximately 130 nm thick, feature a hydrophilic adhesive surface that minimizes surface bleeding. Nonetheless, these flexible nanosheets did not sufficiently suppress motion artifacts arising from body movements in awake mice and were unsuitable for long-term imaging due to limited mechanical durability. To solve these problems, we have proposed a method to create large cranial windows - large enough to cover the entire parietal cortex and cerebellum of mice - using fluoropolymer nanosheets coated with light-curable resin. We call this the "Nanosheet Incorporated into light-curable REsin" or NIRE method [Takahashi et al. *Commun. Biol.*, 2024]. The NIRE method can produce cranial windows that conform to the curved cortical and cerebellar surfaces. NIRE methods-based cranial window suppresses motion artifacts in awake mice and maintains transparency for >5 months. In addition, we demonstrated that the NIRE method can be used for *in vivo* two-photon imaging of neuronal ensembles, single neurons, and subcellular structures such as dendritic spines. In this presentation, we report recent progress of technical improvements. In future, the NIRE method will facilitate large-scale *in vivo* analysis of inaccessible neural processes, such as the neuroplastic changes associated with maturation, learning, and neural pathogenesis.

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Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.24/Z19

Topic: I.04. Physiological Methods

Title: In vivo deep brain microscopy at submicrometer resolution with refractive index-matched prisminterfaces

Authors: T. TAKAHASHI[1,2,3], Y. ZHOU[4], M. TSUTSUMI[1,2], C. ITO[5], H. YUKAWA[5], J. NABEKURA[1], T. NEMOTO[1,2], K. OTOMO[1,6], N. MATSUHISA[4], ***M. AGETSUMA**[1,5]; [1]Natl. Inst. For Physiological Sci., Okazaki, Japan; [2]Exploratory Res. Ctr. on Life and Living Systems, Okazaki, Japan; [3]Tokyo Univ. of Sci., Tokyo, Japan; [4]Res. Ctr. for Advanced Sci. and Technology/ Inst. of Industrial Sci., The Univ. of Tokyo, Tokyo, Japan; [5]Inst. for Quantum Life Sci., Natl. Inst. for Quantum Sci. and Technol. (QST), Chiba Inage-ku, Japan; [6]Grad. Sch. of Med., Juntendo Univ., Tokyo, Japan

Abstract: In cortical circuits, neurons communicate through submicron-scale structures called synapses, which facilitate information processing essential for cognition, learning, and behavior. This is particularly evident in the mouse medial prefrontal cortex (mPFC), a region embedded deep within the brain and essential for these functions. To investigate the underlying neuronal mechanisms in such key brain areas, methods for precise investigation of these deep areas at cellular and synaptic levels are required. Our recent advancements using a right-angle glass microprism implanted along the midline have facilitated minimally invasive optical access and chronic activity imaging from hundreds of neurons in the deep mPFC, uncovering dynamic modulation in neural population coding and functional network structures associated with fear memory (Agetsuma et al., Nat Commun 2023).
However, a technical limitation remains; imaging quality through the glass prism is compromised by spherical aberrations due to refractive index mismatches between the glass and brain tissues, resulting in decreased spatial resolution and brightness. Lower brightness also decreases the signal-to-noise ratio, often necessitating longer exposures or more averaging, which consequently reduces temporal resolution.
Here, we propose a new prism interface, named PRIMISM or Prism with Refractive Index Matched Interface to SpeciMen. The PRIMISM was designed to match the refractive index of brain tissues, reducing spherical aberrations and enabling bright and high-resolution observation in the deep brain tissue. We quantitatively confirmed the enhanced performance of PRIMISM over conventional glass prisms in brain mimetic gel samples. Furthermore, in combination with the two-photon super resolution imaging technique, we succeeded in resolving the sub-micron order synaptic structure in mPFC at more than 1.5 mm depth from the dorsal surface of the mouse brain in vivo. The PRIMISM was also applicable for simultaneous imaging of the multiple layers of somatosensory cortex. We are currently verifying the improvement in recording neuronal population activities.

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Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

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Program #/Poster #: PSTR431.25/Z20

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH U01 NS113873

Title: In vivo optical recordings from mouse lumbosacral dorsal root ganglia

Authors: *N. DEIERLEIN[1], L. CHEN[2], P. SONG[3], G. ZHENG[3], B. FENG[2];
[1]Univ. of Connecticut Biomed. Engin. Dept., Storrs, CT; [2]Biomed. Engin., Univ. of Connecticut, Storrs, CT; [3]Univ. of Connecticut, Storrs, CT

Abstract: Patients with functional gastrointestinal (GI) disorders, such as irritable bowel syndrome (IBS), endure chronic abdominal and visceral pain that is inadequately managed in clinical settings. Both preclinical and clinical data suggest that peripheral sensitization of sensory afferents innervating the distal colon and rectum (colorectum) contributes to the persistence of IBS pain. Consequently, long-term monitoring of colorectal afferent activity holds promise for advancing our understanding of visceral pain chronification and objectively assessing treatment efficacy in reversing sensitization. The majority of lumbosacral innervation to the distal colorectum stems from afferents with somata in the L6, which constitutes the primary pathway for encoding and evoking pseudoaffective reflexes in response to colorectal balloon distension. This preliminary report demonstrates the feasibility of in vivo recordings from mouse L6 dorsal root ganglia (DRG) achieved through surgical implantation of a gradient-index (GRIN) lens into the lumbosacral vertebral region. The GRIN lens (ϕ1mm, 3.4 mm long, Thorlabs) was coupled with a biocompatible adaptor produced via 3-D printing of polylactic acid filament. To express GCaMP6s in colorectal neurons, we crossbred the GCaMP6s floxxed mouse line with the VGLUT2-Cre line. Under anesthesia, we exposed the dorsal foramen of the L6 DRG by gently removing the dorsal vertebra using a high-speed dental drill burr. The GRIN lens was inserted to make gentle contact with the dura mater of the L6 DRG and anchored with tissue glue. The mouse was positioned to minimize respiratory motion artifact by gently lifting up the tail and dorsal skin region with rubber-coated clamps. GCaMP6s signals from individual neurons in the L6 DRG were recorded at 15 frames per second using a custom-built fluorescent microscope capable of simultaneously capturing calcium images from two focal planes. We delivered three different stimuli to evoke neural activity at the L6 DRG: graded colorectal balloon distension (15, 30, 45, and 60 mmHg in 5-second steps), mucosal stroking by longitudinally pulling the balloon inflated at 10 mmHg, and electrical stimulation of the perianal skin with a concentric electrode (ranging from 1 to 3 mA). Recorded GCaMP6s image stacks were post-processed using customized MATLAB algorithms to remove motion artifacts and extract calcium transients from individual neurons.

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Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

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Program #/Poster #: PSTR431.26/Z21

Topic: I.04. Physiological Methods

Support: NSF NCS ECCS 1835278
AFOSR FA9550-19-1-0419

Title: A multiple channel 2D optical interface to 3D engineered neural cultures

Authors: *S. OMIDI, Y. BERDICHEVSKY;
Lehigh Univ., Bethlehem, PA

Abstract: A multiple channel 2D optical interface to 3D engineered neural cultures
Research into neural circuits and their dynamics is vital for decoding the functional and dysfunctional aspects of the brain, especially in relation to neurological disorders such as autism, epilepsy, and schizophrenia. The brain's complex network of neurons processes information via employing both feedforward sensory pathways and recurrent synaptic connections. Traditional 2D in-vitro models, while useful, fail to replicate the three-dimensional structure and connectivity of neuronal networks observed in in-vivo models. Newer 3D in-vitro models generally lack sensory input connectivity. In this work, we developed a novel 3D matrix-free brain-on-a-dish model, enhanced with a compartmentalized 2D optical interface mimicking multi-channel sensory inputs. The ‘sensory’ interface utilizes microchannel confinement of bundles of axons, enabling independent manipulation. Primary rat cortical neurons expressing Channelrhodopsin2 (ChR2) and jRGECO1a were used to form 3D aggregates and axonal bundles. Axonal bundles were independently stimulated by a patterned optical illuminator. This design allows for precise multi-channel control and manipulation of sensory-like inputs. The 3D portion of the model maintained a dense cortical-like structure and supported strong recurrent synaptic connectivity. We delivered different stimulation input patterns and measured the response of the 3D network by imaging changes in jRGECO1a fluorescence. We found that network responses to different input patterns were separable and classifiable, similar to responses evoked by sensory stimulation in the primary sensory cortex. This illustrates the model's capability to combine sensory-like functional inputs with a 3D structure in vitro. This model may have potential use in exploring the mechanisms underlying disorders of the cortex.

Disclosures: S. Omidi: None. **Y. Berdichevsky:** None.

Poster

PSTR431

Advanced Strategies for Neuronal Monitoring and Manipulation

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR431.27/Web Only

Topic: I.04. Physiological Methods

Support: Ministry of Science and ICT Korea 2021R1F1A1062265
Ministry of Science and ICT Korea DGIST R&D Grant 24-NT-01

Title: Hemodynamics of the human spinal region during rhythmic ankle movement

Authors: *H. KIM;
DGIST, Daegu, Korea, Republic of

Abstract: Hemodynamic responses are known to be coupled with synchronous activity of neuronal population in the brain. The study aims to evaluate whether hemodynamic responses are correlated to the synchronous neural activity in the spinal region during lower limb movement. This study presents a noninvasive optical approach that enables tracking human spinal region hemodynamics with functional near-infrared spectroscopy during body movement. A support frame for positioning optical emitters and receivers along the spine was first developed to maximize spatial resolution and identify the optimal distance between them. Then, the methodology was tested at the optimal emitter-detector distance by assessing the hotspots associated with human ankle extension-flexion movement in the spinal region. These spinal region hotspots are demonstrated to be comparable to those identified by intraoperative methods during surgical operations. This study may provide a noninvasive real-time monitoring method for human spinal region activity under normal and abnormal movement conditions.

Disclosures: H. Kim: None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.01/Z22

Topic: I.08. Methods to Modulate Neural Activity

Support: PID2021-122347NB-I00 (MCIN/AEI and ERDF- “A way of making Europe”)
PID2020-114867RB-I00
PID2023-149669NB-I00

Title: Continuous-wave near-infrared laser stimulation non-invasively modulates neural dynamics in sustained and activity-dependent modalities

Authors: *A. GARRIDO-PEÑA[1], P. SANCHEZ-MARTIN[1], M. REYES-SÁNCHEZ[1], R. LEVI[1], F. B. RODRIGUEZ[1], J. CASTILLA[2], J. TORNERO[2], P. VARONA[1];
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Abstract: The effectiveness of NIR laser illumination as a neural stimulation method has been validated in the last decades mostly using high energy pulsed laser. We present here a novel approach using sustained continuous-wave NIR (CW-NIR) laser in a low wavelength (830nm) and an activity-dependent stimulation protocol. In this novel procedure, we manage to intervene at different stages of the action potential generation, delving into the mechanisms responsible for the changes in neural dynamics produced by this optical stimulation. The potential of CW-NIR laser in future clinical applications will depend on a clear understanding of the processes underlying its modulatory effect. We report a reversible effect of CW-NIR laser stimulation in the spike waveform. To this end, we first characterized the intracellular recordings of spontaneous tonic firing neurons of *Lymnaea stagnalis* in a sustained stimulation protocol. We found that the laser stimulation accelerates the dynamics shortening the duration of the spike as a result of a significant increase in the depolarization and repolarization slopes but not in the amplitude. This was supported by a detailed computational study that explored the possible membrane candidates involved in the reported effect (i.e., ionic channels and capacitance). Additionally, the study incorporated an analysis of the role of temperature in the spike waveform, following previous hypotheses that suggested its involvement in the observed effect of NIR laser stimulation. For the experimental study, we developed a novel closed-loop laser stimulation protocol that successfully assessed the evolution of the action potential, dissecting its dynamics at different stages of the spike. We designed open-source real-time algorithms to predict the spikes and stimulate at specific time points (available at github.com/GNB-UAM/spike-predictor). This protocol can have a large impact in clinical applications, since it is adaptable to different time scales and living systems. Our results altogether show that CW-NIR laser stimulation is an effective tool to perform non-invasive neuromodulation. The mechanisms underlying the observed effect point to a combination of membrane elements involving a temperature change in the modulation. The activity-dependent protocol effectively tunes action potential dynamics, with a quick response, as indicated by the short timescale of the effect.

Disclosures: A. Garrido-Peña: None. **P. Sanchez-Martin:** None. **M. Reyes-Sánchez:** None. **R. Levi:** None. **F.B. Rodriguez:** None. **J. Castilla:** None. **J. Tornero:** None. **P. Varona:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.02/Z23

Topic: I.08. Methods to Modulate Neural Activity

Support: National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. RS-2023-00278610)
Convergent Technology R&D Program for Human Augmentation through the National Research Foundation of Korea (NRF) funded by Ministry of Science and ICT (2019M3C1B8090842)
Brain Korea 21 FOUR Project

Title: Magnetothermal brain stimulation modulates synaptic plasticity of the primary somatosensory cortex in adult mice

Authors: M. JEONG[1], K. YOUNGJI[1], ***S. CHUNG**[2];
[1]Dept. of Physiol., Grad. Sch. of Med. Sci., Brain Korea 21 Project, Yonsei Univ., Seoul, Korea, Republic of; [2]Dept. of Physiol., Brain Korea 21 Project, Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Compared to other neuromodulation techniques, magnetic brain stimulation utilizing nanomaterials has garnered considerable interest in recent years because it is wireless, minimally invasive, and capable of precisely regulating a specific neural circuit without substantial attenuation. Previous studies utilizing magnetothermal brain stimulation (MTS) have demonstrated that this technique could induce neural excitation and silence of the targeted brain region, thereby inducing motor behavior in awake rodents. However, whether MTS utilizing nanomaterials can restore synaptic plasticity in the adult cortex remains to be determined. Furthermore, MTS often necessitates genetic alteration by introducing viruses that carry transient receptor potential (TRP) channel proteins on the neuronal membranes to regulate the specific brain area. Nevertheless, using viruses to generate exogenous channel proteins can present a substantial regulatory barrier to clinical implementation. This study aimed to determine whether MTS via nanomaterials reactivates synaptic plasticity in the adult mouse cortex through the innate TRP channels. We administered MTS centered on the targeted brain region after injecting the nanoparticles into the barrel cortex of the adult mice. MTS immediately increased the neuronal activity of the barrel cortex. Furthermore, the long-term MTS for three days increased the amplitude of the field potential of layer 4 of the barrel cortex induced by electrical whisker stimulation. Ex vivo slice patch clamp recording showed that the potency elicited by thalamocortical input was increased following the long-term MTS while the excitation-inhibition balance was preserved. GluN2B-NMDAR was required for MTS to reactivate synaptic plasticity in the adult barrel cortex. This study was the first to show that MTS can cause long-term cortical synaptic plasticity without using exogenously produced channel proteins in the adult cortex.

Disclosures: M. Jeong: None. **K. YoungJi:** None. **S. Chung:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.03/Z24

Topic: I.08. Methods to Modulate Neural Activity

Title: A novel multi-modal magnetic resonance imaging technique to measure the concentration and ratio of iron products in a phantom of cerebral cavernous malformation.

Authors: *O. NGWU-HYACINTH[1], R. WILLOUGHBY[2], M. S. BOLDING[3];
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Abstract: The evolution of magnetic resonance imaging (MRI) has permitted the detection of angiographically occult vascular malformations, including cerebral cavernous malformations (CCMs). Although relatively rare, CCMs are vascular lesions found predominantly in the brain and may cause severe symptoms, including seizure, headache and stroke. CCMs frequently hemorrhage and are permeated or surrounded by iron-rich blood breakdown products (BBPs) whose ratios change in characteristic ways over time. Iron present in BBPs is believed to have a strong positive correlation with CCM disease severity. Additionally, iron in vascular brain lesions disrupts the inhomogeneity of the MRI magnetic field, generating susceptibility artifacts that make these lesions difficult to interrogate with MRI. Quantitative susceptibility mapping (QSM) has shown potential to quantify total lesional iron content by measuring tissue magnetic susceptibility. However, QSM cannot differentiate between various chemical forms of iron. Our team proposes a novel multi-modal MRI technique, *FerroQuant*, for detecting recent hemorrhage within a CCM lesion by measuring the concentration and ratio of BBPs and how they change over time. We hypothesize that combining QSM with imaging techniques sensitive to different forms of iron (T1 and T2 mapping) will allow simultaneous independent measurement of multiple BBPs. **Methods:** Preliminary work was done on a CCM phantom, which consists of jelly beads made from a mixture of red-colored liquid containing 2g of Iron (III) citrate (0.1g Fe^{2+}) and sodium alginate spherified with calcium lactate. Three gelatin molds were embedded with the iron-containing jelly beads to mimic CCM lesions. MRI images were acquired at 3.0T. MRI data were analyzed using FSL. To validate our QSM estimates, the mean susceptibility values of the CCM phantoms were correlated with the QSM-derived iron measurements in human patients. **Results**: The iron-embedded jelly beads appeared hyperintense on QSM relative to the nearby gelatin mold. The susceptibility of iron in these phantoms, as demonstrated by QSM, averages about 2ppm, similar to the iron content of human CCM lesions, as documented in literature (Tan et al., 2014). Therefore, in this experiment, we were able to successfully estimate the iron concentration using QSM. **Limitations and conclusions:** 1). Factors like inflammation, edema, and tissue type that are present in humans and can influence the MRI signal are not present in the phantoms. Future studies will use ferritin and multiple iron products to estimate the iron concentration and ratio of soluble and insoluble iron products with QSM as an index for iron concentration.

Disclosures: O. Ngwu-Hyacinth: None. **R. Willoughby:** None. **M.S. Bolding:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.04/Z25

Topic: I.08. Methods to Modulate Neural Activity

Title: Magnetic nanoparticle characterization for wireless and remote neuromodulation by magneto-mechanical-genetic (MMG)

Authors: *J. SHIN[1], S.-H. CHOI[1], M. KWAK[1], L. JAE HYUN[1], J. CHEON[2];
[1]Yonsei Univ., Seoul, Korea, Republic of; [2]IBS Ctr. for Nanomedicine, Yonsei Univ., Seoul, Korea, Republic of

Abstract: Non-invasive neuromodulation technologies play a crucial role in unraveling neuron functions and brain connectivity. Our nanomaterial-based neuromodulation toolbox harnesses magnetic nanoparticles to enable neuromodulation in freely moving animals. Specifically, the magneto-mechanical-genetics (MMG) technology employs m-Torquer, a nanoscale magnetic particle, to activate neurons expressing the mechanosensitive ion channel Piezo1. In this study, we explored various chemical strategies for surface functionalization of m-Torquer nanoparticles to optimize the binding of Myc antibody and fluorescent markers to m-Torquer surface. Then, we tested various designs of nanoparticles for selective and efficient labeling to neuronal membrane and robust neuronal activation in response to magnetic stimulation. Furthermore, we performed biophysical characterization of MMG-based neuronal stimulation by measuring the torque force (F_τ) generated by MNPs within a rotating circular magnetic array (CMA) and its efficacy in modulating the Piezo1 ion channel for neuronal activation. To demonstrate the capabilities of this technology for *in vivo* neuromodulation in freely behaving animals, we applied MMG to selectively activate VGAT-(GABAergic) neurons in the zona incerta (ZI) area and showed the increased food foraging behaviors by MMG stimulation. Overall, this study offers valuable insights for the development of magnetic nanoparticles for MMG-based neuromodulation and further showcases its potentials for dissecting neural circuitries underlying complex behaviors.

Disclosures: J. Shin: None. **S. Choi:** None. **M. Kwak:** None. **L. Jae hyun:** None. **J. Cheon:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.05/Z26

Topic: I.08. Methods to Modulate Neural Activity

Title: Open-source magnetic system for wireless neuromodulations in vitro and for untethered deep brain stimulation in vivo

Authors: *J.-X. HUANG, P.-H. CHIANG;
Natl. Yang Ming Chiao Tung Univ., Hsinchu City, Taiwan

Abstract: In last decades, significant advances have been made in the development of magnetic neuromodulation technology that allows deep brain neurons to be manipulated without hardware implants. Novel wireless neuromodulation approaches, such as magnetic nanoparticles and magnetic-sensitive proteins, have attracted a great deal of attention. Among them, those approaches that require only low-intensity magnetic fields (< 50mT) and low frequencies (< 20Hz) offers significant promises for wider applications due to their scalability and low power consumption. However, the lack of affordable instruments for various *in vitro and in vivo* studies prevented practical implementation. To facilitate the development of these tecchnologies, we developed a system with open-source hardware and software. The hardware consists of Arduino-based controller, divers of electromagnetic coils, and external feedback sensors to monitor environmental parameters, such as temperature, sound level, vibrations, and magnetic field intensity, in real-time. The feedback system can be used to ensure optimal experimental conditions by interruptting the experiment immediately at specific condition. The Python-based software consists of graphic user interface for editing and controlling the stimulation protocol and for monitoring the feedback signals in real-time. Furthermore, to advance the accuracy of magnetic field application during the behavioral tests. It combines the visual closed-loop system which can be used to control the magnetic stimulation based on the position of subject. Finally, we demonstrate the system in several behavioral tests, including light-dark box test and place preference test. Overall, this study provides a low-cost, user-friendly, and flexible magnetic system for various of application of magnetic neuromodulation technologies. This system can facilitate the development of wireless deep brain stimulations and promote their accessibility in basic and translational studies.

Disclosures: J. Huang: None. **P. Chiang:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.06/Z27

Topic: I.08. Methods to Modulate Neural Activity

Support: ERC Synergy Grant

Title: Real-time brain state-coupled network-targeted dual-site transcranial magnetic stimulation enhances working memory

Authors: *B. JOVELLAR[1], P. BELARDINELLI[2], U. ZIEMANN[3];
[1]Univ. of Tuebingen, Tuebingen, Germany; [2]Eberhard-karls-University, TüBingen, Germany; [3]Eberhard Karls Univ., Tübingen, Germany

Abstract: Working memory impairment is a common affliction in disorders such as Alzheimer's disease, attention-deficit hyperactivity disorder (ADHD), and major depression. Brain stimulation is a promising tool for modulating brain networks and improving cognitive performance. We applied non-invasive brain stimulation—using transcranial magnetic stimulation (TMS)—in a real-time brain state-dependent manner to frontal and parietal cortical regions, incorporating a spike-timing-dependent (STDP) protocol. Using theta oscillation phase as a brain state marker, we compared the effects of brain state-coupled vs. -uncoupled dual-site TMS on: 1) EEG dynamics time-locked to the TMS pulses; 2) working memory performance, & 3) oscillation power during the memory task. We found that: 1) the brain state-coupled protocol caused greater amplitude modifications of evoked activity within 90 ms post-TMS pulse; 2) the brain state-coupled condition enhanced working memory accuracy; and 3) correct vs. incorrect working memory trials had broadband differences in power across encoding, retention, and recall periods at baseline and these were differentially modulated after brain state-coupled vs. -uncoupled stimulation. Our work provides a crucial first step in personalized pathway-targeted brain stimulation in cognitive networks that may minimize potential adverse effects and maximize its cognitive enhancement potential in healthy individuals or in people with network disorders affecting memory such as Alzheimer's disease, major depression, or ADHD.

Disclosures: B. Jovellar: None. **P. Belardinelli:** None. **U. Ziemann:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.07/Z28

Topic: I.08. Methods to Modulate Neural Activity

Support: NIMH Grant 1K23MH125145
NIMH Grant R01 MH132074
Roy J. Carver Trust
the Brain and Behavior Research Foundation Young Investigator Grant 21875
The University of Iowa Sidney R. Baer, Jr. Fellowship

Title: TMS-induced auditory evoked potentials can be effectively masked: Evidence from intracranial EEG

Authors: *E. W. TSANG;
Psychiatry, The Univ. of Iowa, Iowa City, IA

Abstract: Background Combining transcranial magnetic stimulation (TMS) with scalp electroencephalography (EEG) can elucidate cortical mechanisms although TMS-evoked potentials (EPs) are contaminated by auditory artifact. One method to control for auditory artifacts is TMS Adaptable Auditory Control (TAAC) sound masking. Here we evaluate TAAC combining TMS with intracranial EEG (iEEG) in an awake epilepsy patient.
MethodsSingle-pulse intracranial TMS-EPs (iTEPs) with and without TAAC sound masking were recorded in auditory and non-auditory cortices with iEEG (134 electrodes). iTEPs were compared between conditions using cluster-based permutation testing.
ResultsTAAC reduces auditory EPs at sound-responsive sites located in and around auditory cortex. Auditory masking also alters the iTEP morphology in non-auditory regions, suggesting that auditory EPs can contaminate electrophysiological signals beyond auditory regions.
ConclusionsTAAC is an effective method for masking TMS auditory clicks. TMS-iEEG is a feasible and safe method for testing the effectiveness of auditory masking tools in awake human participants.

Disclosures: E.W. Tsang: None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.08/Z29

Topic: I.08. Methods to Modulate Neural Activity

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Japan Society for the Promotion of Science (JP20H05705)
Japan Agency for Medical Research and Development (19dm0207070h0001)
National Institute for Health and Care Research (NIHR) Oxford Health Biomedical Research Centre (to BS). The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care.

Title: Cognitive Control of Insular Cortex Activation and Its Impact on Pain Sensitivity through MEG Neurofeedback

Authors: *Y. WANG[1,2], R. FUKUMA[3,4], B. SEYMOUR[5,6], H. YANG[7], H. KISHIMA[3], T. YANAGISAWA[3,4];
[1]Osaka Univ., osaka, Japan; [2]Department of Neurosurgery, Graduate School of Medicine, Osaka University, Suita, Japan; [3]Dept. of Neurosurg., Grad. Sch. of Med., Osaka Univ., Suita, Japan; [4]Institute for Advanced Co-Creation Studies, Osaka University, Suita, Japan; [5]Ctr. For Information and Neural Networks, Suita, Japan; [6]Wellcome Centre for Integrative Neuroimaging, John Radcliffe Hospital, Oxford, United Kingdom; [7]Inst. for Advanced Co-Creation Studies, Osaka Univ., Suita, Japan

Abstract: The insular cortex, critical in brain pain processing, is a promising target for neuromodulation via neurofeedback, although its direct modulation and causal connection to pain perception remain unclear. Our study explores the impact of magnetoencephalography (MEG) neurofeedback on insular cortex activation and pain thresholds. In a double-blind, randomized, controlled crossover trial, 19 participants engaged in neurofeedback sessions targeting modulation of activity in the right insular cortex. Insula activity levels were calculated from the estimated current values at vertices in the right insula and were visually represented by the size of a circular disk on the screen, providing feedback that enabled participants to learn to either increase or decrease their insula activity. Our findings suggest that participants were able to cognitively alter their insular cortex activation levels during neurofeedback training. Additionally, downmodulation training led to a significant decrease in resting-state insular cortex activity compared to upmodulation. While downmodulation training was associated with an increase in pain thresholds, the analysis did not show a significant interaction effect across different training conditions. These results confirm that insular cortex activity can be effectively modulated through cognitive efforts, reinforcing the potential of MEG-based neurofeedback using estimated currents as a viable tool for clinical therapies.

Disclosures: Y. Wang: None. **R. Fukuma:** None. **B. Seymour:** None. **H. Yang:** None. **H. Kishima:** None. **T. Yanagisawa:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.09/Z30

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant R01NS109885
Swiss National Science Foundation grant P500PB_211119

Title: Elucidating the circuit mechanisms of cortical ultrasonic neuromodulation

Authors: *T. LEMAIRE[1], Y. YUAN[2], A. M. LEMESSURIER[1], J. P. LITTLE[1], R. C. FROEMKE[1], S. SHOHAM[1];
[1]Neurosci. Inst., New York Univ. Grossman Sch. of Med., New York, NY; [2]Yanshan Univ., Hebei, China

Abstract: Transcranial Ultrasound Stimulation (TUS) offers the unique ability to perturb brain circuits in a noninvasive, focal, and reversible manner. Yet, the mechanisms by which ultrasound interacts with heterogenous and interconnected neuronal populations to induce functional effects remain unclear. To elucidate these interactions, we applied TUS to the visual cortex of awake mice and monitored neural activity in three cortical subpopulations with two-photon calcium imaging. We show that TUS evokes focal and stereotypical population responses with cell-type-specific dose dependences. Through independent parametric variations, we demonstrate that evoked responses collectively scale with the time-average intensity of the stimulus. Finally, using a computational model we project that responses are actively modulated by local network inputs and require cell-type-specific intrinsic sensitivities to ultrasound. Our combined experimental and computational approach introduces a new framework to untangle direct vs network-mediated responses to TUS, thereby bringing crucial insight on the complex circuit mechanisms underlying ultrasonic neuromodulation and paving the way for its deployment in clinical settings.

Disclosures: T. Lemaire: None. **Y. Yuan:** None. **A.M. Lemessurier:** None. **J.P. Little:** None. **R.C. Froemke:** None. **S. Shoham:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.10/Z31

Topic: I.08. Methods to Modulate Neural Activity

Support: Alzheimer's Association New to the Field (AARG-NTF)
NIH Maximizing Investigators' Research Award (MIRA-R35)
Human Frontier Science Program Fellowship
UT Austin Proof of Concept Award
University of Texas at Austin Startup Fund

Title: Bioadhesive Hydrogel-Coupled and Miniaturized Ultrasound Transducer System for Long-Term, Wearable Neuromodulation

Authors: *K. TANG[1], J. JEONG[2], J.-C. HSIEH[3], H. WANG[4];
[1]Biomed. Engin., Univ. of Texas, Austin, Austin, TX; [2]Biomed. Engin., The Univ. of Texas at Austin, Austin, TX; [3]Biomed. Engin., Univ. of Texas at Austin, Austin, TX; [4]Biomed. Engin., Univ. Of Texas At Austin Inst. For Neurosci., Austin, TX

Abstract: Disclosures: The authors declare that a patent application relating to this work has been filed.
Abstract: Transcranial focused ultrasound has shown promising approach in providing a non-invasive approach for neuromodulation for neurodegenerative diseases and psychiatric illnesses. However, its application has thus far been limited to the devices' size and need for handheld operators due to challenges related to the dependence of geometrical shape of the transducer in achieving the desired focal depth. Furthermore, the need for ultrasound gel for acoustic coupling between the device and skin limits the viability for long-term use due to its inherent susceptibility to dehydration. Here we report a wearable ultrasound device composing of a miniaturized size comparable to standard EEG/ECG electrodes integrated with bioadhesive acoustic hydrogel to achieve efficient acoustic intensity upon ultrasound stimulation for primary somatosensory cortical stimulation. Specifically, polydimethylsiloxane (PDMS) was patterned using a simplified novel microfabrication process eliminating the need for traditional lithography in developing an air-cavity Fresnel lens (ACFAL) to develop a self-focusing acoustic transducer (SFAT) with center frequency of 650kHz. SFAT-ACFAL was able to achieve an acoustic intensity of up to 30.7W/cm^2 (1.92MPa) in free-field with a focal depth of 10mm, focal width of ~8mm and focal axial size of ~3.5mm. AMPS-Glycerol based bioadhesive acoustic hydrogel was developed to address the need for long-term stability of acoustic couplant for ultrasound application. The hydrogel demonstrated low dehydration rate retaining 76% of its weight with less than 13% attenuation in acoustic intensity and stable adhesion force of 0.961N/cm over 35 days. Moreover, the hydrogel exhibits rehydration characteristics when stored at high humidity conditions. Leveraging both the device and the hydrogel, a single-blinded experiment using functional electrical stimulation (FES) and focused ultrasound stimulation (FUS) was performed on subjects (n=5), where each subject was given a random combination of sham or FUS. Overall, our **Bi**oadhe**s**ive **C**oupled **U**ltrasound **T**ransducer (BisCUT) was able to suppress somatosensory evoked potentials elicited by median nerve stimulation via functional electrical stimulation over 28 days, where post ad-hoc evaluation of P27-N20 complex of the showed significant decrease across EEG signals from C3, CP1, P3, CP5. Thus, demonstrating the efficacy of the miniaturized transducer SFAT-ACFAL and the long-term application of AMPS-Glycerol based bioadhesive acoustic hydrogel.

Disclosures: K. Tang: None. **J. Jeong:** None. **J. Hsieh:** None. **H. Wang:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.11/Z32

Topic: I.08. Methods to Modulate Neural Activity

Title: Exploring Subcortical Neuromodulation with Transcranial Ultrasound Stimulation using EEG source estimation

Authors: *H. SOROUSHI, A. SANSARE, S. BAO, Y. LEI;
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Abstract: Subcortical structures play a crucial role in brain function, yet methods to modulate their activity are limited. Transcranial ultrasound stimulation (TUS) offers a noninvasive approach to influence activity within these areas in humans. In our study, we applied TUS to the thalamus, utilizing an EEG-based algorithm to assess its effects on thalamic activity. This sham-controlled study assessed changes in thalamic activity before, immediately after, and at 10-, 20-, and 30-minutes following TUS. We measured somatosensory evoked potentials (SEPs) induced by median nerve stimulation, which typically elicit a thalamic response approximately 14~17ms after nerve stimulation. To ensure precision in ultrasound application, we used pseudo-CT images from T1-weighted MR scans of each participant to customize sonication parameters specifically for the thalamus. We also ensured that ultrasound energy levels stayed within safe limits. EEG data was collected using an easyCap-M1 with 52 channels, and the activation of subcortical volume sources was analyzed through anatomical reconstructions and estimation techniques. Moreover, we validated our modulation of subcortical activity using temporal interference stimulation specifically targeting the thalamus. Our results indicate significant changes in thalamic activity post-TUS, highlighting its potential as a tool for modulating subcortical functions effectively.

Disclosures: H. Soroushi: None. **A. Sansare:** None. **S. Bao:** None. **Y. Lei:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.12/Z33

Topic: I.08. Methods to Modulate Neural Activity

Support: Hong Kong Research Grants Council General Research Fund (15104520, 15102417 and 15326416)

Title: Non-invasively delivery of ultrasound-sensitive ion channels for sonogenetic neuromodulation of the mouse brain

Authors: *Q. XIAN[1], D. LI[1], S. LEI[2];
[1]The Hong Kong Polytechnic Univ., Hong Kong, China; [2]Biomed. Engin., The Hong Kong Polytechnic Univ., Hong Kong, Hong Kong

Abstract: Sonogenetics uses ultrasound to manipulate cells expressing ultrasound-responsive proteins non-invasively, which can control neuronal functions and modulate animal behaviors. However, sonogenetics requires accurate transduction of ultrasound-sensitive ion channels into the target cells (such as intracerebral localization injections). We introduce a non-invasive

approach for intracerebral gene delivery to achieve non-invasive and higher spatial resolution during the neuromodulation procedure. This approach utilizes the AAV-PHP.B capsids, which can increase gene transfer to the central nervous system and the peripheral system via vasculature. The mutant mechanosensitive channel of large conductance (MscL-G22S) is a well-characterized mechanosensitive channel that rapidly responds to ultrasound stimulation in the brain. We demonstrated this concept in mice using AAV-PHP.B capsids with DIO-MscL-G22S to non-invasively modify and activate neurons within the brain for spatiotemporal neuromodulation. We found that PHP.B with MscL-G22S was successfully expressed in the Cre mice. We specifically targeted the hippocampal region with ultrasound stimulation; the neurons in the targeted region were activated, and the mice's working memory was improved after ultrasound treatment. In conclusion, we achieve non-invasive sonogenetic neuromodulation without surgical intervention and improve the animal's memory. This non-invasive virus delivery method and sonogenetic stimulation can be applied to test gene therapies for diseases that involve the entire nervous system (the central nervous system and the peripheral system) or widely distributed cell populations, such as Alzheimer's disease, Parkinson's disease, spinal muscular atrophy, etc.

Disclosures: Q. Xian: None. **D. Li:** None. **S. Lei:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.13/Z34

Topic: I.08. Methods to Modulate Neural Activity

Support: American Heart Association Innovative Project Award (20IPA35360039).

Title: Low intensity focused ultrasound up to 8 W/cm^2 is safe and tolerable, and enhances corticospinal neurophysiology and motor sequence learning: A preliminary safety and dose escalation study in chronic post-stroke participants

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Abstract: Introduction The central nervous system had been regarded as purely electrochemical, until the discovery that ultrasound can be neuromodulatory. Recently, low intensity focused ultrasound (LIFU) has emerged as a promising neuromodulation tool in treating neurological diseases (e.g., stroke) mainly due to its whole brain-deep and millimeter-focal

stimulation. Yet, the safety and tolerability (i.e., maximum dose) of LIFU have not been established in stroke. Thus, we aimed to establish the safety and tolerability of LIFU in stroke at various dosages.**Methods**We adopted the long-established 3+3 dose escalation paradigm to 0/sham, 1, 2, 4, 6, and 8 W/cm² spatial-peak pulse-average intensity (I_{SPPA}), *in-situ* water bath with skull. Pre-defined safety stopping rules included LIFU-related lesions on structural magnetic resonance imaging (MRI), ≥ 30% (minimally clinical-significant) topical change in apparent diffusion coefficient (ADC) maps, and ≥ 2nd degree scalp burns. Participants underwent a 10-minute single-session LIFU (3 participants per I_{SPPA}) over the ipsilesional cortical hotspot on which transcranial magnetic stimulation (TMS) can elicit the largest motor evoked potential (MEP) on the affected abductor pollicis brevis. Concurrent to LIFU, participants performed three blocks of motor sequence learning (MSL) with the stroke-affected hand. Outcome measures include a) occurrences of pre-defined safety-related adverse events, b) percentage improvements in MSL completion time ($LIFU_{post}$ - $LIFU_{pre}$), and c) percentage differences in corticospinal excitability as measured by TMS-induced MEP amplitudes ($LIFU_{post}$ - $LIFU_{pre}$) at 12, 24, and 36 minutes. Participants were lumped into LOW-dose (N=9; sham, 1, 2 W/cm^2) and HIGH-dose (N=9; 4, 6, 8 W/cm^2) for inter-group comparisons.**Results**Eighteen first-ever chronic stroke participants (6 females, 57±35 years, 24±96 months post-stroke) completed the study. LIFU intensity was escalated from 0 to 8 W/cm^2 with no adverse events in any participant. All participants had less than 30% topical ADC change, and no participant had either LIFU-related lesion seen on structural MRI or ≥ 2nd degree scalp burn. After LIFU, 100% (9/9) of HIGH-dose participants performed ≥ 15% faster on MSL, compared to 22% (2/9) of LOW-dose participants did. Similarly, 89% (8/9) of HIGH- and 63% (6/9) of LOW-dose participants had enhanced corticospinal excitability.**Conclusion**Single session of 10-minute LIFU up to 8 W/cm^2 *in-situ*, transcranial estimated I_{SPPA} is safe and tolerable in chronic stroke. Also, more HIGH-dose participants improved on MSL and corticospinal neurophysiology measures, compared to the LOW-dose group.

Disclosures: Z. Huang: None. **C.C. Charalambous:** None. **M. Chen:** None. **J.A. Feld:** None. **E. Sokhadze:** None. **T. Kim:** None. **X. Jiang:** None. **W. Feng:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.14/Z35

Topic: I.08. Methods to Modulate Neural Activity

Support: Swiss National Science Foundation

Title: Direct neuromodulation of axons by focused ultrasound

Authors: *E. VICARI[1], T. LEMAIRE[2], T. TARNAUD[3], T. PLOVIE[3], O. AKOUISSI[1], O. RIZZO[1], E. TANGHE[3], E. NEUFELD[4], S. P. LACOUR[1], S. MICERA[1,5];
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Abstract: Focused ultrasound is increasingly recognized as a promising non-invasive modality for neuromodulation of brain circuits. However, the question of whether ultrasound can directly excite axons, fundamental for its application to treat peripheral neuropathologies and modulate long-range brain connectivity, remains unresolved. Recent findings disagree on whether the effect of axonal ultrasound stimulation is excitatory or inhibitory, and this uncertainty hinders clinical translation, as the field lacks a standardized approach to achieve predictable neuromodulatory effects.In this study, we investigated the direct excitatory and modulatory effects of ultrasound on isolated axons in an anesthetized rodent model. We targeted exposed spinal roots, which allowed us to monitor both neural and muscular activation of the hindlimb as indicators of effective neuromodulation. We examined the impact of various ultrasound stimulation parameters, including pressure amplitude, pulse duration, pulse repetition frequency, and stimulation duty cycle. To assess the modulatory effects of ultrasound, we electrically stimulated proximal segments of the same targeted axons and analyzed the impact of ultrasound on the amplitude, shape and latency of distally recorded neural responses. Additionally, we performed biophysical simulations to investigate the multiple acoustically-induced mechanical (stretching, bending, transversal compression) and thermal axon exposures that could be responsible for neuronal responses.Our findings demonstrate that axons are directly and robustly excitable by ultrasound. Within a selected parametric regime, ultrasound stimulation of the spinal roots reliably evokes neural activity in the sciatic nerve and induces muscular contractions in the hindlimb antagonist muscles. In this regime, evoked responses show a clear dependence on both pressure amplitude and pulse duration. The simulations of mechanical and thermal effects are compared with the experimentally obtained evoked responses to provide insights into the biophysical mechanisms that may underlie the axonal response to ultrasound, enhancing our understanding of its therapeutic potential and guiding future applications.

Disclosures: E. Vicari: None. **T. Lemaire:** None. **T. Tarnaud:** None. **T. Plovie:** None. **O. Akouissi:** None. **O. Rizzo:** None. **E. Tanghe:** None. **E. Neufeld:** None. **S.P. Lacour:** None. **S. Micera:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.15/Z36

Topic: I.08. Methods to Modulate Neural Activity

Title: Temporal patterning defines the effects of low-intensity focused ultrasound (LIFU)

Authors: *A. STROHMAN, W. LEGON;
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Abstract: Title. Temporal patterning effects of low-intensity focused ultrasound (LIFU) for human neuromodulation Background. Low-intensity focused ultrasound (LIFU) is a rapidly emerging neuromodulation technique that leverages mechanical energy to non-invasively and reversibly alter neural activity with an adjustable depth of focus, providing unparalleled access to the human brain. While a large number of studies have been conducted in humans, the parameters used to deliver LIFU are variable and there is no current consensus on what LIFU parameters lead to robust excitatory or inhibitory effects that last long enough to be considered clinically meaningful. Methods. We stereotaxically targeted LIFU to the left primary motor cortex (M1) at the right first dorsal interosseous (FDI) muscle hotspot identified using single-pulse transcranial magnetic stimulation (TMS) on four separate days in a pre/post design. Single-pulse TMS was then used to elicit 20 motor-evoked potentials (MEPs) at an average baseline peak-to-peak amplitude of 1 millivolt from the right FDI muscle before and after LIFU application. Each day consisted of a different parameter set, matched on the total number of pulses and intracranial intensity, plus an inactive sham. The parameters included: theta burst transcranial ultrasonic stimulation ("tbTUS") based on prior literature (10% duty cycle (DC), 5 Hz pulse repetition frequency (PRF), 80 sec duration), a "standard LIFU" stimulation pattern (5% DC, 50 Hz PRF, 160 sec duration), and a "novel pattern" of LIFU stimulation consisting of a different distribution of pulses over time. Continuous auditory masking was applied across all conditions to mask the participant. MEPs were measured at baseline and at 0 and 10 minutes post LIFU or Sham application. Data are reported normalized to the pre-LIFU baseline. Results. Preliminary results demonstrate the novel pattern of LIFU led to a 41 +/- 12% reduction in normalized MEP amplitudes compared to baseline at 0 and 10 minutes post LIFU. Standard LIFU showed no reduction in MEPs at 0 and 10 minutes. Continued work will directly compare tbTUS, standard LIFU, and the novel pattern with Sham in further subjects for an extended time window at 0, 15, 30, and 60 minutes post LIFU. Conclusions. Our findings point to the importance of how LIFU is delivered with regards to the temporal patterning of energy across time while holding the total amount of energy constant. These results have implications for future studies on the identification of long-lasting, robust LIFU parameters that could translate to clinical therapeutic applications.

Disclosures: A. Strohman: None. **W. Legon:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.16/Z37

Topic: I.08. Methods to Modulate Neural Activity

Support: 5R21AT012247-02

Title: Investigation of the role of the anterior and posterior insula in gating of nociceptive stimuli using low-intensity focused ultrasound

Authors: *G. ISAAC[1], W. LEGON[2];
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Abstract: Central sensitization (CS) is a hallmark feature in several chronic pain conditions, characterized by the amplification of pain. Previous research has demonstrated a disruption in the gating of nociceptive information in chronic pain - a process that normally leads to reduced cortical activation in response to repeated painful stimuli. These findings suggest aberrant nociceptive information processing during CS; however, the specific brain regions involved in this dysregulated processing remains unclear. One candidate brain region is the insular cortex, a key pain processing center whose hyperactivity is associated with an increased experience of pain. The insula can be parsed into the posterior insula (PI) and anterior insula (AI), which differ both structurally and functionally. The PI receives peripheral pain signals before relaying them to the AI. In the AI, these signals are integrated with expectations, awareness, and emotional responses, assigning significance to the painful stimuli. While differences between the AI and PI suggest distinct functions in pain processing, their specific roles in regulating nociceptive gating remain unclear. To address this gap, we leverage the high spatial resolution and deep focal lengths of low-intensity focused ultrasound (LIFU) to selectively inhibit the AI or PI during a nociceptive paired-pulse gating task. Healthy human volunteers received 20 pairs of transient contact heat pain to the dorsum of their hand during either LIFU to the AI, PI, or Sham stimulation. Continuous electroencephalogram (EEG), electrocardiogram (ECG), electrodermal response (EDR), and subjective pain ratings were recorded, and outcome measures included the amplitude of the contact heat-evoked potentials (CHEPs). LIFU applied to AI resulted in increased gating of the second CHEP (49.1%) when compared to both PI (37.5%) and Sham (36.5%) conditions. Through the application of LIFU, we seek to causally interrogate the specific roles of the insular subregions in pain modulation, offering insights into potential therapeutic interventions for FM and chronic pain conditions.

Disclosures: G. Isaac: None. **W. Legon:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.17/Z38

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH R01 NS109794
NIH R21 EY034275

Title: Understanding non-thermal microwave effects on ion permeability of neuronal membranes with a rod-shaped resonator

Authors: *F. YU[1], C. MARAR[1], G. CHEN[1], J.-W. LIN[2], C. YANG[1,3], J.-X. CHENG[1,3,4]; [1]Electrical and Computer Engin. Dept., [2]Biol. Dept., [3]Chem. Dept., [4]Biomed. Engin. Dept., Boston Univ., Boston, MA

Abstract: The neuromodulation effects of microwaves (MWs) have been demonstrated since the 1970s. However, most studies focused on post-treatment analysis following long-term therapies for safety evaluation. There is a very limited number of real-time measurements on neurons during MW or experimental data on mechanism investigation. Previously, our group developed a split-ring resonator (SRR) capable of concentrating MW fields to submillimeter-scale resolution, enabling simultaneous calcium imaging of neuronal activities. Both in vivo and in vitro epilepsy models demonstrated efficient neuronal inhibition with the resonator at MW dosages below FDA safety exposure limits. To meet the future need for implantation with needle injection, we further developed a microwave rod (MWR). The MWR has a spatial precision of ~620 μm and amplifies the electric field by ~36 times, twice the conversion efficiency of the SRR. The resonator allows for high precision targeting with low MW power that does not induce significant effects on the neuronal membrane when applied MW alone. Using this device, we further investigated underlying mechanisms via patch clamp techniques on rat cultured cortical neurons. With a MWR resonating at 2.1 - 2.3 GHz, 1 second of continuous or pulsed MW with a power of 0.3 mW/cm² significantly reduced neuronal excitability for ~10 seconds following microwave cessation. The maximum action potential amplitude during MW dropped by a 20%, associated with a 10% decrease in membrane resistance. Further evaluations with the outside-out patch configuration suggested a modulation of Na^+ and K^+ permeability through tetrodotoxin-sensitive ion channels. Temperature elevation remained minimal, not exceeding 0.2 °C under typical tested parameters, thus supporting a non-thermal mechanism. The reduction in neuronal excitability may explain the inhibition of synchronized firing in epilepsy models, potentially through the suppression of synaptic output. Our study provides insights into the mechanisms of MW neuromodulation and lays the groundwork for exciting potential biomedical applications of the device, particularly in the clinical treatment of epilepsy and neuropathic pain in a drug-free manner.

Disclosures: F. Yu: None. **C. Marar:** None. **G. Chen:** None. **J. Lin:** None. **C. Yang:** None. **J. Cheng:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.18/AA1

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH/NINDS RF1 NS126144
NIH/NIBIB 1U18EB029351

Title: Precision Neuromodulation with Low-intensity Focused Ultrasound: BOLD, LFP, and Spike Dynamics in the Macaque Thalamus

Authors: N. ZHENG[1], P.-F. YANG[2], A. PHIPPS[3], J. KUSUNOSE[4], C. CASKEY[5], ***L. CHEN**[6];
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Abstract: Low-intensity transcranial focused ultrasound (FUS) has shown potential as a therapeutic strategy for neurological and psychiatric disorders by enabling remote modulation of brain regions, including deep structures. When combined with MRI guidance, the FUS array offers high spatial precision in both targeting and modulation. This study aims to test the spatial precision of a frame-based FUS array stimulation system combined with real-time MR-acoustic radiation force imaging (MR-ARFI) for FUS beam location validation, and to explore the neuromodulatory effects on the fMRI Blood Oxygenation Level Dependent (BOLD), local field potential (LFP), and spiking activity in the thalamic ventroposterolateral (VPL) nucleus in two anesthetized macaque monkeys. Initially, we established a 3D FUS beam targeting grid around the VPL nucleus using MR-ARFI feedback inside a 3T MRI scanner. Subsequently, we implanted a linear electrode array at the VPL target for intracranial electrophysiological recording outside the MRI scanner. Delivering 650 kHz ultrasound pulses at 1.05 MPa pressure (in situ estimate) at the VPL target, where BOLD signal changes were detected, elicited significant spiking activity and local field potential (LFP) signal changes, primarily in the low-frequency range (< 12 Hz). Spike width analysis indicated that the FUS pulses predominantly excited narrow-spiking neurons, suggesting a primary influence on inhibitory interneurons. Along the electrode length, LFP responses were highly localized around several channels, demonstrating the focal nature of the FUS beam and its modulation effects. As a positive control, tactile stimulation of fingers evoked distinct LFP and spiking activities from the implanted electrodes, differing from the responses induced by FUS at the thalamic VPL location. Furthermore, ultrasound stimulation of the nearby insular cortex also triggered robust LFP signal changes at the thalamic VPL site. In summary, our study demonstrates that low-intensity FUS can directly modulate LFP and spiking activity in thalamic VPL neurons, and indirectly through the interconnected insular cortex, achieving millimeter-scale spatial precision.

Disclosures: N. Zheng: None. **P. Yang:** None. **A. Phipps:** None. **J. Kusunose:** None. **C. Caskey:** None. **L. Chen:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.19/AA2

Topic: I.08. Methods to Modulate Neural Activity

Support: National Key R&D Program of China 2021YFF0702200
STI 2030-Major Projects 2021ZD0200401
Key R&D Program of Zhejiang Province 2021C03001

Title: Exploring Immediate and Latency Effects of Focused Ultrasound in the Primary Somatosensory Cortex of Nonhuman Primate

Authors: *L. LAN[1,2], M. GAO[2,3], Z. TANG[2,4], M. YE[2,3], T. HE[2,3], Y. XU[2,4], Z. LYU[2,4], H.-Y. LAI[2,3,4,5];
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Abstract: The primary somatosensory cortex (SI) is pivotal in processing sensory information, such as touch, pain, and proprioception, which underpin cognitive and motor functions. Focused ultrasound (FUS) has gained significant interest for its noninvasive approach, precision targeting of deep brain, adjustable parameters, and minimal risk to tissue. Although previous studies using behavioral assessments, electrophysiological recordings, and functional magnetic resonance imaging (fMRI) have shown that FUS can modulate the SI, its offline (latency) effect across the brain remains unclear. This study investigated both online (immediate) and offline effects of FUS targeting in the left SI using a lab-designed MRI-compatible FUS transducer (300 kHz) on the three monkeys. Online effects were assessed via FUS-evoked fMRI blood oxygenation level dependent (BOLD) responses using a 7T MRI system. Offline effects were evaluated by comparing the pre- and post-FUS tactile-evoked BOLD responses, and monitoring changes in functional connectivity (FC) over five hours. The FUS parameter included a single 200 msec pulse of 300-cycle bursts at 500 Hz (50% duty cycle) and a spatial peak temporal average (Ispta) of 0.94 W/cm^2 for 20 sec. Our results showed that FUS evoked a peak 1.5% increase in BOLD responses in SI. Post-FUS measurements revealed no tactile-evoked BOLD responses, suggesting a potential inhibitory effect on SI neural activity. Initial decreases in FC between the left SI and left primary motor cortex (M1) and bilateral anterior cingulate cortex (ACC) were observed 30-min post-FUS. Since the ACC receives inputs from the SI, which regulate pain-aversive behaviors, the reduction in FC might suggest a decrease in nociceptive responses within the ACC. Connectivity began to recover two hours post-FUS for bilateral ACC and 1.5 hours for the right M1, indicating varied latency effects across different brain circuits. This study

demonstrates that FUS can excite neural activity in SI while inhibiting connections within the SI-M1 and SI-ACC circuits. Further exploration of FUS parameters will enhance our understanding of its therapeutic potential for neurological and psychological disorders.

Disclosures: L. Lan: None. **M. Gao:** None. **Z. Tang:** None. **M. Ye:** None. **T. He:** None. **Y. Xu:** None. **Z. Lyu:** None. **H. Lai:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.20/AA3

Topic: I.08. Methods to Modulate Neural Activity

Support: R35GM138173

Title: Development of a 3D Printed Bubble Perfusion System for Ex Vivo Brain Slice Culture

Authors: *G. GUTIERREZ[1], R. J. ORTIZ[1,2], C. BAKER[1];
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Abstract: Microfluidic systems integrate tissue or cell culture with precise fluidic and environmental control to enable organ-on-chip models that improve our understanding of biochemical signaling and accelerate drug discovery and screening. Our lab has developed a 3D printed microfluidic perfusion system that enables real-time calcium imaging of *ex vivo* brain slices while providing on-demand chemical stimuli. Two key technology development efforts are described. First, a solution delivery module was developed to provide on-demand delivery of media/stimulus droplets and O_2 bubbles. The delivery system was calibrated to deliver volumes in the range of 1 - 90 µL, with 4% RSD at typical droplet volumes of 20 µL. Second, since 3D printed resin materials offer poor thermal properties, a water circulation system with on-device media prewarming chambers was developed to achieve mean culture temperatures of 37 ± 0.05 ℃ for circulated water temperatures of 45 °C. Temperature variation within each media droplet was reduced from 1.6 ℃ without the media prewarming chambers to 0.4℃ with prewarming, as measured over a 30 s period. To demonstrate tissue viability in this culture system, brain tissue slices from mice containing the suprachiasmatic nucleus (SCN) were stained with the Ca^{2+}-sensitive dye Calbryte 520 and held in the microfluidic slice culture system for several hours. Integrated fluorescence intensity was measured before, during, and after delivery of a depolarizing stimulus (60 mM KCl). Stimulated Ca^{2+} influx was observed for perfusion experiments lasting from 9-12 hours. Stimulated Ca^{2+} influx was also monitored upon delivery of 50 µM anandamide (AEA), the endogenous ligand for the cannabinoid 1 receptor (CB1R). CB1R, a G-protein coupled receptor, is expressed in various areas of the brain, including the SCN. AEA is an endocannabinoid that acts as a high-affinity partial agonist at the CB1R and has the capacity to induce an influx of intracellular Ca^{2+}. Preliminary data shows a statistically

significant response within the SCN of Ca^{2+} influx in response to 50 µM AEA. Ongoing work is aimed at a quantitative comparison of the Ca^{2+} influx response induced by AEA to that of cannabidiol (CBD) and various other cannabinoids.

Disclosures: G. Gutierrez: None. **R.J. Ortiz:** None. **C. Baker:** None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.21/AA4

Topic: I.08. Methods to Modulate Neural Activity

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G. Harold and Leila Y. Mathers Charitable Foundation
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Damon Runyon Cancer Research Foundation

Title: Sonogenetic stimulation of hsTRPA1-expressing primary hippocampal neurons drives time-locked neuronal firing.

Authors: *C. WALSH, W. WONG, S. CHALASANI;
Salk Inst. for Biol. Studies, San Diego, CA

Abstract: Sonogenetics is a rapidly growing field of study, where the activity of specific populations of neurons is modulated using ultrasound and selective expression of ultrasound-sensitive ion channels. Ultrasound can effectively penetrate biological tissues, and can be focused to sub-millimeter resolution, making it highly suited to use in non-invasive neuromodulation. So far, ultrasound-sensitivity has been demonstrated in cells expressing several mechanosensitive ion channels, including TRP family channels such as TRPA1 and TRPV1, and bacterial MSC family channels MscL and MscS. The discovery of novel sono-channels with increased sensitivity and conductances would provide valuable tools with which to progress the field. Calcium imaging in cultured neuronal and non-neuronal cells is widely used to assay potential sono-channels and test various stimulation parameters. Ultrasound neuromodulation is not well suited to patch-clamp electrophysiology, as ultrasound at frequencies commonly used for ultrasound neuromodulation can disrupt the gigaOhm seal of the patch pipette even at relatively low intensities. Here, we introduce a planar multi-electrode array system and a custom ultrasound stimulation system which can allow extracellular action potential recordings from hundreds of neurons simultaneously, while delivering ultrasound stimulation. Hippocampal primary neurons from embryonic C57BL/6 mice (E18) are cultured on 120 channel multielectrode arrays (MultiChannel Systems) and transduced with AAV encoding the human TRPA1 channel (hsTRPA1), which we have previously shown to be ultrasound-sensitive and effective for sonogenetic neuromodulation in vitro and in vivo. We first show that expression of

this ultrasound-sensitive ion channel does not affect the baseline firing properties of transduced neuron populations. We show that ultrasound stimulation using a custom lithium niobate transducer device (0.6MPa, 7MHz, 100ms every 10s) elicits dramatic increases in action potential firing, which is time-locked to the stimulation, in hsTRPA1-expressing neurons, but not non-transduced neurons. These data demonstrate the potential value of this approach in characterizing future ultrasound-sensitive ion channels, testing a range of ultrasound stimulation parameters without the limitations of patch-clamp techniques, and learning more about the mechanisms underlying the effects of ultrasound on neurons.

Disclosures: C. Walsh: None. **W. Wong:** None. **S. Chalasani:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SonoBac.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.22/AA5

Topic: I.08. Methods to Modulate Neural Activity

Support: NIBIB Grant R56EB031848

Title: Wireless magnetomechanical modulation of cortical neurons

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Abstract: Noninvasive cell-type-specific manipulation of neural signaling is critical in basic neuroscience research and in developing therapies for neurological disorders. Magnetic nanotechnologies have emerged as non-invasive neuromodulation approaches with high spatiotemporal control. Magnetic nanodiscs (MNDs) are suitable for evoking neural activity because they are good transducers of low-frequency and low-amplitude alternating magnetic fields (AMFs). When targeted to the cell membrane, MNDs mechanoactuation triggered by AMFs enhances or inhibits specific cell membrane receptors for the modulation of biological signaling. This research investigates magnetomechanical neuromodulation using MNDs targeted to the cell membrane of primary rat cortical neurons and unveils the key ion channels involved during mechanotransduction. Our MNDs are fabricated using top-down lithography techniques, functionalized with polymers and antibodies, and characterized for their physical properties. Primary cortical neurons co-cultured with MNDs and transmembrane protein chemical inhibitors are subjected to 20s pulses of weak AMFs (18 mT, 6 Hz). Calcium cell activity is recorded during AMFs stimulation. We revealed neuronal activity in primary rat cortical neurons is evoked by the AMFs-triggered actuation of targeted MNDs. Regardless of the size of MNDs, we were able to detect neural activity in cortical cultures within seconds of applying AMFs stimulus.

The applied 20 s of AMFs was sufficient for both 700 nm and 300 nm MNDs to evoke neural activity through magnetomechanical transduction. When mediated by 700 nm MNDs, ~ 50 % of cortical neurons were responsive to the treatment while a higher percentage of responsive neurons (> 60%) was observed when treatment was mediated by 300 nm MNDs. Ion channel chemical inhibition suggests that magnetomechanical neuromodulation results from MNDs actuation on Piezo1 and TRPC1 mechanosensitive ion channels. The actuation mechanisms depend on MNDs size, with cell membrane stretch and stress caused by the MNDs torque being the most dominant. Magnetomechanical neuromodulation represents a tremendous potential since it fulfills the requirements of no heating and weak AMFs, which are limiting factors in the development of therapies and the design of clinical equipment.

Disclosures: A. Gomez: None.

Poster

PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.23/AA6

Topic: I.08. Methods to Modulate Neural Activity

Support: NIG Grant 1R35GM147408
University of Texas at Austin Startup Fund
Robert A. Welsh Foundation Grant (No. F-2084-20210327)
Craig H. Neilsen Foundation Pilot Research Grant

Title: Non-invasive and programmable molecular manipulation for deep brain modulation using focused ultrasound

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Abstract: The precise control of mechanochemical activation within deep tissues via non-invasive ultrasound holds profound implications for advancing our understanding of fundamental biomedical sciences and revolutionizing disease treatments. However, a theory-guided mechanoresponsive materials system with well-defined ultrasound activation has yet to be explored. Here we present the concept of using porous hydrogen-bonded organic frameworks (HOFs) as toolkits for focused ultrasound programmably triggered drug activation to control specific cellular events in the deep brain, through on-demand scission of the supramolecular interactions. A theoretical model is developed to visualize the mechanochemical scission and ultrasound mechanics, providing valuable guidelines for the rational design of mechanoresponsive materials at the molecular level to achieve programmable and spatiotemporal activation control. To demonstrate the practicality of this approach, we encapsulate designer drug

clozapine N-oxide (CNO) into the optimal HOF small particles for FUS gated release to activate engineered G-protein-coupled receptors in the mice and rat ventral tegmental area (VTA), and hence achieved targeted neural circuits modulation even at depth 9 mm with a latency of seconds. This work demonstrates the capability of ultrasound to precisely control molecular interaction and develops ultrasound programmable HOFs to minimally invasive and spatiotemporally control cellular events, thereby facilitating the establishment of precise molecular therapeutic possibilities. We anticipate that this research could serve as a source of inspiration for precise and non-invasive molecular manipulation techniques, potentially applicable in programming molecular robots to achieve sophisticated control over cellular events in deep tissues.

Disclosures: W. Wang: None. **K. Tang:** None. **H. Wang:** None.

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Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.24/AA7

Topic: I.08. Methods to Modulate Neural Activity

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Title: Low intensity focused ultrasound evokes persistent dilation in cortical microvasculature in vivo

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Abstract: Low-intensity focused ultrasound (FUS) has emerged as a promising neuromodulation technique that offers the potential for spatially precise and personalized therapy. While FUS can elicit acute electrical effects such as action potentials in neurons, neuromodulatory effects that persist from hours to days have also been reported. An unresolved question pertains to whether persistent effects might be attributed, at least in part, to vascular mechanisms. The brain's ability to regulate blood flow to active regions involves complex interactions among various non-neuronal cell types and signaling pathways in the cerebral vasculature. Functional magnetic resonance imaging (fMRI) and laser speckle investigations have demonstrated that FUS induces hemodynamic activity in the cortical vasculature. Those measurements, though, have resolved flow in larger vessels. We aimed to explore the impact of FUS on cerebrovascular function at the level of the cortical microvasculature, which has eluded prior investigations. Employing a novel in vivo optical technique, our investigation revealed persistent dilation in microvascular responses, but not in vessel branches of larger diameters. This discovery not only sheds light on a previously unexplored facet of FUS effects *in vivo* but also suggests that concurrent alterations in vascular function may contribute to the observed persistent neuromodulatory effects.

Disclosures: Y. Shen: None. **J. Jethe:** None. **J. Hehir:** None. **J.A. Fisher:** None.

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Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

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Topic: I.08. Methods to Modulate Neural Activity

Support: ICT-36-2020-101016787
945539
PID2019-111493RB-I00
2021-SGR-01410
2021-SGR-00064
BES-2017-082496

Title: Light-activated drugs to restore neuronal activity in neurological disorders

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Abstract: The lack of tissue selectivity of conventional systemic pharmacological agents has long sparked the desire for compounds enabling on demand, localized control. Even drugs with highly selective pharmacological profiles act on their target at unintended organs and locations, with pharmacokinetics that cannot be externally altered. Consequently, the predominant unselective and fixed kinetics of action of conventional pharmacological agents pose significant challenges, particularly in treating neurological disorders such as stroke, traumatic brain injury, Parkinson's and Alzheimer's diseases, and schizophrenia.Photopharmacology has emerged as a promising avenue, providing reversible control of endogenous receptor activity with high spatiotemporal resolution by photoswitching ligands between an active and an inactive isomer.Here, we report novel photoswitchable drugs, named neuroswitches, that remain inactive in the dark but can be activated under orange, red, and infrared light, capable of penetrating the skull. These compounds demonstrate photoreversible efficacy both *in vitro* and *in vivo*, with no observed acute toxicity. Our proposed approach entails systemic delivery of the inactive neuroswitch followed by precise spatiotemporal activation using light patterns within the targeted region (*e.g.*, the area surrounding a neurologically damaged site). This strategy aims to regulate neuronal activity while avoiding systemic adverse effects associated with conventional pharmacological interventions.

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Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.27/AA9

Topic: I.08. Methods to Modulate Neural Activity

Support: University of Utah startup funds awarded to Jan Kubanek

Title: Noninvasive modulation of subcallosal cingulate and depression with focused ultrasonic waves

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Abstract: Introduction:An estimated 30-50% of patients with major depression are resistant to current treatments.Depression is associated with hyperactive subgenual cingulate cortex (SGC), a deep brain regions that has been difficult to modulate with existing approaches.We have applied low-intensity transcranial focused ultrasound to suppress the activity in this target directly, selectively, and non-invasively.
Methods:We have developed an approach that delivers focused ultrasound into confined deep brain targets while compensating for the severe and unpredictable attenuation of ultrasound by the human head and hair.The method delivers into the target controlled ultrasound intensity.Using this approach, we delivered pulsed low-intensity ultrasound into the SGC of 20 patients with treatment-resistant depression within a randomized sham-controlled clinical study (NCT05301036).We validated target engagement using functional MRI.
Results:Low-intensity ultrasound reliably engaged the target, suppressing fMRI BOLD activity specifically at the SGC.Mood and depression scores improved more with real than with sham stimulation.In the per-protocol sample (n = 19), real stimulation was superior to sham for HDRS-6 at 24 hours and for Sadness (both $p < 0.05$, $d > 1$).Non-significant trends were found in the intent-to-treat sample.
Conclusion:Ultrasonic stimulation modulates SGC activity and can rapidly reduce depressive symptoms.The capability to non-invasively and selectively target deep brain regions creates new possibilities for the future development of circuit-directed therapeutics, and for the dissection of deep-brain circuit function in humans.

Disclosures: T. Riis: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SPIRE Therapeutic Inc. **D. Feldman:** None. **B.J. Mickey:** None. **J. Kubanek:** A. Employment/Salary (full or part-time):;

University of Utah. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SPIRE Therapeutic Inc..

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PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

Location: MCP Hall A

Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

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Topic: I.08. Methods to Modulate Neural Activity

Title: Mapping cerebral blood volume (CBV) changes induced by longitudinal focal TMS on awake rats

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Abstract: Transcranial Magnetic Stimulation (TMS) has been FDA-approved for the treatment of neuropsychiatric disorders. However, the mechanism of TMS action remains poorly understood. Rodent models allow for invasive manipulations, thus, back-translating TMS from humans to rats holds great value.We recently developed the high-density theta burst stimulation (hdTBS) paradigm, which enhances the acute aftereffects by 92% compared to the conventional iTBS [Meng et al. 2022]. We conducted two experiments to assess the longitudinal effects of hdTBS. Experiment 1 investigated whether accelerated hdTBS produces distinct effect on cortical excitability; and in experiment 2, we used MRI to map brain networks affected by longitudinal hdTBS. In experiment 1, 3 groups of rats received TMS on hindlimb motor cortex for 5 days: 1) 3 hdTBS sessions/day, inter-session interval of 60 min (n=8); 2) 1 hdTBS session/day (n=9); 3) sham TMS, 1 hdTBS session/day (n=7). Microwire electrodes were implanted on hindlimb muscles to record the motor-evoked potential (MEP) signal. Input-output (IO) curves were measured on day 1 (pre-hdTBS baseline) and on day 7 (1-day post-hdTBS), with the TMS power varying from 75% to 115% of the motor threshold. TMS was administered using a hdTBS stimulator and a rodent-specific focal TMS coil developed in house [Meng et al., 2018]. Results showed significantly increased MEP values in rats receiving 1 session/day, indicating an excitatory effect, while those receiving 3 sessions/day exhibited significantly reduced MEP values, indicating an inhibitory effect.We subsequently performed MRI experiments on 2 additional groups of rats to delineate brain regions impacted by longitudinal TMS: group 1: n=8, 1 hdTBS session/day for 5 days; group 2: n=9, 1 sham hdTBS session/day for 5 days. Resting state fMRI and basal cerebral blood volume (CBV) data were acquired on day 1 and day 7. Iron-oxide contrast agent Feraheme was injected (IV) for CBV measurement. Our results revealed that 1 hdTBS session/day for 5 days significantly enhanced CBV in M1, S1HL, S1BF and in caudate putamen, amygdala, suggesting TMS significantly enhanced basal

brain metabolism in these regions.In conclusion, we have established an awake rat model mimicking human TMS conditions. We found that 1 hdTBS session/day significantly enhanced the excitability of the rat motor cortex, whereas 3 sessions/day significantly reduced it. Moreover, CBV mapping revealed several brain regions, both proximal and distal to the stimulation sites, had heightened basal brain activity after 1 hdTBS session/day for 5 days. This model opens a novel platform for further investigating TMS mechanisms.

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PSTR432

Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

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Time: Wednesday, October 9, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR432.29/AA11

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF NCS-2220677

Title: Transcranial focused ultrasound stimulation of anterior cingulate cortex modulates contingent negative variation and proactive control behavior

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Abstract: The ability to control movements is critical for physical interactions with the environment. Movements can be controlled in an anticipatory manner (proactive) or as a late-corrective action (reactive). Proactive control uses contextual information to prepare possible action choices based on the predicted nature of the interference event before it occurs. The electroencephalography (EEG) literature has reported the contingent negative variation (CNV) as a neural correlate of proactive control. Based on current neuroimaging correlational evidence, CNV could be associated with detecting unique stimuli, motor response anticipation and/or task goal maintenance. However, which of these components of proactive control is encoded by the CNV has yet to be causatively established. Furthermore, neural sites in the cognitive control network whose activity contributes to CNV modulation based on proactive control dynamics need to be better established. The fMRI literature reports the activation of the anterior cingulate cortex (ACC) in the anticipatory response phase during proactive control simultaneous with the CNV. However, the functional role of ACC in proactive control is still debated. To address these gaps, we used an AX-version of the continuous performance task (AX-CPT) to evaluate the behavior and neural correlates of proactive control. Twenty-one participants performed the task while a neural site in ACC, p24', was stimulated with low-intensity transcranial-focused

ultrasound (tFUS). An AX-CPT trial presents a contextual cue stimulus, followed by a probe stimulus with context-based rules guiding the response to the probe. In AX-CPT, the deterministic context has a single action choice warranting proactive control. In contrast, the probabilistic context has multiple action choices dictating different cognitive control strategies based on stimulus combinations. A 500-ms tFUS stimulation was provided on alternate trials 200 ms after cue presentation, corresponding to 300 ms before the appearance of the CNV. The cue response time and probe response error significantly increased in the deterministic context with tFUS ($p<0.05$). Additionally, the fronto-central CNV was more negative in the tFUS condition for the deterministic context. This indicates that tFUS disrupts proactive control by introducing uncertainty in the deterministic context. Hence, we conclude that ACC is associated with proactive control based on the modulation of CNV, which encodes contextually dependent anticipatory responses.

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Neuromodulation Techniques: Thermal, Magnetic, and Ultrasound

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Program #/Poster #: PSTR432.30/AA12

Topic: G.03. Motivation

Support: IBS-R026-D1

Title: In vivo magnetogenetics for cell-type specific targeting and modulation of brain circuits

Authors: *S.-H. CHOI[1], J. SHIN[1], L. JAE HYUN[1], M. KWAK[1], J. CHEON[2];
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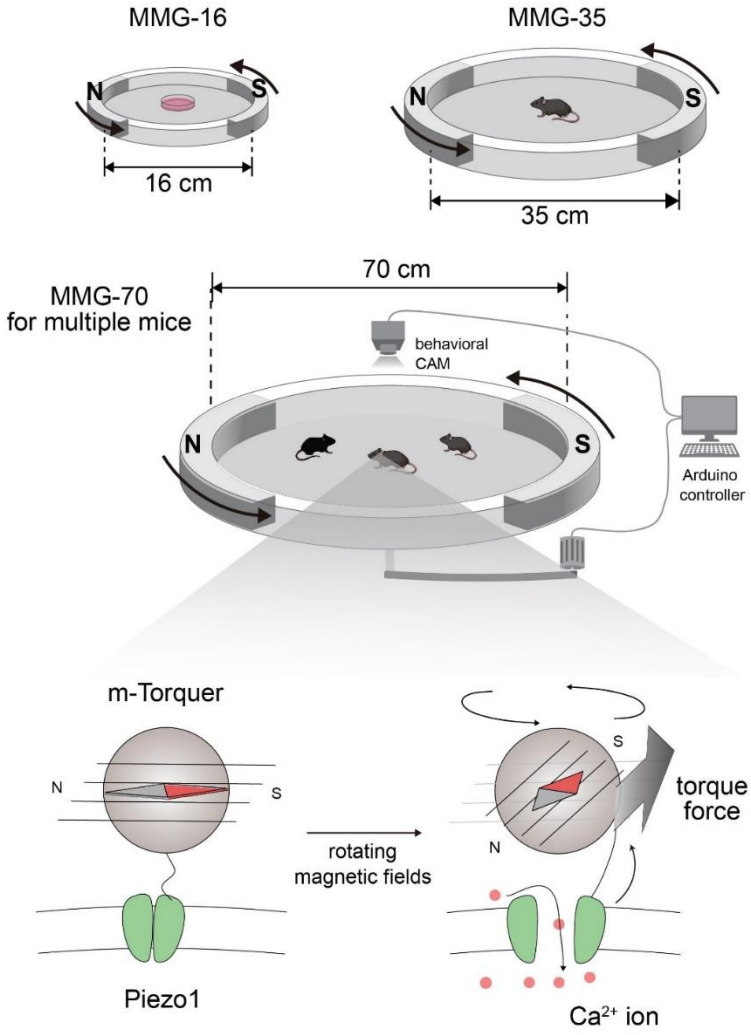
Abstract: Abstract Neuromodulation technologies are crucial for investigating neuronal connectivity and brain function. Magnetic neuromodulation offers wireless and remote deep brain stimulations that are lacking in optogenetic- and wired electrode-based tools. However, due to the limited understanding of working principles and poorly designed magnetic operating systems, earlier magnetic approaches have yet to be utilized. Furthermore, despite its importance in neuroscience research, cell-type specific magnetic neuromodulation has remained elusive. Here, we present a nanomaterials-based magnetogenetic toolbox, in conjunction with Cre-loxP technology, to selectively activate genetically encoded Piezo1 ion channels in targeted neuronal populations via torque generated by the nanomagnetic actuators *in vitro* and *in vivo*. We demonstrate this cell-type-targeting magnetic approach for remote and spatiotemporal precise control of deep-brain neural activity in multiple behaviour models, such as bidirectional feeding control, long-term neuromodulation for weight control in obese mice, and wireless modulation of social behaviours in multiple mice in the same physical space. Our study demonstrates the

potential of cell-type specific magnetogenetics as an effective and reliable research tool for life sciences, especially in wireless, long-term, and freely behaving animals. **Keywords:** Magnetogenetics, Magnetic nanoparticle, Torque-based force, Piezo1, Cell type-specific neuromodulation..

Figure 1. Magnetogenetics (MG) for neuron-specific modulation of brain circuits.MG stimulation induces wireless ion channel gating of specific neurons with a magneto-mechanical-genetics (MMG) apparatus. Under rotating magnetic fields, m-Torquer generates torque force to activate mechanosensitive Piezo1 ion channels.



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