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Poster

PSTR257: Neural Stem Cells

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR257.01/A1

Topic: A.03. Stem Cells and Reprogramming

Support: NSF IOS 2207023
NIH T32 AG044402

Title: Age and sex hormone-based effects on adult neurogenesis in the female brain - a behavioral and histo-stereological perspective

Authors: *S. PILLUTLA[1], M. SKODA[1], A. ISHII[1], M. J. CORENBLUM[1], N. MENAKURU[1], J. R. MEREDITH[4], P. WENE[2], L. MADHAVAN[3];
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Abstract: Adult neurogenesis that occurs in the forebrain subventricular zone (SVZ) and the subgranular zone (SGZ) of the dentate gyrus is influenced by parameters such as age, sex hormones and other cellular and molecular factors. Our previously published research, on male F344 rats, has demonstrated that neurogenesis decline is particularly pronounced in the period between 13 and 15 months of age. We have also established that this particular pattern of age-related decline in neurogenesis is mediated by the reduced expression of the redox-sensitive transcription factor nuclear factor (erythroid-derived 2)-like 2 or NRF2. In this study, we aim to understand the impact of advancing age and the sex hormones, 17β-estradiol (E2) and progesterone (P4), on the NRF2 expression and regenerative function of neural stem progenitor cell (NSPCs) in female rats. In this context, we have determined that the temporal progression of age-related decline in NSPC function is different in female rats and occurs earlier, principally by 7-9 months of age, compared to males. To further investigate this NSPC aging phenomenon, we are analyzing female F344 rats at 2, 6, 9 and 14 months of age. Both intact (Sham) and ovariectomized (OVX) rats were included at each of the 4 aging stages to assess the importance of E2/P4. The following behavioral tasks were performed on the experimental groups to study SVZ and SGZ NSPC function - fine olfactory discrimination, pattern separation and platform reversal in the Morris water maze. Results show significant protection of neurogenesis in the sham animals compared to OVX, especially at 6 and 9 months of age. These results are also supported by findings in the estrous staging where sham rats in estrus or proestrus stages of the cycle (increased circulating E2 and P4) did better compared to other stages. Currently, we are examining the changes in NRF2 expression and activity in NSPCs, in the four age-groups, through double or triple immunostaining of different NSPC subtype markers (specifically GFAP/Nestin, Sox2 and Dcx) and proliferative markers (BrdU, MCM2), with NRF2 and its downstream targets such as NAD(P)H quinone dehydrogenase 1 (NQO1). In conclusion, these

studies will give us a detailed picture of how E2/P4 and age influence NSPC function and NRF2 expression.

Disclosures: S. Pillutla: None. **M. Skoda:** None. **A. Ishii:** None. **M.J. Corenblum:** None. **N. Menakuru:** None. **J.R. Meredith:** None. **P. Wene:** None. **L. Madhavan:** None.

Poster

PSTR257: Neural Stem Cells

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR257.02/A2

Topic: A.03. Stem Cells and Reprogramming

Support: NSF IOS 2207023
NIH T32 AG044402

Title: Evaluating the effects of age and sex hormones on neural stem progenitor cell niches in the female brain

Authors: *M. SKODA[1], S. PILLUTLA[1], A. ISHII[1], M. J. CORENBLUM[1], J. R. MEREDITH[1], P. WENE[2], N. MENAKURU[1], L. MADHAVAN[3];
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Abstract: Neural stem and progenitor cells (NSPCs) in the adult mammalian brain produce new nerve cells throughout life. It is known this neurogenic process is regulated by age- and sex-dependent mechanisms, however, these mechanisms are not fully understood. Our previous work in male rats identified a critical period (CP) of NSPC regenerative decline, between 13 and 15 months of age, and identified the reduced expression of the nuclear factor (erythroid-derived 2)-like 2 (NRF2) transcription factor, as a key mediator of this phenomenon. Based on these findings, we are currently assessing several age-groups of female rats to better understand the roles of age, sex, and NRF2 in regulating NSPC function. We have found the CP of NSPC decline to occur earlier in life in females compared to males— approximating the timeline of reproductive senescence. Specifically, a sharp drop in NSPC activity becomes evident during the rat perimenopausal period (7-9 months of age) when decreases in 17β-estradiol (E2) and progesterone (P4) levels are known to occur. To investigate the influence of E2 and P4 on NSPC function, female F344 rats, aged 2, 6, 9, and 14 months old, were either ovariectomized (OVX) or underwent sham surgery, after which they were assessed through behavioral tests and downstream tissue analyses. Early data indicate significant OVX-induced declines in behavioral task performance relevant to the function of the subventricular zone (SVZ) and hippocampal subgranular zone (SGZ) (assessed via fine olfactory discrimination, pattern separation, and reversal in the Morris Water Maze), at 6 and 9 months of age. Here, we use immunohistochemistry and western blotting to examine changes in estrogen receptor (ER) α/β

and progesterone receptor (PR) A/B expression in these NSPC harboring regions. Previous studies have investigated ER and PR expression in the brain, but have not carefully considered expression specific to the SVZ and hippocampal SGZ. First data indicate a trend towards lower ERβ expression at 6 months of age. Additionally, we are determining the expression of NRF2 and its downstream target genes. Preliminary results indicate downregulation of NRF2 expression after OVX in both the SVZ and SGZ at 2 and 6 months of age. In summary, our studies provide insight into the role of E2/P4 and NRF2 in the regulation of NSPC aging.

Disclosures: M. Skoda: None. **S. Pillutla:** None. **A. Ishii:** None. **M.J. Corenblum:** None. **J.R. Meredith:** None. **P. Wene:** None. **N. Menakuru:** None. **L. Madhavan:** None.

Poster

PSTR257: Neural Stem Cells

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR257.03/A3

Topic: A.03. Stem Cells and Reprogramming

Support: TOA Biopharma Co. Ltd.

Title: Enhancement of Adult Neurogenesis and Restoration of its Attenuation in Germ-free Mice by Three-Combination Probiotics

Authors: *M. NAMIHIRA[1], N. INOUE[2], Y. WATANABE[2], T. HAYASHI[2], K. MUROTOMI[1], K. HIRAYAMA[3], N. SATO[2];
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Abstract: Gut microbiota plays an important role in regulating brain function and adult neurogenesis. Although probiotics have recently been reported as effective against certain psychiatric disorders, the underlying mechanisms remain unclear. In particular, the combination of three probiotic strains, *Bacillus subtilis* TO-A, *Enterococcus faecium* T-110, and *Clostridium butyricum* TO-A, hereafter referred to as ProB3, has been reported to potentially alleviate psychiatric symptoms in patients with schizophrenia. Here, we show that ProB3 promotes adult neurogenesis in mice and restores its dysregulation in germ-free (GF) mice. ProB3 colonization in GF mice enhanced the proliferation of adult neural stem cells compared to specific-pathogen-free (SPF) and GF mice. Furthermore, ProB3 colonization was sufficient to ameliorate the arrest of newborn neuron maturation and the diminution of quiescent neural stem cells in GF mice. ProB3 colonization in mice increased the levels of several metabolites in the blood, such as theanine, 3-hydroxybutyrate, and imidazole peptides, including anserine, which promoted proliferation, neurogenesis, and maturation of newborn neurons in cultured human neural stem cells. Overall, our findings demonstrate that ProB3 colonization promotes adult neurogenesis in

mice and suggest that the promotion of adult neurogenesis may contribute to the alleviation of psychiatric symptoms.

Disclosures: M. Namihira: 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.; TOA Biopharma Co. Ltd. **N. Inoue:** A. Employment/Salary (full or part-time):; TOA Biopharma Co. Ltd. **Y. watanabe:** A. Employment/Salary (full or part-time):; TOA Biopharma Co. Ltd. **T. Hayashi:** A. Employment/Salary (full or part-time):; TOA Biopharma Co. Ltd.. **K. Murotomi:** None. **K. Hirayama:** None. **N. Sato:** A. Employment/Salary (full or part-time):; TOA Biopharma Co. Ltd..

Poster

PSTR257: Neural Stem Cells

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR257.04/A4

Topic: A.03. Stem Cells and Reprogramming

Support: INPER PROTOCOL 2018-1-146

Title: Maternal diabetes alters neural stem cell cycle at early corticogenesis in the rat: transcriptomic analysis

Authors: *R. VALLE-BAUTISTA[1], D. DE LA MERCED GARCÍA[1], D. A. DÍAZ PIÑA[1], N. E. DIAZ[2], A. MOLINA[1];
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Abstract: Maternal diabetes has been related to a deficiency of cognitive processes in tasks that require motor, memory, and visuospatial integration skills during childhood. These cognitive-process difficulties may be related to cytoarchitectonic configuration differences in neocortical layers of pyramidal neurons. The murine models of maternal hyperglycemia showed that the products of diabetic (DB) mothers present an increase in neuronal differentiation and aberrant neuronal migration in the cerebral cortex, likewise changes in regular laminar pattern, and alterations in dendritic arbor polarity establishment in pyramidal neurons of deep layers V-VI, these cytoarchitectonic changes result in less neuronal excitability in young postnatal 21 days (P21), DB products. Due to corticogenesis beginning at the early stages of neural development in rats, around embryo day 12 (E12), as the expansion and differentiation of neural stem cells (NSC), this study aimed to determine if maternal hyperglycemia modifies the transcriptomic profile of cortical neuroepithelium at E12 of embryos without neural tube defects; therefore, total RNA of dorsal prosencephalon from E12 control (Ctrl) and DB groups was extracted to realize RNAseq. Differential expression analysis shows that the neuroepithelium of DB embryos has

111 transcripts upregulated and 136 downregulated, regarding the Ctrl group. Pathway functional enrichment analysis shows that between differentially expressed transcripts, there are genes that have a relevant role in the cell cycle, specifically in mitotic spindle formation, such as Numa1 and Aurkb. These findings suggest modifications in the NSC division pattern to more asymmetric NSC division at early corticogenesis, which would explain the previously reported premature neuronal differentiation in DB embryos.

Disclosures: R. Valle-Bautista: None. **D. De La Merced García:** None. **D.A. Díaz Piña:** None. **N.E. Diaz:** None. **A. Molina:** None.

Poster

PSTR257: Neural Stem Cells

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Topic: A.03. Stem Cells and Reprogramming

Support: AFOSR-FA9550-20-1-0386 (AstroLight)
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EU-Horizon MSCA-ASTROTECH-956325

Title: Nanoscale interfaces modulate adhesion morphology and differentiation selectively promote neurogenesis and gliogenesis in neurospheres adult mice

Authors: *C. LAZZARINI[1], E. POETA[2], G. CONTE[1], R. FABBRI[1], R. ZAMBONI[1], T. POSATI[1], G. P. NICCHIA[3], B. MONTI[2], V. BENFENATI[1];
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Abstract: Neurospheres (NS), derived from embryonic or adult stem cells, are commonly utilized as 3D *in vitro* models for investigating the molecular processes, dynamics, and structure of the brain throughout neural development. In this context, biomaterials engineering and nanomaterials technologies can be helpful to address challenges arising by the need to reproduce *in vitro*, the features of the brain microenvironment (Roth JG, et al., Nat. Rev. Neurosci. 2021). To address this challenge, we proposed using multilayered nanostructured materials called hydrotalcites (HTlc) (Posati T, et al., Scientific Reports. 2016) to interface with NS derived from the subventricular zone (SVZ) of adult mice (8-months-old). Through cell viability assays, confocal microscopy, immunostaining, western immunoblotting and qRTPCR experiments, we identified subpopulations of neural cells (astrocytes, neurons and oligodendrocytes) that adhered to the nanostructures. We found that the chemical and topographic cues of HTlc influenced adhesion at 7 *days in vitro* (DIV) on HTlc that resulted in fewer differentiated cells and more rounded cells compared to NS grown on Poly-D-lysine/Fibronectin (CTRL) (n=3). In addition, at

14DIV, the number of adhering and differentiated cells on HTlc increased, with differentiation spreading between neural and non-neural cells (n=3). Molecular analyses showed that both immature neurons (DCX) and astrocytes (GFAP) significantly increase at both 7 DIV and 14 DIV on HTlc compared to CTRL (n=3). On the other hand, there is a different trend for oligodendrocytes. At 7 DIV, there is a lower concentration of Olig2 positive cells on HTlc compared to CTRL, while at 14 DIV there is an increase in CNPase positive cells on the substrate of interest compared to CTRL (n=3). Furthermore, preliminary functional analysis performed on water transport and calcium signalling suggested the involvement and differential expression of Transient Receptor Potential Vanilloid 4 (TRPV4) and Aquaporin-4 (AQP4) over time, confirming previous results on embryonic NS (Cibelli A, et al., Glia. 2024) (n=2). In conclusion, HTlc substrates have the potential to be a useful tool for selectively promoting the differentiation of NS into both neuronal and non-neuronal cell types and more importantly to understand a possible new mechanism underlying both cell differentiation in NS and cell-substrate interaction.

Disclosures: C. Lazzarini: None. **E. Poeta:** None. **G. Conte:** None. **R. Fabbri:** None. **R. Zamboni:** None. **T. Posati:** None. **G.P. Nicchia:** None. **B. Monti:** None. **V. Benfenati:** None.

Poster

PSTR257: Neural Stem Cells

Location: MCP Hall A

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Support: NRF Grant 2021R1A6A3A01087124
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NRF Grant 2020R1C1C101024514
NRF Grant 2022M3E5E801739512

Title: Anoctamin 1 deficiency alters somatostatin interneurons in the medial ganglionic eminence

Authors: *K. KIM[1], U. OH[2], G. HONG[3];
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Abstract: Corticogenesis is largely dependent on the precise regulation of interneuron neurogenesis, which is mediated by ventral radial glia (vRG) in the medial ganglionic eminence (MGE). Previous research has demonstrated substantial expression of Ano1 transcripts in Fabp7+ and Sox2+ vRGs from E11.5 to E14.5. Ano1-deficient mice exhibited a decreased ratio

of EdU-positive and RC2-positive cells at E14.5, suggesting altered neurogenesis. Further, reduced populations of GABAergic neurons were observed in the cortex of Ano1 knockout (KO) mice at E18.5. The present study seeks to elucidate the impact of Ano1 deficiency on MGE-derived interneuron subtypes. Using immunostaining and fluorescent *in situ* hybridization (FISH), we observed a decreased percentage of Sst+ neurons in the MGE of $Ano1^{-/-}$ embryos at E14.5 and E16.5. Moreover, an increase in the Ascl1+ intermediate progenitor cell population was detected. These findings indicate that Ano1 depletion in vRG leads to a fate shift towards basal progenitors, rather than apical progenitors, during GABAergic neurogenesis, resulting in a reduction of SST+ neurons.

Disclosures: K. Kim: None. **U. Oh:** None. **G. Hong:** None.

Poster

PSTR257: Neural Stem Cells

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Program #/Poster #: PSTR257.07/A7

Topic: A.03. Stem Cells and Reprogramming

Support: NC3Rs Studentship NC/W001675/1
UK Dementia Research Institute

Title: Two-photon calcium imaging reveals local propagating calcium waves in human cerebral organoids

Authors: *F. K. W. LAM[1], S. S. HARRIS[1], C. ARBER[3], J. ROWLAND[1], S. WRAY[2], M. BUSCHE[1];
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Abstract: Cerebral organoids (COs) are widely used to model aspects of human brain development, function and disease. However, little is known about the longitudinal neuronal network dynamics present in COs. Here, we aimed to characterise these dynamics during the development of COs, by recording neuronal activity at the single-cell level using two-photon Ca^{2+} imaging. We recorded at different depths within the spherical three-dimensional cellular structure of iPSC-derived COs, labelled with the Ca^{2+} indicator Cal520-AM, between 80-300 days *in vitro* (DIV). We identified three distinct modes of spontaneous activity: unsynchronous activity, synchronous activity and local propagating waves. Unsynchronous activity, *i.e.* sparser, uncorrelated activity, was the baseline activity present throughout and the most prominent type of activity from ~100 DIV onwards. Synchronous activity, *i.e.* events of activity that are correlated across the majority of cells within the field of view at the same time, was the

predominant mode of activity during early developmental stages (DIV 80-100). Surprisingly, our recordings also revealed local propagating waves that appeared from ~100 DIV onwards. These waves consisted of a Ca^{2+} transient event which initiated within a single neuron, before propagating to adjacent neurons, and was followed by an onset of multiple Ca^{2+} transients in connected neurons within the propagating radius of the initial wave. To understand the underlying mechanisms of the waves, we conducted a series of pharmacological experiments. The waves persisted following application of the voltage-gated sodium channel inhibitor, tetrodotoxin (TTX), but completely ceased following application of Thapsigargin, an inhibitor of the sarco/endoplasmic reticulum Ca^{2+} ATPase, which blocks the release of internal Ca^{2+} stores. Furthermore, the waves persisted in the presence of glutamatergic synaptic transmission inhibitors (CNQX and APV), albeit we observed reduced propagating distances. These experiments suggest that the waves are not primarily initiated in individual neurons by action potentials but, rather, as a result of spontaneous Ca^{2+} release from internal stores, and propagate to surrounding neurons via synaptic connections. In summary, by employing two photon Ca^{2+} imaging, we reveal that COs develop complex and functionally interconnected networks of neurons which display multiple types of spontaneous activity, including local propagating waves initiated through Ca^{2+} release from internal stores. To the best of our knowledge, these waves have not been described previously and may be significant in the early development of human neuronal networks.

Disclosures: F.K.W. Lam: None. **S.S. Harris:** None. **C. Arber:** None. **J. Rowland:** None. **S. Wray:** None. **M. Busche:** None.

Poster

PSTR257: Neural Stem Cells

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR257.08/A8

Topic: A.03. Stem Cells and Reprogramming

Title: Assay development for functional analysis of iPSC-derived neural organoids

Authors: B. STREETER, D. SULLIVAN, P. J. ELLINGSON, A. PASSARO, S. A. CHVATAL, *D. MILLARD;
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Abstract: The flexibility and accessibility of induced pluripotent stem cell (iPSC) technology has allowed complex human biology to be reproduced in vitro at high throughput scales. Indeed, rapid advances in stem cell technology have led to widespread adoption for the development of in vitro models of neuron electrophysiology to be used in screening applications in drug discovery and safety. Furthermore, advanced cell preparations, such as spheroids or organoids, are under intense investigation with aims toward establishing mature physiologically relevant

phenotypes in vitro. The objective of this work is to develop and optimize a functional assay of neural organoids in vitro. To that end, a customized multiwell microplate (SpheroGuide) was designed specifically for neural organoid assays. The SpheroGuide MEA plate utilizes a funnel design to target the neural organoids to a planar grid of microelectrodes embedded in the substrate of each well of the culture plate. Impedance measurements were used to quantify the attachment of the organoids to the substrate and microelectrodes, while functional activity was quantified via electrophysiological measurements. Acute (no attachment) and chronic (surface coating-mediated attachment) recording protocols were evaluated and compared. Specifically, the use of surface coatings (no coating, PEI, Matrigel) and centrifugation were evaluated for chronic recording protocols with regular measurements of attachment and network function over four weeks. PEI-coated wells exhibited the best performance, with neural organoid attachment in 100% of wells and the development of synchronous network activity over 2 weeks in culture. Matrigel and no coating conditions displayed fewer active electrodes overall, but consistent results across wells due to the funnel design targeting organoids to the array. The optimized attachment protocol using PEI was then used to plate and monitor the electrophysiology of 48 dorsal forebrain organoids, with one individual organoid plated in each well of a SpheroGuide MEA plate. Impedance measurements showed robust attachment of all 48 organoids over a 21-day time course. Activity of the organoids peaked at Day 14 post-seeding, while synchrony increased over the full 21 days in culture. These results support the continued development of quantitative assays of neural function with increased throughput for in vitro 3D neural organoid models.

Disclosures: B. Streeter: A. Employment/Salary (full or part-time):; Axion Biosystems. **D. Sullivan:** A. Employment/Salary (full or part-time):; Axion Biosystems. **P.J. Ellingson:** A. Employment/Salary (full or part-time):; Axion Biosystems. **A. Passaro:** A. Employment/Salary (full or part-time):; Axion Biosystems. **S.A. Chvatal:** A. Employment/Salary (full or part-time):; Axion Biosystems. **D. Millard:** A. Employment/Salary (full or part-time):; Axion Biosystems. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Axion Biosystems.

Poster

PSTR257: Neural Stem Cells

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR257.09/A9

Topic: A.03. Stem Cells and Reprogramming

Title: Combining 3D-Bioprinting and iPSC cells for human brain modeling and pathophysiology

Authors: *S. SORRENTINO[1,2], S. WENDT[3], C. J. GROTEN[3], B. MAC VICAR[3], H. B. NYGAARD[3];

[1]The Univ. of British Columbia, Vancouver, BC, Canada; [2]University of British Columbia, Vancouver, BC, Canada; [3]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The difficulties in translating preclinical findings to human studies have pointed out the inadequacy of current research models and underscored the need for alternative approaches to studying brain physiology and pathology. Recently, human induced pluripotent stem cell (hiPSC) 3-dimensional (3D) models, such as organoids and neurospheres, have emerged as powerful tools for modeling human nervous tissue in vitro. However, these systems rely on hiPSC self-organization and are therefore characterized by low reproducibility and homogeneity.3D bioprinting is an innovative bioengineering technique that combines biomaterials and live cells to shape 3D structures in a layer-by-layer fashion. The bioink provides mechanical support for the growing cells and allows a better exchange of nutrients, oxygen, and drugs, which makes it ideal for drug screening applications. However, the manufacturing of exceedingly soft structures, such as the brain, represents a significant bioengineering challenge often resulting in printing failures, lack of structure or short culturing time, and poor functional characterization.Here, we generated a 3D-Bioprinted brain model suitable for long-lasting culturing (8 months) of iPSCs-derived cortical neurons and astrocytes starting from neuronal precursor cells (NPCs). NPCs were successfully bioprinted in a defined multilayer wood-pile structure to mimic the human cerebral cortex architecture. This was accomplished using high spatial resolution, low-pressure extrusion, and high speed while maintaining cell viability and proliferation. In maintenance medium, NPCs express SOX2, PAX6, and Nestin and show no action potential firing and spontaneous synaptic currents. Induction of cell maturation resulted in neurite elongation within the first days of differentiation and the appearance of glutamatergic synaptic currents and action potential firing. Further analysis with calcium imaging on AAV9-synapsin-GCAMP6f transfected cells demonstrated that neurons formed intricate and functional networks with inter-layer connections. 3D neuronal cultures were efficiently maintained for more than 8 months with astrocytes spontaneously differentiating after two months of culturing. Moreover, hiPSC-derived microglia could be administered to the bioprinted constructs, after which these cells infiltrated and integrated with other cell types. Overall, our data indicate the potential of hiPSCs-derived 3D bioprinted cultures for brain modeling and future possible drug screening applications.

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Poster

PSTR257: Neural Stem Cells

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Program #/Poster #: PSTR257.10/A10

Topic: A.03. Stem Cells and Reprogramming

Title: Investigating Intracellular Dynamics in Human iPSC-Derived Neurons: A Fast Kinetic High-Throughput Imaging Approach

Authors: *J. TRASK;
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Abstract: In the field of neuroscience and neurodegenerative diseases, understanding intracellular processes is pivotal. Specifically, two critical events—mitochondrial functions and calcium signaling in neurons play fundamental roles. Leveraging high-content imaging (HCI) with advanced image analysis, we employed fast kinetic imaging to visualize, quantify, and assess these dynamic events in a human in vitro based model using iPSC-derived neurons with exceptional temporal resolution using a high-throughput confocal microscopy system. Our approach involved two key studies. In our first study, we investigated mitochondrial dynamics using an uncoupler of mitochondrial oxidative phosphorylation, carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP), and glutamate as the reference controls. We conducted confocal imaging with a 40x objective lens at 2 frames per second (fps). From analysis we found evidence of mitochondria fractions in both neuronal bodies and neurites following image analysis segmentation, leading to questions about mitochondria dysfunction processes. In our second study, we investigated calcium signaling. Using an on-board liquid handling device integrated in the HCI imager, we injected Glutamate (agonist) or Gabazine (antagonist of GABAα receptors) to measure real-time calcium responses using confocal imaging with a 20x lens at 5 fps. At the single-cell level, we segmented and compared calcium flux responses of individual neuronal cell bodies and neurites. Notably, calcium response remained more active in neuronal cell bodies than in extended neurites. Our precise assessment of calcium flux in individual neurons and neurites provides insights into potential cell behavior, underlying mechanisms, and fundamental neuronal communication processes. This approach will allow the potential to identify disruptions associated with neurodegenerative conditions, neurotoxic insults, or neuronal maturation. Additionally, our comprehensive HCI approach generates tens to thousands of high-content imaging morphological phenotypic features. These features enable further exploration of untapped mechanisms or modes of action resulting from perturbations. Ultimately, this high-dimensional multivariate data enhances our understanding of the intricate interplay between calcium dynamics and mitochondrial function in neurons. The real-time integration of fast kinetic imaging with HCI offers an automated and robust method for investigating intracellular dynamics within the context of neurons at the single cell level.

Disclosures: J. Trask: None.

Poster

PSTR257: Neural Stem Cells

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Program #/Poster #: PSTR257.11/A11

Topic: A.03. Stem Cells and Reprogramming

Support: NIH R01NS100514

Title: Direct neuronal reprogramming of glial progenitor cells using a regulatable sequential gene expression system for improved neuronal subtype specification

Authors: *A. BARAL, E. REISENBIGLER, R. A. MARR, D. A. PETERSON;
Neurosci., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: During development, cell fate is determined through sequential expression of instructive transcription factors (TFs) in combination with epigenetic influences. Subsequent to development, it is possible to directly reprogram the lineage of somatic cells without inducing pluripotency. Direct reprogramming may have therapeutic use in the CNS, as neurons are long-lived, post-mitotic cells that are not replaced when lost due to injury or disease. However, one limitation to current direct reprogramming approaches is controlling the temporal expression of instructive TFs. When multiple TFs are needed to guide lineage respecification, they are delivered through coinfection and are expressed simultaneously. This can result in low efficiency of cellular reprogramming to the final cell fate subtype. Here, we describe an improved strategy using NG2 glial progenitor cells (oligodendrocyte progenitor cells- OPCs) for neuronal reprogramming that allows for a temporal separation of an initial, pioneering TF and a subsequent instructive TF to guide neuronal subtype specification. Retroviral co-delivery and simultaneous expression of separate vectors for the pioneering TF Ascl1 and the instructive TF Dlx2 resulted in the detection of the early neuronal marker beta-III-tubulin by seven days. However, the generation of induced neurons from the OPC population was much less efficient than parallel studies using single expression of either Ngn2 or NeuroD1. We hypothesize that the lower efficiency may result from the expression of Dlx2 too early in the reprogramming process. To test this, we have generated a switch vector construct where proliferating OPCs are retrovirally coinfected with 1) a regulator construct containing a tamoxifen inducible Cre-recombinase system (CreERT), and 2) an effector construct containing two gene sets, designated Gene Set 1 and Gene Set 2. In this inducible switch system, Gene Set 1 is initially expressed while Gene Set 2 is silent. Upon tamoxifen administration, Gene Set 1 is excised and Gene Set 2 is expressed. Delivery of a control effector where Gene Set 1 expresses destabilized GFP and Gene Set 2 expresses dsRed resulted in the initial detection of green cells that disappeared within 24 hours of tamoxifen addition coincident with the emergence of red cells validating the switch approach. We expect that delivery of an experimental effector construct with Gene Set 1 expressing Ascl1-GFP and Gene Set 2 expressing Dlx2-dsRed will result in improved neuronal induction and subtype specification into GABAergic neurons. This approach represents an advance for efficient and specific lineage reprogramming of glial progenitor cells into neurons.

Disclosures: A. Baral: None. **E. Reisenbigler:** None. **R.A. Marr:** None. **D.A. Peterson:** None.

Poster

PSTR257: Neural Stem Cells

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR257.12/A12

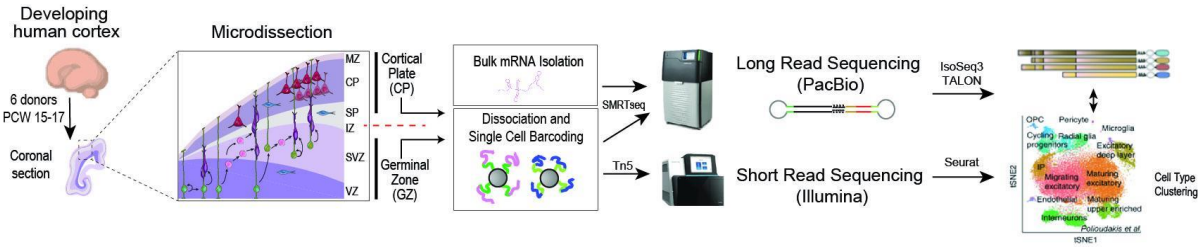
Topic: A.03. Stem Cells and Reprogramming

Support: R01MH124018
R01MH125252
R01MH121521
R01MH123922
SFARI 957585

Title: Gene-isoform diversity in the developing human neocortex

Authors: *L. DE LA TORRE-UBIETA[1], A. PATOWARY[1], P. ZHANG[1], C. T. JOPS[2], C. K. VUONG[1], X. GE[3], K. HOU[1], N. N. GONG[4,5], X. WANG[6], C. LIU[7], J. LI[1], M. J. GANDAL[8,9]; [1]UCLA, Los Angeles, CA; [2]Univ. of Pennsylvania, Philadelphia, PA; [3]Oregon State Univ., Corvallis, OR; [4]Duke Univ., Durham, NC, ; [5]Psychiatry, Perelman Sch. of Med., Philadelphia, PA; [6]Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; [7]Upstate Med. Univ., Syracuse, NY; [8]Psychiatry, Ucla-Semel Inst., Los Angeles, CA; [9]Psychiatry, Perelman Sch. of Medicine, Univ. of Pennsylvania, Philadelphia, PA

Abstract: RNA splicing is highly prevalent in the brain and has strong links to neuropsychiatric disorders, yet the role of cell-type-specific splicing and transcript-isoform diversity during human brain development have not been systematically investigated. Here, we leveraged single-molecule long- read sequencing to deeply profile the full-length transcriptome of the germinal zone (GZ) and cortical plate (CP) regions of the developing human neocortex at tissue and single-cell resolution. We identified 214,516 unique isoforms, of which 72.6% were not annotated in Gencode-v33, and uncovered a substantial contribution of transcript-isoform diversity, regulated by RNA binding proteins, in defining cellular identity in the developing neocortex. We leveraged this comprehensive isoform-centric gene annotation to re-prioritize thousands of rare de novo risk variants and elucidate genetic risk mechanisms for neuropsychiatric disorders.



Disclosures: L. De La Torre-Ubieta: None. **A. Patowary:** None. **P. Zhang:** None. **C.T. Jops:** None. **C.K. Vuong:** None. **X. Ge:** None. **K. Hou:** None. **N.N. Gong:** None. **X. Wang:** None. **C. Liu:** None. **J. Li:** None. **M.J. Gandal:** None.

Poster

PSTR258: CNS Regeneration

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR258.01/A13

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant EY031403
Maine INBRE

Title: Axon pathfinding in induced retinal ganglion cells generated during the first postnatal week

Authors: A. REARDON[1], K. NEY[2], K. BURNHAM[3], *M. B. WOODWORTH[4], D. GOODMAN[3];
[1]Biochem., [2]Biol., [4]Neurosci., [3]Bates Col., Lewiston, ME

Abstract: Retinal ganglion cells (RGCs) are the projection neurons that connect the retina with visual processing areas in the brain. he adult mammalian retina has no ability to regenerate RGCs, and, therefore, the loss of these neurons via injury or disease causes irreversible visual impairment. In previous work, we identified a set of developmentally expressed transcription factors sufficient to induce the generation of functional RGCs in the mouse retina outside the window of endogenous RGC development. Axons of these induced RGCs extend into the optic nerve, then progressively reach central visual targets including the thalamus and superior colliculus. In this work, we aim to understand how these axons navigate in the early postnatal nervous system, when cues that guided their endogenous counterparts are no longer available. We examine axon guidance molecules expressed by both endogenous RGCs and induced RGCs to determine which factors contribute to temporally ectopic pathfinding. Overall, we aim to investigate the generation and development of RGCs from existing cells intrinsic to the retina, which could enable restoration of vision in human patients.

Disclosures: A. Reardon: None. **K. Ney:** None. **M.B. Woodworth:** None.

Poster

PSTR258: CNS Regeneration

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR258.02/A14

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant EY031403
Maine INBRE

Title: Developmental transcription factors critical for the induction of retinal ganglion cells from late retinal progenitors

Authors: *E. BARNES, M. B. WOODWORTH;
Neurosci., Bates Col., Lewiston, ME

Abstract: Retinal ganglion cells (RGCs) are output neurons that relay visual information from the retina to the brain. The adult mammalian retina cannot regenerate, so RGC injury or death results in irreversible visual impairment. In previous work, we found that in vivo electroporation of a set of candidate transcription factors induces the generation of RGC-like neurons outside the window of endogenous RGC development. These transcription factors were drawn from a list of genes expressed in the retina and with functions in retinal neurogenesis and regeneration. Some can instruct non-neural cells to adopt neural fates and generate induced neurons. In this study, we identify the expression and function of individual transcription factors within this group in order to characterize the contribution they make to the development of induced RGCs. Overall, we aim to understand the expression and function of the individual transcription factors that affect the induction of these RGCs, which could provide insight into the restoration of vision in blind patients using progenitors resident within the adult retina.

Disclosures: E. Barnes: None. **M.B. Woodworth:** None.

Poster

PSTR258: CNS Regeneration

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR258.03/A15

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant EY031403
Maine INBRE
Gilbert Vision Restoration Initiative
Knights Templar Eye Foundation

Title: Verification of Axon Guidance in a Novel Murine Model of NF1 Gliomas

Authors: *L. COSTEA[1], B. K. YOUNG[2], M. B. WOODWORTH[3];
[1]Bates Col., Lewiston, ME; [2]Ophthalmology, Stanford Univ., Palp Alto, CA; [3]Neurosci., Bates Col., Lewiston, ME

Abstract: Neurofibromatosis type 1 (NF1) is a somatic genetic disease affecting many parts of the body. In the optic nerve of pediatric patients, gliomas grow, causing intractable childhood blindness. In hopes of developing better clinical diagnosis and treatment approaches for NF1, several mouse models have been generated, but it remains unclear how well existing models recapitulate human clinical pathology. We describe a new mouse model created via breeding of two published models of the disease. In this model, NF1 mutant mice develop gliomas on their optic nerves prior to two months of age. In this work we use adeno-associated virus and fluorophore-conjugated cholera toxin B tracing to image the projection patterns of retinal ganglion cells (RGCs). In the future, calcium imaging will be conducted to verify functional blindness phenotypes. Clinical tracking of NF1 symptoms has started to better describe the disease, but the need for an accurate mouse model is paramount to future research.

Disclosures: L. Costea: None. **B.K. Young:** None. **M.B. Woodworth:** None.

Poster

PSTR258: CNS Regeneration

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR258.04/A16

Topic: A.04. Transplantation and Regeneration

Support: R01NS119472

Title: Characterizing polysynaptic connectivity between human brain organoid grafts and the rat motor system using an HSV tracer

Authors: *S. SINGH[1], R. BLUE[2,3], D. JGAMADZE[4], B. REES[1], M. CASTELLANOS[1], J. LEE[5], S. KUMAR[6], A. SINGH[7], P. HARARY[9], J. KIM[4], Z. ZHANG[8], H. SONG[8], G.-L. MING[8], H.-C. I. CHEN[4];
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Abstract: Axonal projections from neural grafts in the brain are often used to establish the degree of connectivity between the graft and host tissue. However, the possibility that grafts can more substantially integrate with host tissue through polysynaptic connections has not yet been explored. Here, we characterize the ability of H129ΔTK-TT, a polysynaptic, anterograde, and Cre-dependent HSV tracer, to establish the connectivity patterns of transplanted human brain organoids with rat motor pathways. Human brain organoids in this study were generated using a Cre-GFP human induced pluripotent stem cell line. The presence of Cre protein was required for

replication of the H129ΔTK-TT tracer, which limited the origin of tracing to the organoid graft. Sixteen rats underwent organoid transplantation immediately after an aspiration cavity was made in the right-sided rat motor cortex. Two months post-transplantation, organoids were injected with 600 nl of H129ΔTK-TT ($9x10^9$/ml). Post-injection, animals were sacrificed at 7 days or when symptoms of systemic toxicity developed. Brain and spinal cord tissue were analyzed histologically to localize tdTomato signal using a combination of publicly available software packages and atlases. We found GFP signal, representing organoid grafts and their projections, in bilateral primary motor cortex as well as ipsilateral corpus callosum and the mediofrontal cortex/septal region. In comparison, tdTomato signal, representing downstream polysynaptic partners of the organoid grafts, localized to a much broader territory, including the bilateral primary motor cortex, caudate, putamen, globus pallidus, subthalamic nucleus, ventral tegmental area, substantia nigra, zona incerta, nucleus accumbens, thalamic nuclei (ventro/central medial, medio/laterodorsal, ventro/central lateral, ventral posterior, reticular, parafascicular), and pontine nuclei. Moreover, $tdTomato^+$ cells were found in the ventral horn of the cervical spinal cord. Utilization of the H129ΔTK-TT polysynaptic tracer demonstrated successful integration of transplanted organoid grafts with host motor systems, with increased polysynaptic connectivity into native motor pathways compared to direct growth of graft projections. Notably, polysynaptic organoid connectivity to spinal cord ventral horn motor neurons of the host animal was identified through H129ΔTK-TT tracer signal in the absence of GFP organoid signal. This study thus highlights the ability of organoids to exploit intact host motor pathways after transplantation into injured motor cortex and potentially allow for meaningful cortical repair in various brain injury etiologies.

Disclosures: S. Singh: None. **R. Blue:** None. **D. Jgamadze:** None. **B. Rees:** None. **M. Castellanos:** None. **J. Lee:** None. **S. Kumar:** None. **A. Singh:** None. **P. Harary:** None. **J. Kim:** None. **Z. Zhang:** None. **H. Song:** None. **G. Ming:** None. **H.I. Chen:** None.

Poster

PSTR258: CNS Regeneration

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR258.05/A17

Topic: A.04. Transplantation and Regeneration

Support: NSF Grant 1754340
NSF Grant 1120796
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NIH-NINDS Grant R01NS113859
Shriners Hospital for Children Grant 86700-NCA

Title: Molecular mechanisms of Hedgehog signaling-dependent neural and muscular regeneration in Xenopus laevis larvae

Authors: *A. M. HAMILTON[1], L. N. BORODINSKY[2];
[1]Res., Shriners Hosp. For Children, Room 631, Sacramento, CA; [2]Physiol. and Membrane Biol., Univ. of California Davis, Sch. of Med., Sacramento, CA

Abstract: Regeneration within the central nervous system remains one of modern medicine's greatest challenges. Fortunately, Hedgehog (Hh) signaling holds great promise for improving regeneration in a wide variety of tissues, and has been manipulated to improve axon outgrowth and sensory and motor recovery in models of nervous system injury. Dysregulated Hh signaling, however, is a hallmark of many types of cancer, necessitating a deeper understanding of the mechanisms underlying regenerative Hh signaling. We previously demonstrated that non-canonical Hh signaling is necessary after injury for regeneration of muscle and spinal cord in the *Xenopus laevis* tail amputation model, but the underlying mechanisms and target cell types have not yet been elucidated. Here, we demonstrate that Hh signaling promotes regeneration of motor neuron axons, while enhancing the pruning of sensory axons in the regenerating tail. In addition, we show that Hh signaling biases Pax7+ muscle satellite cells towards proliferation over differentiation into Myf6+ immature myocytes. To identify regenerative non-canonical Hh signaling partners, we performed single cell RNA sequencing analysis of larval regenerate and tail stump tissues at 24 hours post-amputation, and found that while treatment with the Hh signaling inhibitor vismodegib has minimal effects on mature cell types, stem/progenitor cells in both muscle and neural lineages are significantly changed by Hh inhibition during regeneration. Inhibition of Hh signaling results in downregulation of a wide range of neurotransmitter receptors and calcium signaling regulatory genes in both muscle satellite cells (MSCs) and neural progenitor cells (NPCs). However, the canonical downstream Hh signaling targets Gli1, HHIP and Ptch1/2 are only consistently downregulated in MSCs, while their expression in NPCs varies highly by cluster, suggesting a non-canonical Hh signaling response in some types of NPC. To assess whether Hh signaling controls regenerative calcium activity, we monitored calcium transients via expression of GCaMP6s during regeneration, and found that inhibiting Hh signaling reduces the persistent post-amputation increase in calcium transient frequency in the regenerating tail at 24 hour post injury. Overall, these results identify potential mechanisms of Hh-dependent regeneration, offering new therapeutic avenues for enhancing spinal cord and muscle regeneration.

Disclosures: A.M. Hamilton: None. **L.N. Borodinsky:** None.

Poster

PSTR258: CNS Regeneration

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR258.06/A18

Topic: A.04. Transplantation and Regeneration

Support: KAKENHI 18K15367
KAKENHI 20K16496
KAKENHI 22K07349
Salon de K Grant (2023)

Title: Different capability to regenerate the spinal cord between zebrafish and medaka

Authors: S. AOKI, M. HORI, H. ZHANG, ***H. TSUJIOKA**, T. YAMASHITA;
Osaka Univ., Osaka, Japan

Abstract: Functional recovery from spinal cord injury is limited because glial scar formed at the injured site prevent axonal regeneration in the central nervous system. By contrast, zebrafish have remarkable ability to regenerate many tissues including the spinal cord. In zebrafish, immune response is observed after complete transection of the spinal cord, processes of ependymoradial glia and axons of neurons form glial and axonal bridge after that, then finally the injured spinal cord remodels into almost healthy morphology. Although comparison between mammals and zebrafish is potentially promising to reveal regeneration specific mechanisms, far evolutional distance hinders direct comparison of them. Recent studies found that medaka, which shares many biological features with zebrafish, possess lower regenerative ability in several tissues such as the heart or the retina, and comparison of them is becoming an attractive model to reveal regeneration specific mechanisms. However, capability to regenerate the spinal cord of them has not been compared yet. Here, we compared regenerative ability of the spinal cord of adult zebrafish and medaka. Recovery of free swimming distance after spinal cord injury was significantly lower in medaka compared to zebrafish. Thickness of glial and axonal bridge was significantly thinner in medaka compared to zebrafish. Regeneration of axons labeled by tracer was not clear in medaka whereas it was observed in zebrafish. We also performed RNA-seq using transected spinal cord tissue of them 2 weeks after injury, and found that genes related to axonal regeneration were upregulated in zebrafish. The above results suggest that regenerative ability of the spinal cord is lower in medaka compared to zebrafish, and it accompanies different gene expression profiling. This study suggests that comparison of zebrafish and medaka will become an attractive model to reveal regeneration specific mechanisms after spinal cord injury.

Disclosures: S. aoki: None. **M. Hori:** None. **H. Zhang:** None. **H. Tsujioka:** None. **T. Yamashita:** None.

Poster

PSTR258: CNS Regeneration

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR258.07/A19

Topic: A.04. Transplantation and Regeneration

Support: JSPS KAKENHI grant 20K08163
JSPS KAKENHI grant 23K07338

Title: Intravenous infusion of mesenchymal stem cells elicits therapeutic efficacy in rat perinatal brain injury.

Authors: *K. TERADA[1,2], M. SASAKI[1], H. NAGAHAMA[1], Y. KATAOKA[1], S. OKA[1], R. UKAI[1], T. YOKOYAMA[1], Y. IIZUKA[1], T. SAKAI[1], S. FUKUMURA[1], T. TSUGAWA[1], J. D. KOCSIS[3], O. HONMOU[1];
[1]Sapporo Med. Univ., Sapporo, Japan; [2]Pediatrics, Tomakomai City Hosp., Tomakomai, Japan; [3]Neurol., Yale Univ. Sch. of Medicine, Dept. of Neurol., Cheshire, CT

Abstract: The pathogenesis of perinatal brain injury (PBI) is multifocal, and one of the major causes in PBI is a combination of in utero chorioamnionitis and perinatal asphyxia. Although recent advances in perinatal medicine have improved the survival rates of preterm infants, neurodevelopmental disorders including cerebral palsy, intellectual impairment and behavioral disorders remain significant complications. We tested whether the intravenous infusion of mesenchymal stem cells (MSC) had therapeutic efficacy against PBI in a rat model. Pregnant Sprague Dawley rats at embryonic day (E) 18 received a low dose of lipopolysaccharide (100 μg/kg) intraperitoneally and the pups were born on E21 via spontaneous vaginal delivery. On postnatal day (PND) 7, the left common carotid artery of each pup was double-ligated and they were exposed to 8% oxygen for 2 h. Total fifty-six rats were randomized on PND10, and MSCs (1.0×10^6 in fresh DMEM) or vehicle (fresh DMEM without MSC) were intravenously infused. We conducted behavioral analyses with the Rotarod test (RT), Cylinder rearing test (CRT), and Morris water maze test (MWM), measured brain volume with 7T-MRI, and performed histological assessments on PND49. In RT and CRT, we observed significant motor and sensorimotor improvement in MSC-treated rats. In MWM, escape latency and path length were shorter, and the percent time in the quadrant where the platform was located was longer in the MSC group than in the vehicle group. In addition, the vehicle group swam faster (average speed) than the MSC group in MWM. These results in MWM support hypotheses that MSC improves cognitive function and might also have the potential to inhibit hyperactivity disorder in PBI. In vivo MRI revealed that MSC infusion increased residual (non-ischemic) brain volume compared to the vehicle group. Histological analyses showed that cortical thickness, the number of $NeuN^+$ and $GAD67^+$ cells, and synaptophysin density in contralesional (right) hemisphere in the MSC group were greater than the vehicle group. In conclusion, infused MSCs improve neurological functions with alleviation of behavioral disorder and might stimulate growth or inhibit cell death in the residual brain tissue. Intravenous administration of MSCs might be suitable for the treatment of PBI.

Disclosures: K. Terada: None. **M. Sasaki:** None. **H. Nagahama:** None. **Y. Kataoka:** None. **S. Oka:** None. **R. Ukai:** None. **T. Yokoyama:** None. **Y. Iizuka:** None. **T. Sakai:** None. **S. Fukumura:** None. **T. Tsugawa:** None. **J.D. Kocsis:** None. **O. Honmou:** None.

Poster

PSTR258: CNS Regeneration

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR258.08/A20

Topic: A.04. Transplantation and Regeneration

Title: Chemogenetic Activation of Human Neurons Accelerates Axonal Growth and Reconstruct the Corticospinal Tract in Ischemic Mice

Authors: *D. ZHENG[1], Z. WANG[2], S.-C. ZHANG[3];
[1]Duke-Nus Grad. Med. Sch. Singapore, Singapore, Singapore; [2]Duke-National Univ. of Singapore, Singapore, Singapore; [3]Waisman Ctr., Univ. of Wisconsin, Madison, WI

Abstract: Stroke is the second leading cause of death and third leading cause of disability worldwide. It is caused by cerebral hypoxia, leading to irreversible neuronal death. There are currently no effective therapeutic strategies to replace the lost neurons and reconstruct the disrupted neural circuit. Cell therapy to replace the lost cells and reconnect disrupted neural circuits especially the corticospinal tract (CST) offers a potential therapy. We have established a strategy to enable the survival of neural progenitors that were transplanted into the ischemic cavity, which resulted in reconstitution of the damaged brain, vascularization of the graft, and axonal growth into the host brain. A major challenge is the long distance for the axons of the transplanted human cortical neurons to travel to their targets in the brain stem and spinal cord. Here we develop a chemogenetic strategy to promote the axonal growth by transplanting DREADD (Designer receptor exclusively activated by designer drugs)-expressing cortical neural progenitor cells (NPCs) into the lesion core of the stroke mice. Without DREADD activation, it took six months for the axons to grow to the brain stem, and one year to reach the spinal cord. The activation of DREADD enabled the axons to grow to the brain stem in three months, and to the spinal cord in six months. Correspondingly, the stroke mice with DREADD activation exhibited earlier motor behavioral recovery. These results demonstrate the accelerated axonal growth and reconnection of the CST by grafted cortical neurons. Ongoing studies are aiming at identifying the downstream pathways that mediate the faster axonal growth.

Disclosures: D. Zheng: None. **Z. Wang:** None. **S. Zhang:** None.

Poster

PSTR258: CNS Regeneration

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR258.09/A21

Topic: A.04. Transplantation and Regeneration

Support: TWU Research Enhancement Program
Mission Connect

Title: Cortical grafts derived from induced pluripotent stem cells integrate specifically into the motor cortex and improve forelimb motor movement.

Authors: *M. D. GLADEN, Z. R. LYBRAND;
Dept. of Biol., Texas Woman's Univ., Denton, TX

Abstract: Traumatic brain injury often results in a permanent loss of neuronal tissue that causes longer-term defects in cognitive and motor abilities. A study done by the Center for Disease Control looking at 5-year outcomes of patients that received inpatient treatment found that 74% of patients had not seen any improvements which is likely due to this permanent loss of neuronal tissues, specifically the loss of neurons. The goal of this project is to engineer an in-vitro cortical graft that can be transplanted into the brain and establish a de novo neural network to overcome the neuronal loss associated with TBI. Cortical grafts will be engineered from induced pluripotent stem cells using a guided 3D cerebral organoid protocol. These grafts replicate cortical development with glutamatergic and GABAergic neurons, neural progenitor cells, and glial cells. The grafts were grown for at least 2 months and then infected with an adeno-associated virus (AAV) that would express green fluorescent protein (GFP) under the human synapsin (hSYN) promotor. One week following the infection the grafts were transplanted into NOD-SCID mice. Before surgery, male mice were randomly placed into three groups Sham, TBI, and TBI + transplant. The TBI + transplant received a TBI in the left motor cortex from a controlled cortical impactor. Bleeding was controlled and the immediate necrotic tissue was excised to make room for the graft and then the graft was transplanted. The TBI group procedure the same procedure without the graft, while the sham group only received a craniometry. Following surgery, all mice underwent modified neuro severity score for 7 days then once weekly for the remainder of the study. To test specific forelimb motor ability, a water drop test was performed at the timepoints previously described. 28 days after surgery the mice were perfused, and the brains were collected for histological analysis. We observed significant right forelimb motor ability recovery fourteen days following surgery when comparing TBI + transplant and TBI groups. We observed no statistical difference in left forelimb motor ability among all groups. Using serial histological analysis, we identified efferent projection from the graft local and distant brain regions. Migratory cells from our graft were found. This research lays the groundwork for understanding synaptic integration from cortical grafts, offering a transformative approach to treating traumatic brain injury by restoring neural connectivity and function.

Disclosures: M.D. Gladen: None. **Z.R. Lybrand:** None.

Poster

PSTR258: CNS Regeneration

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR258.10/A22

Topic: A.04. Transplantation and Regeneration

Support: JSPS KAKENHI grant 21K11194

Title: Rehabilitation enhances functional recovery after intravenous infusion of mesenchymal stem cells for chronic cerebral ischemia in rats

Authors: *T. YAMASHITA[1,2], M. SASAKI[1], Y. SASAKI[3], H. NAGAHAMA[1], S. OKA[1], Y. KATAOKA[1], R. UKAI[1], T. YOKOYAMA[1], M. KOBAYASHI[3], M. KAKIZAWA[3], J. D. KOCSIS[4], O. HONMOU[1];
[1]Sapporo Med. Univ., Sapporo / Hokkaido, Japan; [2]Rehabilitation, Sapporo Medical University Hospital, Sapporo, Japan; [3]Rehabil., Sapporo Med. Univ. Hosp., Sapporo, Japan; [4]Neurol., Yale Univ. Sch. of Med., Dept. of Neurol., Cheshire, CT

Abstract: Introduction: We recently showed that intravenous infusion of mesenchymal stem cells (MSCs) for chronic cerebral ischemia provides functional improvements via induced neural plasticity. Intravenous infusion of MSCs enhances interhemispheric connectivity through the corpus callosum (CC), indicating a potential enhancement of neural plasticity, even in the chronic phase of cerebral stroke. Rehabilitation may augment the enhanced neural plasticity induced by the infused MSCs and guide neural plasticity in the appropriate direction. Thus, we tested the hypothesis that a combination of intravenous infusion of MSCs and daily rehabilitation may exert enhanced therapeutic efficacy compared with MSC or rehabilitation therapy alone in the chronic phase of cerebral stroke. We investigated the synergistic effects on the behavioral function of a combination rehabilitation strategy (daily treadmill exercise) and intravenous infusion of MSCs eight weeks after permanent occlusion of the unilateral middle cerebral artery to induce chronic cerebral ischemia in rats. **Material and Methods:** Permanent middle cerebral artery occlusion (MCAO) was induced in Sprague-Dawley rats. Eight weeks after MCAO induction, the rats were used as a chronic cerebral ischemia model. Four experimental groups were studied: Vehicle group (medium only, no cells), Rehab group (vehicle + rehabilitation), MSC group (MSC only), and Combined group (MSC + rehabilitation). Rat MSCs were intravenously infused eight weeks after MCAO induction, and the rats received daily rehabilitation through treadmill exercise for 20 min. Behavioral testing, lesion volume assessment using magnetic resonance imaging (MRI), and histological analysis were performed during the observation period until 16 weeks after MCAO induction. **Results:** All treated animals showed functional improvement compared with the Vehicle group; however, the therapeutic efficacy was greatest in the Combined group. The combination therapy is associated with enhanced neural plasticity, as shown by histological analysis and MRI diffusion tensor imaging. **Conclusion:** The study results indicate that combined therapy consisting of MSC therapy and rehabilitation therapy has a positive effect on behavioral performance and structural changes in the CC during the chronic phase of cerebral ischemia. These findings provide

behavioral evidence for enhanced recovery by combined therapy with rehabilitation and intravenous infusion of MSCs and may form the basis for the development of clinical protocols in the future.

Disclosures: T. Yamashita: None. **M. Sasaki:** None. **Y. Sasaki:** None. **H. Nagahama:** None. **S. Oka:** None. **Y. Kataoka:** None. **R. Ukai:** None. **T. Yokoyama:** None. **M. Kobayashi:** None. **M. Kakizawa:** None. **J.D. Kocsis:** None. **O. Honmou:** None.

Poster

PSTR258: CNS Regeneration

Location: MCP Hall A

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Program #/Poster #: PSTR258.11/A23

Topic: A.04. Transplantation and Regeneration

Support: NINDS R01NS058784 (GKS)
German Research Foundation HA 9566/1-1 (PH)

Title: Deciphering stem cell transplant-mediated recovery in stroke-injured brains- A longitudinal MRI and multiomics study

Authors: *P. HABIB[1], X. LIANG[1], R. T. NORISTANI[1], N. JOHNSTON[1], J. KIM[2], S. EWBANK[3], R. AIRAN[3], T. BLISS[1], G. K. STEINBERG[1];
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Abstract: Intraparenchymal transplantation (tx) of human neural stem cells (hNSCs) shows therapeutic potential for patients with chronic ischemic stroke. However, the underlying mechanisms of how hNSCs drive recovery remain elusive. A crucial clue to deciphering the mechanisms of recovery might be the recent observation of a transient T2-FLAIR (Fluid-Attenuated Inversion Recovery) MRI signal in the premotor cortex after intraparenchymal hNSCs transplantation in chronic stroke patients. The magnitude of the T2-FLAIR positively correlated with the extent of clinical recovery. Translating from bedside back to bench, we replicated the transplantation-induced FLAIR signal following a chronic stroke in adult male Sprague Dawley rats subjected to 30 min of stroke (using the transient middle cerebral artery occlusion model) followed 1 month later by transplantation of embryonic-derived neural stem cells (NR1) or buffer into the ipsilesional striatum. For a multimodal investigation of the FLAIR signal and regenerative responses of stem cell transplantation into the stroke-injured brain on regional, cellular, and molecular levels we combined longitudinal MRI sequences (T2w, FLAIR, DWI, SWI, DTI) with neurobehavioral assessments (whisker paw, cylinder test, EBST, corner test, grip strength, T-maze) and spatiotemporal multiomics performed at various time points in an observation period of 3-month post-tx. We observed that rats do not exhibit significant

recovery within the first month after stroke but demonstrate improvement following transplantation surgery. Specifically, NR1 treatment increased the number of rats that recovered compared to the vehicle group, with FLAIR signal peaking at 1-day post-surgery and correlating with enhanced recovery likelihood. Additionally, NR1-induced FLAIR was more hyperintense and detectable till later (7d- post-surgery) than vehicle-induced FLAIR. DTI analysis revealed decreased fractional anisotropy (FA) and mean diffusivity (MD) metrics in both ipsi- and contralesional striatum and motor cortex after NR1 treatment, indicating structural alterations associated with recovery. Furthermore, a pilot spatial transcriptomics analysis identified enhanced immune responses in the transplant-induced FLAIR region. Our study provides evidence that altered immune responses in the stem cell transplant-induced FLAIR region may promote recovery after chronic stroke and suggests the potential of T2-FLAIR and DTI metrics as a marker for therapeutic efficacy.

Disclosures: P. Habib: None. **X. Liang:** None. **R.T. Noristani:** None. **N. Johnston:** None. **J. Kim:** None. **S. Ewbank:** None. **R. Airan:** None. **T. Bliss:** None. **G.K. Steinberg:** None.

Poster

PSTR258: CNS Regeneration

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR258.12/A24

Topic: A.04. Transplantation and Regeneration

Title: Exploring Human Glia-Neuron Interactions in Co-Transplantation-Based Human-Mouse Chimeric Brain Models

Authors: *Z. MA, A. PAPETTI, M. JIN, P. JIANG;
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Abstract: The human pluripotent stem cell-based human-mouse chimeric mouse brain models serve as a useful tool for studying the development and function human neural cell developed in vivo. Understanding glia-neuron interactions is crucial for unraveling brain function complexities and developing treatments for neurological disorders. To investigate the interactions between human neuron and glia within an intact brain environment, we employed a co-transplantation strategy. This involved transplanting human induced pluripotent stem cell (iPSC)-derived neural progenitor cells (NPCs) together with primitive macrophage progenitors (PMPs) into the neonatal mouse brain, creating human-mouse chimeric brains containing human microglia, macroglia (astroglia and oligodendroglia), and neurons. At two months post-transplantation, we observed interactions between human glia and neurons, such as human microglia pruning human neuronal synapses. Different from prior studies suggesting that human microglia require human colony-stimulating factor 1 (hCSF1) for survival in cerebral organoids, our findings demonstrate that human microglia can survive in organoids without hCSF1

supplementation when combined with ventralized NPCs and differentiation conditions that promote macroglial cell growth. This observation was also validated in vivo, as microglia persisted without needing hCSF1 expression in the host brain when co-transplanted with ventralized NPCs. Single-cell RNA sequencing (scRNA-seq) of the co-transplanted chimeric brain recapitulated human glial progenitor cell (GPC) population and revealed a dynamic stage in astroglial development resembling that in the human brain. Cell-cell communication analysis highlighted significant neuron-glia and glia-glia interactions, especially the interaction between adhesion molecules neurexins (NRXNs) and neuroligins (NLGNs) within and between neurons and astrocytes. This new co-transplantation model offers opportunities for investigating intricate pathophysiological processes associated with human neurological diseases, particularly those where glia-neuron interactions and non-cell-autonomous effects play crucial roles.

Disclosures: Z. Ma: None. **A. Papetti:** None. **M. Jin:** None. **P. Jiang:** None.

Poster

PSTR258: CNS Regeneration

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR258.13/A25

Topic: A.04. Transplantation and Regeneration

Support: Japan Agency for Medical Research and Development (AMED)
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Bilateral Open Partnership Joint Research Projects, and Core-to-core Program “Neurogenesis Research & Innovation Center”)
Cooperative Study Programs of National Institute for Physiological Sciences
Takeda Science Foundation
Nitto Foundation

Title: Polysialic acid-mediated adhesion inhibition promotes the collective migration of neurons and recovery of brain function

Authors: *M. MATSUMOTO[1,2], K. MATSUSHITA[4], M. HANE[5], C. KUREMATSU[1], H. OTA[6,1], V. HERRANZ-PÉREZ[7], M. SAWADA[1,2], J. GARCIA-VERDUGO[8], K. KIMURA[9], T. SEKI[10], C. SATO[5], N. OHNO[11,3], K. SAWAMOTO[1,2];
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Functional Biol. and Physical Anthrop., Univ. of Valencia, Burjassot, Spain; [8]Univ. de Valencia, Paterna (valencia), Spain; [9]Nagoya City University, Grad. Sch. of Sci., Nagoya, Japan; [10]Dept. of Anat. and Life Structure, Dept. of Anat. and Life Structure, Juntendo Univ. Grad. Sch. of Med., Tokyo, Japan; [11]Dept. of Anatomy, Div. of Histology and Cell Biol., Jichi Med. Univ., Shimotsuke, Japan

Abstract: It has been reported that new neurons produced from endogenous stem cells in the brain contribute to the regeneration of lost neurons, but these findings have not yet been applied to the treatment of brain diseases. Here, we show that chains of migrating new neurons maintain unexpectedly large non-adherent areas between neighboring cells, allowing for efficient migration. In the injured brain, polysialic acid—which negatively regulates adhesion—is decreased by neuraminidase in mice and primates, resulting in increased adhesion and reduced migration efficiency. Moreover, administration of zanamivir, a neuraminidase inhibitor used to treat influenza, promoted neuronal migration toward injured areas, regeneration of lost neurons and functional recovery. Together, these results reveal a novel mechanism of efficient neuronal migration in the adult brain under physiological conditions, identify the cause of disruption of this mechanism during brain injury, and provide a potential definitive treatment for brain diseases through drug repositioning.

Disclosures: M. Matsumoto: None. **K. matsushita:** None. **M. Hane:** None. **C. Kurematsu:** None. **H. Ota:** None. **V. Herranz-Pérez:** None. **M. Sawada:** None. **J. Garcia-Verdugo:** None. **K. Kimura:** None. **T. Seki:** None. **C. Sato:** None. **N. Ohno:** None. **K. Sawamoto:** None.

Poster

PSTR258: CNS Regeneration

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR258.14/A26

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

Title: Modelling neurotoxicity, neuroprotection, and neurodevelopment for drug discovery applications

Authors: T. MODEBADZE, M. PATERSON, B. HALL, H. SCOTT, L. NIXON, M. BARBOUR, ***E. L. V. MALAVASI**;
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Abstract: Developing neurons form new network connections by extending processes (neurites) that give rise to dendrites and axons, and, eventually, functional synapses. Neurite outgrowth is a fundamental aspect of both neuronal development and regeneration after injury. The correct establishment, maturation, and maintenance of the neurite arbor, and of all the synaptic connections embedded within it, is essential for normal neuronal function and plasticity. Indeed,

many disorders of the Central Nervous System (CNS), including neurodevelopmental and neurodegenerative diseases, are in part attributed to alterations in neurite development or maintenance, neurite morphology, and/or synaptic density. In addition, some molecules are known to either positively (neurotrophins) or negatively (neurotoxins) interfere with this process, whilst others (neuroprotectants) can shield neurons from damaging insults. Any molecule that permeates the blood-brain barrier has the potential to directly affect neuronal health, whether that is the molecule's intended therapeutic purpose, or potential side effect. Measuring a molecule's impact on neurite outgrowth in vitro is an effective way to reveal its neuroprotective, neurodegenerative, or neurotoxic properties before it is first introduced in a living organism. Therefore, robust assays that enable accurate and reproducible quantification of neurite outgrowth, neurite complexity and synaptic density can be applied not only to the study of the pathophysiology of several CNS disorders, but also to neurotoxicity screening, and to the evaluation of candidate therapeutics that aim to counteract the effect of damaging mutations or toxic exposures. Leveraging our expertise in CNS modelling, we have developed in vitro assays that enable kinetic assessment of neurite outgrowth and reveal how this is impacted by exposure to neurotoxic or neuroprotective molecules. Primary mouse cortical neurons are isolated from embryonic day 18 animals and treated with a neurotrophin (Brain Derived Neurotrophic Factor) or with neuroprotectants (donepezil or dimethyl fumarate) before being exposed to excitotoxicity induced by glutamate treatment. Changes in neurite length are recorded in real time in IncuCyte® over several days, and the effect of the different exposures is quantified. At the end of the live recording, dendrites, synapses and organelles are immunolabelled and imaged on a CellInsight CX7 LED Pro high-content imaging platform. Comparative analysis of kinetic and high-content imaging data is presented, and its ability to unlock in-depth insight into the efficacy and mechanism of action of candidate drugs is discussed.

Disclosures: T. Modebadze: A. Employment/Salary (full or part-time):; Concept Life Sciences. **M. Paterson:** A. Employment/Salary (full or part-time):; Concept Life Sciences. **B. Hall:** A. Employment/Salary (full or part-time):; Concept Life Sciences. **H. Scott:** A. Employment/Salary (full or part-time):; Concept Life Sciences. **L. Nixon:** A. Employment/Salary (full or part-time):; Concept Life Sciences. **M. Barbour:** A. Employment/Salary (full or part-time):; Concept Life Sciences. **E.L.V. Malavasi:** A. Employment/Salary (full or part-time):; Concept Life Sciences.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.01/A27

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

Support: KAKENHI 24K03266
KAKENHI 22H05094

Title: Correlation between neuronal activity and dendritic dynamics in neonatal barrel cortex layer 4

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Abstract: Spontaneous neuronal activity has an important role in the formation of neuronal circuits during development. However, the relationship between spontaneous activity pattern and circuit formation is still elusive. To elucidate this, we observed neuronal activity and dendritic development of the layer 4 neurons in the somatosensory barrel cortex as a model. It has reported that the neurons show spontaneous activity patterns derived from thalamocortical axons in the developing period. In this period, spiny stellate neurons which are located on the edge of barrel acquire highly biased basal dendritic patterns oriented toward center. To visualize the activity and morphology of single layer 4 neurons, we transfected plasmid vectors using in utero electroporation-mediated gene transfer on embryonic day 14. The genetically-encoded calcium indicator (GCaMP7s) was expressed using Flpe-FRT recombination system and the membrane binding red fluorescent protein (GAP-tagRFP) was expressed using Cre-loxP recombination system. By combining these systems, GCaMP7s and GAP-tagRFP were expressed in different sparse populations of layer 4 excitatory neurons. In order to observe the developing brain in vivo, we attached a cranial window to the skull at postnatal day 5, and performed in vivo two-photon time-lapse imaging. As a result, we succeeded in simultaneously observing the activity synchronized with surrounding neurons and dendritic morphology dynamics. We found that the frequency of the spontaneous activity correlated with the dynamics of dendritic tips. We also analyzed correlation between the dendritic dynamics and synchronicity with the neurons belonging to the same barrel. In the conference, we would like to discuss the role of synchronous spontaneous activity in the layer 4 circuit formation.

Disclosures: T. Egashira: None. **H. Mizuno:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.02/A28

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

Support: NIH T32 GM008042
NIH T32 NS048004
R01 HD108370

Title: Excitatory and inhibitory cortical dynamics during developmental desynchronization are reproduced in a novel model of cortical network activity.

Authors: *M. WU[1], T. JAIN[4], M. DIPOPPA[2], C. PORTERA-CAILLIAU[3];
[2]Ctr. for Theoretical Neurosci., [3]Neurol., [1]UCLA, Los Angeles, CA; [4]UCSF, San Francisco, CA

Abstract: Understanding how cortical circuits mature is critical as proper wiring of these circuits enables more complex abilities such as sensory processing to develop. Initially, spontaneous neuronal activity in the developing neocortex is characterized by intermittent, brief bursts of synchronous network events that interrupt much longer periods of silence. Network activity then undergoes a crucial transition (during the second postnatal week in mice) from intermittent network-wide synchronous events to asynchronous, sparse neuronal firing that is energetically and computationally more efficient. However, 1) the evolution of excitatory and inhibitory population dynamics during desynchronization and 2) the mechanisms driving desynchronization, have not been fully elucidated. To address this, we used in vivo longitudinal 2-photon calcium imaging in mice from postnatal days (P) 9 to 14 to characterize the evolution of network dynamics. We expressed GCaMP8s in Nkx2.1Cre;Ai14 mice to simultaneously record from excitatory (Exc) neurons and inhibitory (Inh) neurons derived from the medial ganglionic eminence in layer 2/3 of the primary somatosensory cortex (S1). As previously reported, we found that the frequency of synchronous network events increases significantly, while their duration shortens between P9 and P14, indicating that network activity is becoming continuous and sparse. Unexpectedly, we found that the trajectories of Exc and Inh activity patterns diverge during development, such that correlations between Exc-Exc neuron pairs decrease monotonically and plateau by P13 while the correlation between Inh-Inh neuron pairs only decreases slightly. In order to gain mechanistic insight, we generated a new computational model that incorporates different classes of Inh interneurons and imposed values of their density and synaptic strength across development based on the published literature. Our data-driven model reproduced the pairwise correlations of Exc and Inh neurons observed experimentally. We then applied *in silico* perturbations to the model. As a result, the model predicted that manipulation of somatostatin and parvalbumin neuron activity influenced the desynchronization trajectory in opposite ways. We will present results of experiments that test such predictions by chronically manipulating Inh neuron subtypes in vivo and testing the effects on the trajectory of desynchronization using longitudinal 2-photon calcium imaging.

Disclosures: M. Wu: None. **T. Jain:** None. **M. Dipoppa:** None. **C. Portera-Cailliau:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.03/A29

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

Support: NIH R35 NS127232
NIH/NIGMS T32 GM145440
NIMH F30 FMH136683A

Title: Impact of synaptic neoteny on cortical circuit development

Authors: *A. J. RECUPERO, F. POLLEUX;
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Abstract: A salient feature of human brain development is the prolonged, or neotenic, timing of cortical neuron maturation, and most strikingly synaptic maturation, taking years in humans compared to months in non-human primates and weeks in rodents. This extension of the timing of synaptic maturation has been hypothesized to extend critical periods of learning in humans, while its disruption in neurodevelopmental disorders is believed to interfere with multi-sensory integration. However, until recently, the molecular mechanisms underlying this striking degree of synaptic neoteny were largely unknown, hindering our ability to experimentally test the causal relationship between the species-specific timing of synaptic maturation and the tempo of circuit maturation. One molecular candidate is *Slit-Robo Rho-GTPase activating protein 2 (SRGAP2).* The ancestral copy of this gene (*SRGAP2A*), present in all vertebrates, has undergone two large segmental gene duplications (*SRGAP2B* and *SRGAP2C*) in the human genome only. These human-specific genes inhibit all known functions of SRGAP2A, resulting in a significant delay of excitatory and inhibitory synaptic maturation in cortical pyramidal neurons (CPNs). Genes associated with autism spectrum disorder (ASD) in humans, such as *Synaptic GTPase Activating Protein 1 (SynGAP1),* have also been shown to modulate synaptic maturation timing and interestingly, SynGAP1 exerts a function opposite to SRGAP2A, slowing the rate of excitatory synaptic maturation. Recently, in collaboration with Dr. Pierre Vanderhaeghen's group, we discovered that SynGAP1 mediates its function by inhibiting SRGAP2A both in human and mouse CPNs. These results provide us with a unique opportunity to test the impact of accelerated ($SynGAP1^{+/-}$) or prolonged ($SRGAP2^{+/-}$) synaptic maturation on the timing of circuit maturation. To quantitatively evaluate the timing of cortical circuit maturation, we are taking advantage of the fact that during postnatal cortical development, neuronal activity transitions from highly correlated activity to 'adult-like' decorrelated activity. Using longitudinal *in vivo* two-photon Ca^{2+} imaging in the barrel cortex of mice from postnatal day (P) 7 through P21, we are evaluating if accelerated ($SynGAP1^{+/-}$) or delayed ($SRGAP2^{+/-}$) synaptic maturation alters the timing of circuit maturation by quantifying the decorrelation rate of activity in CPNs. This approach will provide critical insights into (1) the causal links between the timing of synaptic maturation and circuit development and (2) the impact of human-specific synaptic neoteny on the phenotypic expression of neurodevelopmental disorders in humans.

Disclosures: A.J. Recupero: None. **F. Polleux:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.04/A30

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

Support: Samsung Science and Technology Foundation SSTF-BA1501-52
National Research Foundation of Korea Grant 2019R1A6A1A10073437 funded by the Korean government (MEST)
AI-Bio Research Grant 0409-20230153 through Seoul National University

Title: Computational and experimental analysis of neural circuit dynamics for stage-specific behaviors in C. elegans.

Authors: *T. CHOE[1], A. BAE[2,3], M. CHOI[4], J. LEE[1,3];
[1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Neurosci. Inst., Seoul Natl. Univ., Seoul, Korea, Republic of; [3]Research Institute of Basic Sciences, Seoul, Korea, Republic of; [4]Res. Inst. of Basic Sci., Seoul, Korea, Republic of

Abstract: The nematode *C. elegans* serves as an ideal model organism for investigating two key areas: 1) neuronal plasticity at the network level, and 2) the connection between the nervous system and behavior, owing to the availability of the connectome of the entire nervous system throughout all developmental stages. Among these stages, the dauer represents an alternative state that worms enter when encountering environmental stress, exhibiting distinct behaviors not observed in other reproductive stages. To unravel the mechanisms underlying stage-specific behaviors, we recently reconstructed the nervous system of the dauer stage and discovered extensive rewiring of downstream signals from sensory neurons in a stage-specific manner. Building upon this discovery, our focus shifted towards elucidating the link between circuit changes, behavioral alterations, and signal modifications in response to sensory stimuli in the dauer stage. In this study, we present novel data on electrical synapses, complementing existing knowledge of the dauer nervous system, and confirm dimorphism in a sub-circuit composed of a sensory neuron and downstream interneurons. Furthermore, we demonstrate stage-specific change in behavior in response to relevant sensory cue, particularly in terms of the reversal response. To gain insight into which dauer-specific connectivity might be pivotal in driving dimorphic behavior in the dauer stage, we conducted simulations on the sub-circuit. By evaluating the estimated neuronal response after swapping dimorphic connectivity between the dauer and adult stages, we identified a dauer-specific electrical synapse as a primary candidate responsible for dimorphic behavior. Introducing this single dauer-specific electrical synapse into the adult stage was sufficient to induce behavior change resembling that observed in the dauer stage. Since there were no notable differences in the physiology of the sensory neuron, we compared the calcium activity of downstream interneurons following optogenetic stimulation of the sensory neuron. In summary, our findings highlight the significance of a crucial dauer-specific electrical synapse, as evidenced by behavioral changes upon its ectopic expression in adults, along with calcium imaging of interneurons in both dauer and adult stages.

Disclosures: T. Choe: None. **A. Bae:** None. **M. Choi:** None. **J. lee:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.05/A31

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

Support: NIH DP1 Director's Pioneer Award
NIH Neurosurgeon Research Career Development Program
DUMC Holland Trice Scholars Program
Klingenstein-Simons Fund
Whitehall Foundation

Title: Probing the functional design of human cortical circuits with CellREADR

Authors: *E. A. MATTHEWS[1], J. B. RUSS[2], P. THOMPSON[3], S. ZHAO[4], M. METHANI[3], T. KEARSE[3], Z. HUANG[5], D. G. SOUTHWELL[6];
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Abstract: Characterizing the functional properties and circuit organization of human neurons is fundamental to understanding brain function and treating neurological disorders. However, due to a lack of experimental tools for accessing, manipulating, and monitoring defined cell populations in the human brain, it has been impossible to unravel the functional cellular design of human neural circuits. To address this critical gap, we have deployed and advanced a programmable RNA sensing tool for cell type-specific targeting, CellREADR (Qian et al., 2022), in organotypic cortical tissues collected from neurosurgical patients. We designed CellREADR constructs to drive the expression of various effectors in human interneurons (*SLC32A1*, *SST*, *CALB2*, and *UNC5B*) and glutamatergic projection neurons (*FOXP2*), and then delivered CellREADRs to human brain slices using adenoviral vectors. From 3-15 days after virus application, CellREADR live fluorophore expression enabled targeted patch clamp studies of human neurons (250 cells, 27 patients), including multimodal PatchSeq characterization of cellular morphological and transcriptional features. To probe the functional synaptic connectivity of interneurons in human cortex, we also used CellREADR to drive the optogenetic effector ChIEF (Lin et al., 2009) in CALB2+ cells. Light activation of CALB2+ interneurons elicited inhibitory post-synaptic potentials in slice pyramidal neurons and interneurons labeled by expression of a Dlx2.0 enhancer virus (Addgene plasmid #163505), indicating both inhibitory and disinhibitory circuit functions for this population. Finally, by using CellREADR to target expression of the calcium indicator, GCaMP7f, in CALB2+ and FOXP2+ populations, we performed live imaging of human cortical microcircuits and observed functional network connectivity that varied between the CALB2+ and FOXP2+ populations. Altogether, by

leveraging CellREADR scalability and programmability in *ex vivo* human brain, these experiments have advanced valuable tools for human neuroscience while furthermore providing early insights into the functional cellular design of human circuits.
Lin, J. Y., Lin, M. Z., Steinbach, P., & Tsien, R. Y. (2009). Characterization of engineered channelrhodopsin variants with improved properties and kinetics. *Biophysical Journal*, *96*(5), 1803-1814.Qian, Y., Li, J., Zhao, S., Matthews, E. A., Adoff, M., Zhong, W., An, X., Yeo, M., Park, C., Yang, X., Wang, B.-S., Southwell, D. G., & Huang, Z. J. (2022). Programmable RNA sensing for cell monitoring and manipulation. *Nature*, *610*(7933), 713-721.

Disclosures: E.A. Matthews: None. **J.B. Russ:** None. **P. Thompson:** None. **S. Zhao:** None. **M. Methani:** None. **T. Kearse:** None. **Z. Huang:** None. **D.G. Southwell:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.06/A32

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

Support: JPMJSP2104
KAKENHI 23KJ1001
KAKENHI 21K18245
KAKENHI 20H03346
KAKENHI 16H06459
KAKENHI 24H00586
KAKENHI 24H02310

Title: Temporal roles of NMDA receptors for dendritic refinement in the mouse barrel cortex unveiled by rapid protein knockdown

Authors: *A. NIHASHI[1,2], N. NAKAGAWA[3,2], M. YAMAMOTO[4], T. SATO[3], R. AJIMA[5,6], Y. SAGA[7], Y. YOSHIMURA[4,6], M. KANEMAKI[8,2], T. IWASATO[9,2];
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Abstract: Dendritic refinement of layer 4 spiny stellate neurons (barrel neurons) in the mouse barrel cortex is a useful model for understanding mechanisms of activity-dependent circuit maturation in the postnatal brain. Mature barrel neurons located at the barrel edge have basal

dendrites asymmetrically expanded primarily within a single barrel, which underlies a precise one-to-one functional relationship between whiskers and barrels. Single-cell knockout of NR1, the essential NMDA receptor (NMDAR) subunit, impairs dendritic asymmetry of barrel neurons (Mizuno et al., Neuron 2014), suggesting the importance of NMDAR-mediated neural activity for dendrite refinement. However, due to the lack of proper technology, temporal roles of NMDARs in dendritic refinement remain largely unclear. We showed that the newly developed auxin-inducible degron 2 (AID2) technology (Yesbolatova et al., Nat. Commun. 2020) can knockdown target proteins quickly in the neonatal mouse brain (Nihashi et al., SFN2023). By combining the AID2 and Supernova (Mizuno et al., 2014, Luo et al., Sci. Rep. 2016) systems, we knocked down the NR1 protein in single barrel neurons between postnatal day 3 (P3) and P6, which is the core period of dendritic refinement (Nakazawa et al., Nat. Commun. 2018). We found that NMDARs from P3 to P6 are indeed required for formation of barrel neuron dendrite asymmetry. We then asked whether NMDARs are required even after barrel neurons form clear dendrite orientation bias. AID2-mediated NR1 knockdown starting at P6 quickly abolished dendrite asymmetry of barrel neurons. These results suggest that NMDARs are required not only for formation but also for maintenance of dendrite asymmetry of barrel neurons. We also found that the Golgi lateral polarity once formed in barrel neurons (Nakagawa and Iwasato, Cell Rep. 2023) was also lost by NR1 knockdown starting at P6. Thus, a novel inducible protein knock-down approach revealed temporal roles of NMDARs for dendritic refinement during postnatal development.

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Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.07/A33

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

Support: NTU (113L893104)
NSTC (NSTC 112-2311-B-002-007-MY3)

Title: Synaptotagmin III regulates the stage 2 retinal waves by interacting with SNAP-25 in developing retinal ganglion cells

Authors: C.-C. TSENG, M. SUNG, S.-H. CHEN, H.-Y. WEN, *C.-T. WANG;
Natl. Taiwan Univ., Taipei, Taiwan

Abstract: During the first postnatal week in rodents, developing retinal neurons display stage 2 retinal waves (also termed cholinergic waves), which are initiated by the spontaneous release of

starburst amacrine cells (SACs) to neighboring SACs and retinal ganglion cells (RGCs), essential for the refinement of retinogeniculate synapses. Our preliminary results suggested that the expression level of Synaptotagmin III (Syt3), a calcium-binding protein coupling exocytosis, is up-regulated in the optic nerves at P6 (the developmental critical period of eye-specific segregation), indicating its potential role in regulating retinogeniculate projection. However, how Syt3 may affect visual circuit development remains elusive. In this study, we aim to delineate the mechanism underlying Syt3 regulation of stage 2 waves. First, we examined the localization of Syt3 in developing rat retinas (P2 and P6) by immunofluorescence. Syt3 was expressed in the inner plexiform layer and RGCs at P2 and P6, with abrupt expression in P6 optic nerves. Next, we employed the cell-type specific promoter (the Brn3b promoter for RGCs) to specifically overexpress Syt3 or its mutant with weakened calcium-binding ability (Syt3-C2AB*) in RGCs. By using live calcium imaging, we further analyzed the stage 2 wave properties following molecular perturbation. We found that overexpressing Syt3 in RGCs, but not overexpressing Syt3-C2AB* in RGCs, increased wave frequency compared to control, suggesting that Syt3 in RGCs may regulate stage 2 retinal waves by affecting calcium-dependent exocytosis. Further, using proximity-ligation assays, we found that overexpressing Syt3 in RGCs increased the interaction with SNAP-25 (a t-SNARE protein in exocytosis), but overexpressing Syt3-Y468N in RGCs did not. Finally, we found that overexpressing SNAP-25 in RGCs dampened the frequency of stage 2 retinal waves and impaired retinogeniculate projection. Our results suggest that Syt3 up-regulation of stage 2 waves may be mediated via interaction with SNAP-25 in RGCs, therefore regulating RGC exocytosis during visual circuit refinement.

Disclosures: C. Tseng: None. **M. Sung:** None. **S. Chen:** None. **H. Wen:** None. **C. Wang:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.08/A34

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

Support: Helse Sør-Øst
Anders Jahres Fund

Title: Development and differential expression of perineuronal nets in specific spinal motoneuron subpopulations

Authors: M. MAVROVIC[1], B. VAGASKA[2], *J. C. GLOVER[3];
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Abstract: The adaptive plasticity of spinal cord (SC) motor circuits is regulated in part by the molecular environment surrounding the constituent neurons. Dynamic changes in synaptic plasticity during development and after injury are linked to the presence of a dense network of extracellular matrix molecules known as perineuronal nets (PNNs) that surround certain populations of neurons. Here we have studied the developmental pattern of PNN expression and synaptic stabilization in selected motoneuron (MN) subpopulations in the mouse spinal cord. Using Western blotting, immunohistochemistry, qPCR and RNA-seq we show that PNN expression increases gradually over the first three postnatal weeks and that Aggrecan is a major PNN component. Earlier studies have shown that spinal MNs express PNNs but have not assessed differential expression in MN subtypes or relationship of PNNs to innervation pattern. To this end we have used retrograde viral labeling following intramuscular injection of a Cre-bearing retro-AAV2 to target homonymous MNs. We find that by the end of the 3rd postnatal week about 80% of hindlimb flexor *Tibialis anterior* MNs express PNNs, of which about 30% show strong expression, whereas about 95% of hindlimb extensor *Gastrocnemius* MNs express PNNs, of which about 50% show strong expression. Vestibulospinal neurons control posture and balance during movement through differential inputs on extensor and flexor MNs. Our results indicate a differential expression of PNNs in extensor and flexor MNs by the 3rd postnatal week, which parallels the postnatal maturation of vestibulospinal inputs. In ongoing work we are testing the role of PNNs in the development of selective vestibulospinal inputs to extensor versus flexor MNs.

Disclosures: M. Mavrovic: None. **B. Vagaska:** None. **J.C. Glover:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.09/A35

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

Support: NTU (113L893104)
NSTC (NSTC 112-2311-B-002-007-MY3)

Title: Investigating the roles of ZNRF1 in regulating retinal waves

Authors: *T.-J. CHEN, L.-C. HSU, C.-T. WANG;
Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Ubiquitination influences many aspects of neuronal function, such as the prevalence of protein turnover during neural circuit development. Previous studies have indicated that activity-dependent homeostasis of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) is crucial for synaptic plasticity. ZNRF1, a RING-type E3 ubiquitin ligase,

is localized to the presynaptic site and contributes to the maintenance of synaptic protein homeostasis. However, how ZNRF1 regulates neural circuit development remains elusive. During the first postnatal week in rodents, immature rodent retinas display spontaneous calcium waves termed retinal waves, essential for establishing functional visual circuits. Retinal waves originate from starburst amacrine cells (SACs), spreading to retinal ganglion cells (RGCs) and central brain targets. Our preliminary results have discovered that exocytotic molecules regulate the patterns of rat stage 2 waves within SACs or RGCs, such as SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins or ionotropic glutamatergic receptors (iGluR). In this study, we aimed to investigate how ZNRF1 may regulate stage 2 retinal waves. First, we found that ZNRF1 was expressed in the postnatal retinas of wild-type C57BL/6 mice, suggesting that ZNRF1 is involved in retinal development. Using live calcium imaging to detect retinal waves in isolated whole-mount retinas of postnatal mice, we observed a reduction in wave frequency but an increase in wave interval in *Znrf1*-deficient (*Znrf1*$^{-/-}$) compared to wild-type retinas, suggesting that ZNRF1 up-regulates wave rhythm during development. Next, similar levels were found in SNARE proteins (Synaptobrevin-2, Syntaxin-1, and Synaptosome-associated protein of size 25 kDa/SNAP-25), Synaptotagmin-1, and Synaptotagmin-3 suggesting that depletion of ZNRF1 may not significantly alter the homeostasis of these exocytotic proteins in developing retinas. Furthermore, by bath-applying the iGluR blocker cocktails, we found that blocking the iGlu transmission dampened the rhythms and propagation of stage 2 retinal waves in the developing mouse retina. Further immunofluorescence showed that ZNRF1 deficiency decreased the overall levels of GluA2 expression and influenced the recycling of AMPARs in mouse RGCs. Thus, our results shed light on the role of ZNRF1 in regulating retinal waves via the regulation of dynamics of synaptic transmission.

Disclosures: T. Chen: None. **L. Hsu:** None. **C. Wang:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.10/A36

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

Support: NIH Grant R01NS131486
NIH Grant DP2MH132943
McKight Scholar Award
Rita Allen Scholar Award
Klingenstein-Simons Fellowship in Neuroscience

Title: Developing a bioorthogonal chemistry approach for the identification of neuronal cues facilitating glial phagocytosis in the brain

Authors: *D. J. VITA[1], L. CHEADLE[2,3];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Neurobio., Cold Spring Harbor Lab., Cold Spring Harbor, NY; [3]Howard Hughes Medical Institute, Chevy Chase, MD

Abstract: Synaptic refinement is a conserved process from *c. elegans* to humans by which the nervous system restructures its connectivity typically leading to a reduction in circuit complexity. During early development neurons over-proliferate and form excessive, weak connections which must be later refined through the selective strengthening and maintenance of some synapses and the elimination of others. Substantial efforts have been made to understand the mechanisms involved in synaptic refinement, revealing essential roles for glial phagocytosis, with more recent work demonstrating glial-dependent, non-phagocytic mechanisms as well. Failure to remove excessive synapses is often associated with developmental disorders including autism, while reactivating developmental refinement mechanisms later in life can result in neurodegenerative states. Identifying the molecular mechanisms underlying synaptic remodeling therefore holds great potential for understanding nervous system function and revealing potential therapeutic targets for treating numerous diseases. Detecting neuronal cues that lead to glial engulfment, however, has been challenging with relatively few “eat me” signals known for the nervous system. This is due in part to a lack of methodologies with the resolution to examine molecular changes occurring at the cell surface of neurons where neuron-glial signaling is actively occurring to recruit glial engulfment, leading the field to test candidate cues in a piecemeal fashion. To circumvent this, we are developing Bioorthogonal Identification of Targeted Engulfment (BITE), an unbiased proteomics-based strategy to reveal novel cell surface cues presented by neurons to instruct glial phagocytosis of neuronal material. Utilizing recent advances in bioorthogonal chemistry to examine a cell specific proteome *in vivo*, BITE incorporates the noncanonical amino acid azidonorleucine into the neuroproteome affectively labeling nearly all proteins from this cell population. This in turn facilitates the identification of neuronal proteins found within glia following cell sorting. In short, by labeling the neuroproteome during glial phagocytic events, we can then isolate glial cells and pull down internalized, labeled, neuron-derived proteins for proteomic analysis. Using BITE as a screening tool, we can detect neuronal cues specialized to elicit glial phagocytosis of neural material in an unbiased and context specific way. The development of this method has the potential to reveal entirely new mechanisms of glial phagocytosis and can be applied to a variety of disease models.

Disclosures: D.J. Vita: None. **L. Cheadle:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.11/A37

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

Support: CIHR (PJT 399790)
Human Frontier Science Program Organization (CDA00009/2018 and RGY0072/2019)
SickKids Foundation and Canadian Institutes of Health Research (CIHR) – Institute of Human Development, Child and Youth Health (NI19-1132R)
Natural Sciences and Engineering Research Council of Canada (RGPIN-2017-06344)

Title: Septotemporal differences in hippocampal-prefrontal cortex circuit maturation inform a sensitive period for cognitive flexibility

Authors: *A. CRUZ-SANCHEZ[1], A. ABDUSALOM[1], H. CHASIOTIS[1], R. GUGUSTEA[3], M. HASANTASH[4], C. ANACKER[5], M. ARRUDA-CARVALHO[2];
[1]Univ. of Toronto Scarborough, Scarborough, ON, Canada; [2]Dept. of Psychology, Univ. of Toronto Scarborough, Toronto, ON, Canada; [3]Univ. of Toronto, Toronto, ON, Canada; [4]Dept. of Psychiatry, [5]Psychiatry, Columbia Univ., New York, NY

Abstract: The hippocampal (HPC)-medial prefrontal cortex (mPFC) circuit is vital for the processing and execution of higher order cognitive functions. Anatomical studies suggest a topographical distribution in which more ventral regions of the HPC project to more ventral regions of the mPFC. Although this circuit has been extensively studied for its synaptic and behavioral signatures in adulthood, very little is known about the maturation of the HPC-mPFC pathway and whether septotemporal differences in connectivity exist throughout development. Here, we used viral tracing, optogenetic-assisted patch clamping and behavior in mice to examine changes in hippocampus-mPFC projections along the septotemporal axis from postnatal day (P)10 to P60. We first examined mPFC projections stemming from the dorsal, intermediate and ventral CA1 (dCA1, iCA1 and vCA1, respectively) in adult mice, then mapped age-dependent changes in projections specifically to the prelimbic (PL) and infralimbic (IL) cortices. We found topographical differences in projection patterns with adult-like vCA1 terminal density already present at the earliest age tested of P15 for both mPFC subregions, and an increase in iCA1 terminal density only in the PL at P30. Investigation of optically-evoked CA1 responses onto mPFC subdivisions showed that vCA1-PL synapses display a relatively delayed sex-specific increase in presynaptic efficacy starting at P30, in contrast to vCA1-IL synapses which show stable paired pulse responses from P15. iCA1-PL presynaptic efficacy increases from P21, with iCA1-IL synapses showing a slightly delayed increase at P30. At the postsynaptic level, both vCA1-PL and -IL synapses display equivalent AMPA:NMDA ratios from P15, but iCA1-PL and -IL synapses show increased AMPA:NMDA ratios starting at P30. These data identify distinct topographical signatures in the anatomical and synaptic maturation of the CA1-PFC pathway. Finally, the timing of vCA1-mPFC presynaptic maturation marked a sex- and pathway-specific sensitive period in behavior, as chronic inhibition of the vCA1-mPFC pathways during juvenility led to a deficit in extradimensional set shifting exclusively in females, whereas a similar manipulation in adulthood did not affect cognitive flexibility. Our data establish a timeline for the postnatal maturation of HPC efferents in a subdomain- and target-defined manner, disambiguating the contributions of iCA1 and vCA1 to mPFC subdivisions. Critically,

these findings identify a sex- and subprojection-specific developmental sensitive period with important implications for cognitive function.

Disclosures: A. Cruz-Sanchez: None. **A. Abdusalom:** None. **H. Chasiotis:** None. **R. Gugustea:** None. **M. Hasantash:** None. **C. Anacker:** None. **M. Arruda-Carvalho:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.12/A38

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

Support: CIHR (PJT 399790)
Human Frontier Science Program Organization (CDA00009/2018 and RGY0072/2019)
SickKids Foundation and Canadian Institutes of Health Research (CIHR) – Institute of Human Development, Child and Youth Health (NI19-1132R)
Natural Sciences and Engineering Research Council of Canada (RGPIN-2017-06344)

Title: Maturation of intrinsic and synaptic properties of the mouse Dorsal Peduncular cortex

Authors: A. KHLAIFIA[1], J. J. BOTTERILL[3], ***A. CANELLA**[1], F. VIOLI[1], D. ZAIDI[1], A. PATEL[1], M. ARRUDA-CARVALHO[1,2];
[1]Psychology, [2]Cell and Systems Biol., Univ. of Toronto, Scarborough, ON, Canada; [3]Anatomy, Physiology, and Pharmacol., Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: The dorsal peduncular cortex (DP), located within the ventral limit of the medial prefrontal cortex (mPFC), plays a key role in anxiety-like behavior, fear and emotional learning, as well as in stress responsivity and sympathetic responses. Deficits in mPFC synaptic maturation and neuronal development early in life have been linked to several neurodevelopmental and mental disorders, including changes in anxiety and emotional function. Yet, how early life changes in DP synaptic maturation and connectivity may affect anxiety, emotional learning and stress responsivity in adulthood is unknown. Here we examined the development of synaptic and intrinsic properties of DP pyramidal neurons in both superficial (layer 2/3) and deeper layers (layer 5) of C57BL/6J mice throughout the first postnatal month using whole cell patch clamp recordings. We found that the second postnatal week is marked by a shift into a mature cellular profile, encompassing the hyperpolarization of the resting membrane potential and action potential threshold, a decrease in input resistance and membrane time constant, an increase in rheobase and a decrease in after-hyperpolarization amplitude across layers. Excitatory spontaneous responses of DP layer 2/3 and 5 pyramidal neurons plateau during the second and third weeks of age, respectively, whereas spontaneous inhibitory responses show

a gradual increase, plateauing by the fourth postnatal week. Overall, our data show similar maturation of intrinsic and synaptic properties of pyramidal neurons in both DP layers. Ongoing experiments aim to characterize the upstream and downstream connectivity of DP pyramidal neurons. This work will provide a blueprint for the developmental trajectory of the DP, highlighting potential sensitive periods of synaptic maturation when external factors such as stress may potentiate anxiety-like and fear behavior.

Disclosures: A. khlaifia: None. **J.J. Botterill:** None. **A. Canella:** None. **F. Violi:** None. **D. Zaidi:** None. **A. Patel:** None. **M. Arruda-Carvalho:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.13/A39

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

Title: Revealing activity signatures of early life adversity in the basolateral amygdala driving behavior

Authors: *C. CODY, M. FANIKOS, H. C. BRENHOUSE;
Psychology, Northeastern Univ., Boston, MA

Abstract: Traumatic events experienced early in life can shift an individual's behavioral repertoire in adolescence, causing increases in risk seeking and anxiety-like behaviors. Understanding the ways in which early life adversity (ELA) increases adolescent vulnerability to increased risk taking and anxiety-like behaviors is essential for preventing maladaptive mental health outcomes. ELA alters cellular function and neuronal activity in regions controlling these behaviors, including the basolateral amygdala (BLA), ventral hippocampus (vHipp) and prefrontal cortex (PFC). The connections between these areas are responsible for assessing threat, responding to novel stimuli, and assessing valence. Prior work from our lab has shown that ELA leads to hyper-innervation of glutamatergic BLA projections to the PFC in adolescence. The BLA is particularly poised to control risk and anxiety related behaviors, and is also vulnerable to ELA-related changes including increases in neuronal excitability, making the BLA an incredibly valuable target for investigating ELA-induced pathologies. Thus, examining the trajectory of ELA-induced activity changes in the BLA and its projection sites may uncover a targetable neural mechanism underpinning adolescent maladaptive behavior. Here we utilized a 20-day model of maternal separation (MS) ELA, in which pups are separated from their mothers for 4 hours/day from postnatal day (PND) 2-20. In Experiment 1, MS-induced changes to cFos levels were measured to identify changes in activity in the BLA, vHipp, and PFC at various time points of MS or control rearing. This study elucidates the time course of both typical neuronal activity maturation, as well as activity changes induced by MS within these regions. In

Experiment 2, BLA cells activated by MS rearing were tagged with an activity dependent viral vector on the final days of MS exposure. These cells were then chemogenetically inhibited in the adolescent timeperiod during a prepulse inhibition (PPI) task, which has been shown to rely on coordinated activity between the BLA, vHipp, and PFC. We hypothesize that MS over activates BLA cells, resulting in increased connectivity with the PFC and altered vHipp-PFC connectivity, and that inhibiting these tagged cells will restore typical behavior in PPI testing. The results of these studies contribute to the understanding of how ELA alters pathological behaviors in adolescence and inform strategies to develop treatments for disordered behaviors involving increased risk seeking and anxiety.

Disclosures: C. Cody: None. **M. Fanikos:** None. **H.C. Brenhouse:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.14/A40

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

Support: DC007695
DC007695-S1

Title: Spontaneous Activity Drives the Synchronous Growth of the Calyx of Held and Maturation of Principal Neurons in the Medial Nucleus of the Trapezoid Body

Authors: *D. HELLER[1], N. BENITES[1], E. AMICK[1], A. A. DAGOSTIN[3], S. M. YOUNG, Jr.[4], H. P. VON GERSDORFF[5], G. A. SPIROU[2];
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Abstract: The formation of neural circuits during early development can occur independent of neural activity, but the maturation and refinement of nascent connections is an activity-dependent mechanism. In sensory systems, patterned spontaneous activity (SA) originates in the peripheral sense organ and propagates through the central nervous system. Interestingly, SA in the auditory system occurs prior to the onset of external stimuli (in mice, ear canal opens after postnatal day (P)10), highlighting the importance during neural circuit formation. Globular bushy cells located in the ventral cochlear nucleus (VCN) project contralaterally and innervate principal neurons (PNs) in the medial nucleus of the trapezoid body (MNTB) forming the calyx of Held (CH) nerve terminal. The CH:MNTB synaptic connection is utilized as a model system for studying the role of SA during neural circuit formation, in part because growth of the CH occurs rapidly (P2-P6) resulting in mono-innervation, and key biophysical properties have been

characterized. The effects of removing cochlear SA on formation of the CH have proven inconclusive, perhaps due to homeostatic compensation by central neurons. To address this confounding factor, we directly manipulated synaptic transmission at the CH:MNTB connection through viral vector mediated, rapid-onset expression of tetanus neurotoxin (TeNT). Following unilateral viral injections into the VCN at P0, mCherry fluorescence (co-expressed with TeNT) was detectable within 48 hours in CHs innervating the contralateral MNTB. Whole-cell patch-clamp recordings were performed at P4, P6, and P9 to investigate the effects of TeNT mediated synaptic silencing. Efficacy of TeNT expression was assessed through recordings of spontaneous excitatory postsynaptic currents (EPSCs) with reduced frequency and slower decay kinetics compared to non-transduced ipsilateral MNTB (iMNTB) PNs (control). Paired recordings simultaneously patching a transduced CH and associated PN were performed with presynaptic depolarizing current injections failing to elicit a postsynaptic spike or EPSC (P6; n = 3). Blocking SA at the CH:MNTB resulted in delayed maturation of PNs with increased input resistance, slower membrane time constant and delayed transition from tonic to phasic firing continuing into the second postnatal week. Immunostaining shows impaired growth of the CH expressing TeNT with reduced volume compared to iMNTB PNs (P6; $580 \pm 278\ \mu m^3$ vs $702 \pm 256\ \mu m^3$; $p = 0.32$; $n = 10$ and P9; $447 \pm 345\ \mu m^3$ ($n = 11$) vs $1619 \pm 505\ \mu m^3$ ($n = 7$); $p < 0.05$). This study highlights an important role for SA triggering growth of the CH and the synchronous maturation of PNs biophysical properties.

Disclosures: D. Heller: None. **N. Benites:** None. **E. Amick:** None. **A.A. Dagostin:** None. **S.M. Young:** None. **H.P. von Gersdorff:** None. **G.A. Spirou:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); syGlass.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.15/A41

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

Support: NRF 2023R1A2C200621711

Title: Computational Modeling of Activity-Dependent Plasticity in Neural Populations Based on Spike Timing-Dependent Hebbian Plasticity

Authors: *Y. LEE[1], D. LEE[1], H.-J. PARK[1,2,3];
[1]Dept. of Nuclear Medicine, Grad. Sch. of Med. Science, Brain Korea 21 Project, Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [2]Dept. of Nuclear Med., Severance Hosp., Seoul, Korea, Republic of; [3]Dept. of Cognitive Sci., Yonsei Univ., Seoul, Korea, Republic of

Abstract: This study explores the relationship between activity-dependent plasticity and Hebbian plasticity, both crucial to learning and memory yet distinct in their scope and scale. Activity-dependent plasticity broadly describes changes in brain structure or function triggered by neural activity, while Hebbian plasticity is a more specific form of synaptic plasticity characterized by significant synaptic strengthening resulting from simultaneous cell activation. We hypothesize that activity-dependent plasticity is facilitated through mechanisms of Hebbian plasticity, as demonstrated by our computational models that utilize spike timing-dependent Hebbian plasticity (STDP) within a spiking neural network (SNN). Our research involved constructing a small group of SNN populations comprising an input population (source) generating Poisson spike trains and a receiving population (target) that receives these stimuli. During the simulations, we dynamically varied the frequency of spatiotemporal bursts from the source that varied in timing when reaching the target. As the number of synaptic connections from source to target was dynamically adjusted based on relative weight intensity, the network learned efficient pathways and rewired by pruning. We monitored long-term modifications in target neurons influenced by the relative timing of stimulus arrival and neuron spiking, thus providing a context for understanding changes due to STDP. We also observed changes in excitatory and inhibitory synaptic currents, classified as early or late, depending on the timing of synaptic input relative to postsynaptic activity. In such dynamic environments, networks showed a tendency to differentiate between familiar stimuli and novel patterns, responding accordingly. They displayed a more efficient response when familiar stimuli were presented. Based on the long-term statistical history of stimuli, the STDP rule determined whether the synchronous or asynchronous interactions resulted in potentiation or depression. This STDP rule alters the sensitivity of the target population, thus causing activity-dependent plasticity in the population. Additionally, we conducted experiments to manipulate the timing of postsynaptic spikes in response to incoming stimuli. By pairing continuous subthreshold stimuli with postsynaptic current injections, we noted potentiation or depression based on their relative timing intervals. These simulations underscore that STDP can effectively underpin activity-dependent circuit refinement, effectively bridging the gap between broad neural population adaptability and specific synaptic adjustments.

Disclosures: Y. Lee: None. **D. Lee:** None. **H. Park:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.16/A42

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

Support: NIMH R01 MH124695-01

Title: Striatal indirect pathway regulates the postnatal maturation of parvalbumin but not somatostatin inhibition in the prefrontal cortex.

Authors: *M. JANECEK[1], A. D'AGOSTINO[2], R. PEIXOTO[1];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]UPMC, Pittsburgh, PA

Abstract: Prefrontal cortex (PFC) and the basal ganglia are interconnected through a series of cortico-striato-thalamo-cortical (CSTC) loops that are critical for higher-order cognitive and motor functions, and whose dysfunction is implicated in multiple neurodevelopmental disorders. While cortical activity is known to regulate striatal development, comparatively little is known about the role of the striatum—the main input nucleus of the basal ganglia—in upstream cortical maturation. We previously found that partial ablation of spiny projection neurons of the indirect pathway (iSPN) disrupts the maturation of prefrontal GABAergic synapses. However, the interneuron cell types sensitive to loss of iSPN output underlying the loss of prefrontal inhibition were unknown. Since early cortical circuits are under the outsized influence of early-born somatostatin- (SST) and later parvalbumin- (PV) expressing interneurons, we investigated whether SST or PV connectivity onto principal pyramidal (PYR) cells is affected by iSPN ablation. We used transgenic SST-Flp mice and viral strategies to selectively express a light-sensitive excitatory opsin in either cortical SST or PV interneurons and intracellularly recorded optically evoked inhibitory postsynaptic currents (oIPSCs) onto PYR cells in P14-15 prefrontal sections. We did not observe any changes in SST interneuron connectivity onto PYR synapses following iSPN ablation. However, we found a striking reduction of oIPSC amplitude at PV-to-PYR synapses. Paired-pulse ratio measurement further revealed a facilitation in release probability, suggesting presynaptic alterations at PV-PYR synapses in response to iSPN ablation. We further characterized PV interneurons and while PV excitability and firing rate remain typical, we observe that inhibitory connectivity onto PV interneurons may be disrupted by iSPN ablation. Taken together, we elucidate the instructive role of CSTC loops in prefrontal cortical synapse maturation and demonstrate that early striatal imbalance caused by partial iSPN ablation disrupts PV-PYR but not SST-PYR synapses. These findings are consistent with our previously reported decrease in overall inhibitory synaptic input onto PYRs and indicate that PV interneurons play a role in shaping prefrontal synaptic plasticity in response to the output of CSTC loops. Intriguingly, PV interneuron firing remains preserved, suggesting that CSTC output might not instruct intrinsic PV maturation by the second postnatal week.

Disclosures: M. Janecek: None. **A. D'Agostino:** None. **R. Peixoto:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.17/A43

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

Support: R00NS112604

Title: Sodium channel kinetics in human developing cortex and biophysical properties of sodium channel variants associated with brain malformations

Authors: *S. GOLINSKI[1], R. S. SMITH[2];
[2]Pharmacol., [1]Northwestern Univ., Chicago, IL

Abstract: Voltage-gated sodium channels (VGSCs) are critical for brain development and genetic variants in VGSCs are associated with a spectrum of neurodevelopmental phenotypes spanning fetal to adolescence periods. Here, we characterize the biophysical properties of pathogenic variants in VGSC subtypes SCN3A and SCN2A associated with malformations in cortical development (MCDs) in humans. Using patch clamp and pharmacology in in vitro models, we isolate sodium conductances and analyze biophysical features of VGSC activation/inactivation in several developing cell types isolated from the midgestational human cortex. We further identify splice isoforms of VGSCs and their potential functional contributions in developing neurons. These results demonstrate the biophysical pathophysiology of VGSC variants resulting in early brain malformations, and offer a key insight into how baseline sodium currents facilitate prenatal development. Additionally, our results suggest potential therapeutic targets within these channels that could ameliorate or prevent the progression of related developmental disorders.

Disclosures: S. Golinski: None. **R.S. Smith:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.18/A44

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

Support: Simons Foundation SCPAB
Stanley Center at the Broad Institute

Title: Protracted maturation of frontal cortex drives changes in decision making in mouse and marmoset

Authors: *K. J. MASTRO[1], W.-C. LEE[2], W. WANG[3], Y. LIN[4], M. B. JOHNSON[5], B. A. STEVENS[6], B. L. SABATINI[7];
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Abstract: Age-related changes in behavior must be due, in part, to the changing neural architecture that occurs across the lifespan. During adolescence, there is a significant enhancement in cognitive capabilities that parallels the maturation of frontal cortical circuits, but how the development of frontal cortex drives cognitive development is still an area of active exploration. The protracted development of the frontal cortex during adolescence represents a vulnerable period for genetic and environmental insults that may drive brain structure and function into the disease states. Understanding the developmental trajectory of disease-relevant circuits across this vulnerable period of development can provide tractable means for therapeutic interventions. To tackle these questions, we have established a multi-systems approach that unpacks the genetic, synaptic, circuit and behavioral changes that occur over the course of adolescence across both mice and a non-human primate, the Common Marmoset. Firstly, we have identified synaptic, cellular, and behavioral changes that occur across the neurotypical adolescent development of the mouse which extends the period for cognitive maturation from weeks to months. Most notably, there is a prolonged and significant enhancement of the inhibitory maturation that alters cognitive performance in a reward-based decision-making in the two-armed bandit task (2-ABT). Consequently, these circuit-level changes drive age-related differences in cognition far beyond the traditional window of development. Secondly, marmosets display similar age-related changes in behavioral strategy during the 2-ABT and display anatomical changes in thalamic input that mirror changes in the mouse. Lastly, we performed single-nucleus RNA sequencing across similar periods of development and have mapped the shared cell-state changes that occur over this developmental period. In the future, these experiments will nominate shared and divergent pathways that may drive these age-related changes in both brain structure and function. These experiments establish the foundation for a multi-systems level approach to understanding how genetic and environmental risk factors may alter these developmental trajectories with the goal of identifying tractable targets for therapeutic intervention.

Disclosures: K.J. Mastro: None. **W. Lee:** None. **W. wang:** None. **M.B. Johnson:** None. **B.A. Stevens:** None. **B.L. Sabatini:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.19/A45

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

Support: NIMH Grant P50MH112491
NIMH Grant R21MH126374

Stanley Center for Psychiatric Research
Howard Hughes Medical Institute

Title: Transcriptional development and psychiatric risk in the adolescent mouse, marmoset, and human prefrontal cortex

Authors: K. J. MASTRO[1], Y. LIN[2], J. HOMMAN-LUDIYE[3], J. A. BOURNE[4], B. A. STEVENS[5], ***M. JOHNSON**[2];
[1]Harvard Med. School/Broad, Boston, MA; [2]Broad Inst. of MIT and Harvard, Cambridge, MA; [3]Monash Univ., Clayton, Australia; [4]Section on Cell. and Cognitive Neurodevelopment, NIMH, Bethesda, MD; [5]Harvard Med. Sch. Neurobio., Boston Children's Hosp., Boston, MA

Abstract: The adolescent brain undergoes significant developmental changes in structure and function. Neuroimaging, histology, and transcriptomics define a developmental trajectory that extends through the second into the third decades of human life. Symptoms of severe mental illness, including psychosis and mood disorders, most commonly emerge during this same period, arising from the interplay of genetic predisposition, environmental risk factors, and the protracted development of the adolescent brain. The prefrontal cortex is among the brain regions displaying particularly extended adolescent maturation, is critical for cognitive functions disrupted in psychiatric disorders, and is a site of proposed pathological hallmarks of schizophrenia, namely decreased synaptic density and gray matter volume. To enable the generation of mechanistic hypotheses regarding the impact of genetic and environmental psychiatric risk factors on adolescent prefrontal and cognitive development, we have generated a single-cell transcriptome atlas of the marmoset dorsolateral prefrontal cortex across postnatal development, as well as a comparison dataset from mouse frontal cortex that aids in defining the relevant age range in this most common model species. We have integrated our data with analogous human data for a comparative analysis of molecular and cellular changes in the prefrontal cortex during the adolescent critical period of psychiatric vulnerability. We have annotated these data with the latest schizophrenia and bipolar disorder genetic associations, testing for heritability enrichment to identify cell types and states - including key stages of astrocyte and oligodendrocyte maturation - most likely to mediate psychiatric genetic risk. This resource and our analyses provide new insights into glial support of synapse maturation and circuit refinement during postnatal development and help bridge rodent functional studies to non-human primate models and human therapeutic target nomination.

Disclosures: K.J. Mastro: None. **Y. Lin:** None. **J. Homman-Ludiye:** None. **J.A. Bourne:** None. **B.A. Stevens:** None. **M. Johnson:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.20/A46

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

Support: NTU (113L893104)
NSTC (NSTC 112-2311-B-002-007-MY3)

Title: Dysbindin regulates the spatiotemporal properties of stage 2 retinal waves via the nucleocytoplasmic shuttle in developing retinal ganglion cells

Authors: *Y.-Y. HSIEH, T.-L. CHENG, Y.-H. TING, C.-T. WANG;
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Abstract: Dysbindin is encoded by a major susceptible gene of Schizophrenia (SCZ). SCZ patients exhibited neurodevelopmental defects such as impaired primary sensory processing, including visual processing. Previous studies indicate that correct refinement of rodent visual circuits requires stage 2 retinal waves, initiated by the release from starburst amacrine cells (SACs) to neighboring SACs and retinal ganglion cells (RGCs). However, whether and how Dysbindin regulates visual system development in rodents remains unknown. In this study, we aimed to investigate the mechanism underlying Dysbindin regulation of retinal development. We found that the expression level of Dysbindin was down-regulated in developing rat retinas upon the completion of stage 2 waves (~P10). Dysbindin was highly expressed in the developing inner plexiform layer and RGCs, with significantly higher expression in P8 RGCs than in P2 or P6 RGCs. By combining the cell-type specific promoter (the Brn3b promoter for RGCs) and live calcium imaging, we found that overexpressing Dysbindin in RGCs, but not SACs, decreased wave frequency and spatial propagation compared to control. Since Dysbindin has been shown to regulate transcription by affecting the nucleocytoplasmic shuttle, we further determined whether Dysbindin regulation of stage 2 retinal waves may be mediated by the nucleocytoplasmic shuttle in RGCs. To do this, we introduced a Dysbindin mutant harboring the defective nuclear export signal (Dysbindin-mNES; Dysbindin-L243,246,252,256A) in RGCs. Wave frequency and spatial propagation were significantly increased by overexpressing this mutant (Dysbindin-mNES) compared to control or wild-type Dysbindin in RGCs, suggesting that a reduction in the nuclear export of RGC's Dysbindin may increase the spatiotemporal properties of stage 2 retinal waves. To further detect the developmental changes of the Dysbindin nucleocytoplasmic shuttle in RGCs, we analyzed Dysbindin immunoreactivity in the nucleus of developing RGCs at P2, P6, or P8. We found that Dysbindin immunoreactivity in the RGC's nucleus was increased at P8 compared to P2 or P6. Thus, Dysbindin may fine-tune the rhythm and propagation of stage 2 waves via nucleocytoplasmic shuttle in RGCs, involved in regulating visual circuit refinement.

Disclosures: Y. Hsieh: None. **T. Cheng:** None. **Y. Ting:** None. **C. Wang:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.21/A47

Topic: F.04. Neuroimmunology and Neurovirology

Support: 5R01MH052716-27

Title: Natural Killer Cells in the Developing Cerebellum: Shepherds or Poachers of Neurogenesis?

Authors: *A. ALLEE[1], A. A. MAXIMOVA[2], M. M. MCCARTHY[3];
[1]Univ. of Maryland Baltimore, Baltimore, MD; [2]U of Maryland Baltimore, Baltimore, MD;
[3]Dept. of Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Neurodevelopmental disorders have been firmly and repeatedly linked to early life inflammation, sex, and gender, with males 2-4 times more likely to be diagnosed with a neuropsychiatric disorder such as autism spectrum disorder (ASD). Despite the robust nature of these observations, the underlying mechanisms are largely unknown and the potential contribution of peripheral immune cells little considered. Natural killer (NK) cells are peripheral immune cells that can embody the immune dysregulation so frequently implicated. Peripheral NK cells isolated from individuals with ASD demonstrate a heightened basal activation state and spontaneous degranulation compared to NK cells from neurotypical counterparts. Canonically described as anti-viral and anti-tumor cells, past work from our lab has established their enriched presence in the developing rat brain compared to peripheral blood, a previously undescribed niche. When assessing the structural localization of NK cells in the brain during early life, we find they are most numerous in the developing cerebellum. Leveraging these findings, this project seeks to characterize this peripheral immune cell's niche in the developing brain to better assess their contributions to typical neurodevelopment, and reciprocally, understand how this normal function might become deleterious in the context of early life inflammation. We begin our interrogation by asking if we can manipulate NK cell frequency in the developing brain. Measured by flow cytometry, we found that we can deplete NK cells within the developing brain using an anti-NK1.1 antibody administered directly to the brain in vivo. Complementarily, we found that when stimulating this population using an activating anti-IL15 receptor α subunit antibody, male NK cells increased 2-fold compared to females. We hypothesize that the intrinsic sex difference observed in this population following activation has functional consequences for neuroanatomy that begin in early development and contribute to the sex-bias in neurodevelopmental disorder diagnosis. To assess this question, we have ongoing experiments quantifying neurogenesis in the developing hippocampus and cerebellum following NK cell manipulation. Future studies will describe the chemoattractants responsible for recruitment and determine if they are resident and proliferate upon activation, or if they are recruited from the periphery. These studies will lay the foundation for understanding how NK cell sex differences may contribute or hinder typical neurodevelopment in the cerebellum while also elucidating the crosstalk ongoing between the innate immune and central nervous systems.

Disclosures: A. Allee: None. **A.A. Maximova:** None. **M.M. McCarthy:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.22/A48

Topic: F.04. Neuroimmunology and Neurovirology

Support: NIMH 1F30MH135570-01

Title: Investigating hematopoietic origins of peri-hippocampal mast cells in postnatal neurodevelopment

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

Abstract: Crosstalk between peripheral immune cells and the brain occurs in healthy conditions and during the progression of neurodevelopmental diseases. Identifying the role peripheral immune cells play during critical periods in brain development will lend insight into neuroimmune interactions at baseline and how this communication could go awry in neuropsychiatric diseases. Mast cells (MCs) are innate immune cells capable of releasing histamine, cytokines, and growth factors during host defense, allergy, and tissue remodeling. During neurodevelopment, a small MC population drives synaptic patterning and sexual differentiation of the preoptic area (POA) (Lenz et al., 2018 J. Neurosci), but little is known about other populations of brain MCs during development. We found a robust MC population adjacent to the hippocampus, peaking in cell number around postnatal (PN) day 7 and rapidly declining by PN14, a pattern reminiscent of a critical period. The temporally limited presence of these peri-hippocampal MCs leads to questions about their functional importance. Previous data shows that these MCs are not actively recruited from either blood or bone marrow sources and are proliferating to maintain this population (Blanchard et al., under review). Recent discoveries have shown that MCs possess dual hematopoietic ontogeny, as MCs from different peripheral tissues can be derived from either “definitive” hematopoiesis or earlier extraembryonic yolk sac hematopoiesis. Different ontological origin confers diverse functions to MCs in various tissues. We hypothesize that the developmentally restricted, peri-hippocampal MCs we observe are primarily yolk-sac derived, showcasing a unique hematopoietic signature. We will use the tamoxifen-inducible Cdh5-CreERT2-eYFP mouse line to temporally activate Cre in hemogenic endothelium, the earliest cell type to produce hematopoietic stem cells. Tamoxifen injection at either embryonic day 7.5 (E7.5) or E10.5 will label cells exclusively originating from either yolk sac or definitive hematopoiesis respectively. We predict that peri-hippocampal MCs will be primarily yolk-sac derived during their limited residence near the hippocampus in postnatal development, similarly to the primary myeloid cells of the brain, microglia, which are solely yolk-sac derived. Comparisons include MCs from the POA as well as various peripheral tissues like skin, tongue, and peritoneum to verify yolk sac vs. definitive hematopoietic fate.

Understanding the hematopoietic origins of brain-resident, peri-hippocampal MCs will help unravel the functional significance of these peripheral immune cells in a neurodevelopmental niche.

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

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.23/A49

Topic: F.04. Neuroimmunology and Neurovirology

Support: 2R01MH052716-26 to MMM

Title: Peri-hippocampal mast cells in neonatal post-mortem human tissue.

Authors: *K. ENGEL[1], A. F. CIESINSKI[2], M. M. MCCARTHY[3];
[1]Univ. of Maryland, Baltimore, Baltimore, MD; [2]Wake Forest Univ., Baltimore, MD, ; [3]Dept. of Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Our lab has been focusing on mast cells (MCs), innate immune cells mostly known for their involvement in allergic responses. We are concentrating on a large population transiently clustered in the peri-hippocampal region lining the choroid fissure during the first two weeks of life in rodents, peaking in numbers by post-natal day 7 (PN7), and absent by PN20. We have found that the MCs undergo “piecemeal” degranulation during this period, potentially releasing growth factors that are necessary for neurodevelopment. In adult human post-mortem tissue, MCs line cortical meninges and thalamic blood vessels, but the developmental peri-hippocampal population has, to our knowledge, not been characterized in newborn human brain. To fill this gap, we hypothesize that this transient MC population exists in the choroid fissure adjacent to the hippocampus in newborn human brain and undergoes “piecemeal” degranulation, similarly to the rodent population. To test this hypothesis, we’ve obtained post-mortem human hippocampal tissue from individuals ranging from 8 to 300 days of age from the Neuro BioBank at the University of Maryland School of Medicine. Samples have been paraffin embedded, cut at 20um on a cryostat, and stained with MC specific stains carboxypeptidase 3 (CPA3) and Avidin, in addition to Human Nuclear Antibody (HNA) to confirm the CPA3/Avidin signal is nucleus positive. In addition to counting the number of MCs per donor, we will be measuring MC degranulation by applying our 1-5 degranulation scale where fully granular MCs are a 1 and completely degranulated MCs/ghost cells are a 5. We have observed substantial co-staining of CPA3, Avidin, and HNA in the choroid fissure surrounding the hippocampus of human donor tissue and expect to see a similar variety of MC degranulation states, but fewer MCs compared to the rodent model. Our goal is to determine if the peri-hippocampal mast cell population

transiently present in the rodent brain is similarly evident in the neonatal human brain. If so, this would suggest a conserved function across mammalian development and will be informative into the role of these innate immune cells in both health and disease.

Disclosures: K. Engel: None. **A.F. Ciesinski:** None. **M.M. McCarthy:** None.

Poster

PSTR259: Neural Circuit Development

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR259.24/A50

Topic: F.04. Neuroimmunology and Neurovirology

Support: NIMH Grant 5R01MH052716-27

Title: Exploring the potential of mast cell-induced B-cell class switching to regulate CNS immunoglobulin diversity during early postnatal brain development

Authors: *M. R. BRUCE[1], A. A. MAXIMOVA[2], M. M. MCCARTHY[3];
[1]Pharmacol., Univ. of Maryland, Sch. of Med., Baltimore, MD; [2]U of Maryland Baltimore, Baltimore, MD; [3]Dept. of Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Immune cells and associated secreted factors play a poorly understood role in central nervous system (CNS) development. For example, both immunoglobulin M (IgM) and IgG can directly enhance glial maturation in the brain (PMIDs: 37296298 & 29507409). However, a mechanistic understanding of Ig diversification during early development remains incomplete. Mast cells are competent to induce B-cell class-switching in both human and murine model systems, but this concept has yet to be explored in the brain. Interestingly, a historically under-studied population of mast cells exists within the CNS in the peri-hippocampal region during the first two weeks of postnatal life in rodents. Appearance of this distinct mast cell population coincides with a decrease in IgM+ B-cells in the brain. This inverse relationship suggests either death/efflux of B-cells from the CNS or, alternatively, induction of B-cell class-switching. Preliminary data support the latter hypothesis, with the finding that levels of IgG in the cerebrospinal fluid (CSF) fluctuate across the early postnatal period in rats, exhibiting distinct developmental peaks. Therefore, we seek to investigate whether peri-hippocampal mast cells (phMCs) exert effects on local B-cell Ig-receptor diversity. To examine this, we will use flow cytometry to characterize the surface Ig expression (IgD/IgM/IgG/IgE/IgA) of rat brain B-cells during the early postnatal period. Analysis of the presence/absence of various Ig isotypes will determine the extent of B-cell class-switching in the brain. Additionally, we will evaluate whether phMC activation may affect B-cell Ig expression profiles using intracerebroventricular injection of the mast cell degranulating compound 48/80 and subsequent regional flow cytometric analysis. Finally, we will examine the levels of secreted immunoglobulin in

cerebrospinal fluid (CSF) as well as from regional tissue lysates across experimental conditions using ELISA-based methods. These data will provide insight into the extent of B-cell Ig receptor diversity in the neonatal brain, as well as a potential role for phMCs in regulating this process, the results of which may reveal novel mechanisms involving the role of immunoglobulin in CNS development.

Disclosures: M.R. Bruce: None. **A.A. Maximova:** None. **M.M. McCarthy:** None.

Poster

PSTR260: Neurodevelopmental Disorders I

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR260.01/A51

Topic: A.07. Developmental Disorders

Title: Quantitative analysis of an in vitro Rett syndrome model based on MeCP2 knockdown in iPSC derived neurons.

Authors: *T. HAZAMA[1], M. NAKAO[2], R. YAMOTO[3], T. HOSOYA[4];
[1]Ricoh Co., Ltd., Kawasaki-shi, Japan; [2]Ricoh Co. Ltd., Kawasaki, Japan; [3]Ricoh Co. Ltd.,, Kawasaki, Japan; [4]Biomed. Reseach Dept., Ricoh Co. Ltd., Kawasaki, Japan

Abstract: Rett Syndrome (RTT) is a progressive and pervasive X-linked neurodevelopmental disorder that predominantly affects girls by the early childhood. The detailed disease mechanisms remain unclear and fundamental treatments are yet to be established. The vast majority of RTT cases are triggered by sporadic mutations in the methyl CpG-binding protein 2 (MeCP2) gene. Among many different symptoms, MeCP2 mutations affect multiple stages of the brain development. For the analysis of the disease mechanisms and drug development, in vitro models based on iPSC-derived cells carrying diseased MeCP2 are being investigated. We have developed an in vitro RTT model using shRNA knockdown of MeCP2. iPSC-derived neurons and human primary astrocytes were co-cultured and transduced with lentiviral vectors encoding MeCP2 shRNA. We also developed a method to quantitatively analyze the neurite structure using unstained bright-field images. The results showed that the neurons exhibited neurite atrophy defined as the reduction in the complexity of neural arborization, which is a hallmark of RTT. Furthermore, brain-derived neurotrophic factor (BDNF), an enhancer of neurite arborization in RTT, increased the neurite density in the MeCP2 knockdown co-cultures. Therefore, MeCp2 knockdown induces neurite atrophy that is rescued by a drug for RTT, suggesting that the RTT model exhibits a disease-related neurite phenotype. Detailed analyses further suggested the model also exhibited other cellular phenotypes of the RTT. Therefore, the model system provides a unique opportunity for the understanding of the disease mechanisms and drug discovery.

Disclosures: T. Hazama: None. **M. Nakao:** None. **R. Yamoto:** None. **T. Hosoya:** None.

Poster

PSTR260: Neurodevelopmental Disorders I

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR260.02/A52

Topic: A.07. Developmental Disorders

Support: The JPB Foundation - Picower Postodoctoral Fellowship (G.F.)
NATIONAL INSTITUTE OF MENTAL HEALTH / NRSA T32MH112510 (R.M.L)
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NIH - R01NS130361 (M.S.)
NIH - R01MH133066 (M.S.)
Simons Foundation Autism Research Initiative (M.S.)

Title: The role of altered neuromodulation in motor dysfunction in Rett Syndrome

Authors: *G. FERNANDES[1], H. SUGIHARA[1], R. M. LAM[2], Y. OSAKO[1], M. SUR[2]; [1]Picower Inst. for Learning and Memory, MIT, Cambridge, MA; [2]Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Rett Syndrome is a severe neurodevelopmental disorder caused by loss-of-function mutations in the Methyl-CpG-binding protein-2 (MeCP2) gene. One of the most devastating symptoms of Rett Syndrome is the disruption of motor function. Patients lose purposeful use of their hands and develop repetitive movements, rigidity, and dystonia. The primary motor cortex, crucial for voluntary movement and motor learning, is modulated by the norepinephrine system, through projections from the Locus Coeruleus (LC). Phasic LC activity increases before movement execution and following reinforcement to promote motor learning via adaptive circuit gain. MeCP2 loss reduces global norepinephrine release, yet the impact on phasic LC activity and its relation to motor learning and function remains unexplored. To assess the impact of MeCP2 deficiency in the LC on motor function, we employed a go/no-go motor task. In this task, mice must swiftly decide to either execute or withhold a lever press based on the delivered cue tone, to obtain a reward or avoid a punishment. The precise spatiotemporal activity of LC projections to distinct targets including the motor cortex is known to facilitate movement execution and encode a reinforcement signal to facilitate accuracy of behavioral performance in this task. Mice with LC-specific loss of MeCP2 (LC-MeCP2) learned to execute the lever press

in response to the go tone but failed to integrate the negative reinforcement signal and distinguish between the go and no-go tone. In addition, wildtype mice developed a stereotypical trajectory of their motor movements (i.e. lever presses) across the training period. While the trial-to-trial correlation of lever presses in WT mice increased with learning, that of LC-MeCP2 mice did not. This indicates a role for LC-MeCP2 in both the accuracy of behavioral performance as well as the execution of goal-driven, reproducible motor movements. We performed *in vivo*, 2-photon calcium imaging of motor cortical neurons during learning of the motor task. Learning of goal-driven motor behaviors is known to correlate with synaptic plasticity and the emergence of reproducible, spatiotemporal neuronal activity in the motor cortex. Hence, we predict that loss of MeCP2 in the LC will lead to aberrant modulation of plasticity and disrupt correlated neuronal activity in the motor cortex. Together with measurements of phasic norepinephrine release, we expect these findings to elucidate a role for the norepinephrine system in the development of motor control and extend our understanding of the neuromodulatory systems that underlie motor dysfunction in Rett Syndrome.

Disclosures: G. Fernandes: None. **H. Sugihara:** None. **R.M. lam:** None. **Y. Osako:** None. **M. Sur:** None.

Poster

PSTR260: Neurodevelopmental Disorders I

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR260.03/A53

Topic: A.07. Developmental Disorders

Support: Ramon y Cajal 2022
MSCA-IF-2020

Title: Deciphering alterations in cerebellar parvalbumin-neurons in Rett Syndrome

Authors: Z. HUSSON[1], C. JUANES[2,3], A. QUINTANA[2,3], E. VALJENT[1], ***L. CUTANDO RUIZ**[2,3];
[1]IGF, Univ. Montpellier, CNRS, Inserm, Montpellier, France; [2]Neurosci. Inst. and Dept. of Cell Biol., Physiol. and Immunology. Univ. Autòno, Bellaterra (Barcelona), Spain; [3]Universitat Autònoma de Barcelona (UAB), Bellaterra (Barcelona), Spain

Abstract: Mutations in the X-linked gene encoding methyl-CpG binding protein 2 (Mecp2) can lead to the progressive neurodevelopmental disorder known as Rett syndrome, characterized by regression in motor, social, and cognitive skills. The cerebellum, a brain region with high energy demands, particularly in the molecular layer where fast-spiking Parvalbumin (PV)-neurons are located, may be vulnerable to energy impairments. Mitochondria, responsible for regulating cellular energy production, exhibit abnormal morphology in cerebellar biopsies from Rett

syndrome patients, though the specific cellular mechanisms underlying these changes remain unclear. To investigate it, we analyzed the molecular identity of Mecp2-containing cells in the cerebellum of wild-type mice using the Pvalb-Cre;RiboTag mouse line. Cell type-specific transcriptomic analysis revealed predominant Mecp2 expression in PV-neurons. Immunoprecipitation of cerebellar extracts confirmed Mecp2 mRNA enrichment in Purkinje cells and molecular layer interneurons, evidenced by increased expression of GABAergic and Purkinje cells-related transcripts (Pcp2, Gad1, Slc32a1, Pvalb) and by a reduction in glutamatergic and glial markers. Histological analysis in control and Mecp2-null mice showed decreased Purkinje cells somata area and reduced PV expression in Rett syndrome cerebellums, indicative of Purkinje cell atrophy due to Mecp2 deficiency. To further assess whether cerebellar PV-neurons are particularly vulnerable in Rett Syndrome mice, a transcriptomic analysis of this neuronal population was performed in control and Rett Syndrome mice. The analysis of differentially expressed genes (DGE) and the over-representation analysis (ORA) conducted with the RNA-sequencing data, revealed changes in genes encoding for mitochondrial complex proteins and genes related to mitochondrial membrane organization, suggesting impaired mitochondrial functionality in the cerebellar PV-neurons of Rett syndrome mice. Ongoing proteomic and functional studies performed in mitochondrial immunoprecipitates from Pvalb-Cre;MITO-Tag;Mecp2 mice will validate these findings and provide novel insights into the mitochondrial dysfunctionality in Rett Syndrome cerebellum. Additionally, significant changes in transcripts encoding GABAergic signaling components were observed in the cerebellum of Rett Syndrome mice, suggesting impaired GABAergic transmission in PV-neurons. Forthcoming experiments would be focused on targeting these proteins to ameliorate the motor and social symptoms present in Rett syndrome.

Disclosures: Z. Husson: None. **C. Juanes:** None. **A. Quintana:** None. **E. Valjent:** None. **L. Cutando Ruiz:** None.

Poster

PSTR260: Neurodevelopmental Disorders I

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR260.04/A54

Topic: A.07. Developmental Disorders

Support: Innosuisse Grant 101.366 IP-LS

Title: Exploring the pathophysiology of Rett Syndrome: insights from in vitro models an electrophysiological analysis

Authors: *M. PASCUAL-GARCIA[1], C. NUNES[1], M. FRANCISCO[1], A. CHERNOV[1], E. HARDE[2], A. HIERLEMANN[1], M. SCHRÖTER[1];
[1]ETH Zurich, Basel, Switzerland; [2]F. Hoffmann-La Roche, Basel, Switzerland

Abstract: Rett syndrome (RTS) is a rare neurodevelopmental disorder occurring in one of 10000 girls. RTS patients primarily show alterations in psychomotor performance, cognitive abilities, and comorbidities, such as epilepsy. The majority of RTS cases are caused by sporadic loss-of-function mutations in the Methyl-CpG-binding Protein 2 (MeCP2) gene on the X chromosome, which is critical for normal neuronal development and function. As a consequence of the aberrant expression, there is an imbalance in the excitation/inhibition (E/I) ratio in RTS patients. Potentially contributing to this imbalance is a delay in the hyperpolarization shift of the chloride gradient in GABAergic interneurons. However, the contribution of GABA shifts to the E/I imbalance in RTS patients is still unclear. In this study, we characterize the developmental trajectory of neuronal networks from RTS patient-derived iPSCs (isogenic pair) and an engineered MeCP2 knockout line in vitro and thoroughly investigate their electrophysiological phenotypes. We employ high-throughput high-density microelectrode array (HD-MEA) measurements, patch clamp recordings, and immunohistochemical analyses to assess neuronal functionality and differentiation. Preliminary results suggest that the loss of the MeCP2 protein leads to deficiencies in neuronal maturation and differentiation, accompanied by abnormalities of GABAergic cells. Developmental tracking of neuronal networks indicates subtle differences in the electrophysiological phenotype. Furthermore, network activity changes seem to be highly correlated with the presence of astrocytes, highlighting their significance in this neurodevelopmental disorder. Our results confirm impaired functionality of MeCP2-deficient neurons, indicative of a deficit in their E/I ratio.

Disclosures: M. Pascual-Garcia: None. **C. Nunes:** None. **M. Francisco:** None. **A. Chernov:** None. **E. Harde:** None. **A. Hierlemann:** None. **M. Schröter:** None.

Poster

PSTR260: Neurodevelopmental Disorders I

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR260.05/A55

Topic: A.07. Developmental Disorders

Title: In vitro neuronal phenotyping using live imaging in genetic epilepsies and neurodevelopmental disorders

Authors: *H. NAMI, J. ROBERTS, R. CONRAD, E. SHARON, A. KAYKAS, J. SERRATS, R. BAROR;
insitro, South San Francisco, CA

Abstract: Microscopy, and imaging in particular is a widely used tool to identify disease phenotypes across various cellular disease models. The most common type of imaging, immunofluorescence, requires the fixation of the cells and the addition of antibodies to identify relevant targets. Fixed staining, though useful, has a longer turnaround time, requires the

validation of antibody specificity, and is a terminal assay. In contrast, phase contrast live imaging can be used continuously without causing any harm to cultured cells, enabling the assessment of specific phenotypes over time. For this study, we chose to use these tools to gain further insight into genetic epilepsies and neurodevelopmental disorder disease models in iPSC derived cortical neurons. Using digital phase contrast imaging (DPC), we imaged cells over multiple time points to quantify the progression of cell lines with different genetic backgrounds in an arrayed format. Using continuously label-free DPC imaging, we identified a significant fitness effect for MECP2-KO lines when compared to WT or other disease lines, as MECP2-KO showed increased proliferation when compared to other lines. We validated these findings using a Cell Titer Glo biochemical assay to validate ATP abundance. We further hypothesized that this increase in proliferation is due to inhibited neuronal differentiation of these cells. We validated this by measuring neural-progenitor marker expression in these lines using imaging. In summary, we show here an example for phenotypic discovery which is solely based on label free DPC live imaging. We further intend to continue developing our abilities to extract more complex phenotypes using this imaging method.

Disclosures: H. Nami: A. Employment/Salary (full or part-time):; insitro. **J. Roberts:** A. Employment/Salary (full or part-time):; insitro. **R. Conrad:** A. Employment/Salary (full or part-time):; insitro. **E. Sharon:** A. Employment/Salary (full or part-time):; insitro. **A. Kaykas:** A. Employment/Salary (full or part-time):; insitro. **J. Serrats:** A. Employment/Salary (full or part-time):; insitro. **R. Baror:** A. Employment/Salary (full or part-time):; insitro.

Poster

PSTR260: Neurodevelopmental Disorders I

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR260.06/A56

Topic: A.07. Developmental Disorders

Support: NIH Grant NS121542
NIH Grant NS108508
NIH Grant NS120315

Title: Impaired motor learning in Rett syndrome mice with Mecp2 deletion in Purkinje cells of the cerebellum

Authors: P. SHEN[1], M. JACKSON[1], L. POLEPALLI[1], T. LI[1], A. HAMKI[1], K. DHEERAVATH[1], C. LI[1], *W. LI[2];
[1]Neurobio., The Univ. of Alabama at Birmingham, Birmingham, AL; [2]Univ. of Alabama at Birmingham Dept. of Neurobio., Birmingham, AL

Abstract: Rett syndrome, also known as RTT, is a severe case of autism spectrum disorder that is caused by mutations in the X-linked *MECP2* gene. The outcome of this genetic abnormality carries many neurological impairments with no current cure. Various clinical manifestations occur with this syndrome such as seizures, motor disability, weakness of the musculoskeletal system, communication deficit, and respiratory abnormalities. Among these symptoms, motor dysfunction is the most profound, as manifested by stereotypic hand movement, loss of acquired motor skills, and gait abnormalities. The mouse model of RTT, with the constitutive deletion of *Mecp2*, exhibits similar motor dysfunction. It is well-known that the cerebellum plays a crucial role in motor coordination and learning. Here, to study the role of MeCP2 in the cerebellar-related motor function, we deleted the *Mecp2* gene specifically from the major cerebellar neuronal type, Purkinje cells (PCs). We characterized aspects of motor impairment in this model following examination of several motor learning paradigms including an accelerating rotarod test, trace and delay classical eyeblink conditioning, and a skilled pellet reaching task. Furthermore, to dissect the underlying cellular mechanisms, we performed Ca^{2+} imaging, *ex vivo* slice electrophysiology, and *in vivo* single unit recordings to assess PC intrinsic properties and synaptic transmission and plasticity at afferent input to PCs from parallel fibers and climbing fibers. To complement this study, we also evaluated expression of presynaptic and postsynaptic proteins and examined PC dendritic arborization and spine morphology. Our data show that *Mecp2* deletion in PCs results in impaired synaptic function and morphology, providing a mechanistic explanation for the deficits of motor learning. Altogether, this study clearly reveals the significance of impaired cerebellar function in RTT, which contributes to motor aspects of this brain dysfunction.

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Poster

PSTR260: Neurodevelopmental Disorders I

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR260.07/A57

Topic: A.07. Developmental Disorders

Support: NIH Grant NS134246
IRSF Grant 3901
NIH Grant NS031373

Title: Mecp2 deficiency enhances the cellular stress response in Rett syndrome mouse models

Authors: *S. GONZALEZ[1], C. HOLT[1], J. CIKOWSKI[1], M. SMITH[2], G. DODIS[1], A. M. VANDERPLOW[2], Y. RHEE[1], C. M. NISWENDER[3], R. G. GOGLIOTTI[1];

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Abstract: Rett syndrome (RTT) is a neurodevelopmental disorder that is associated with loss-of-function mutations in the *Methyl CPG Binding Protein 2* (*MeCP2*) gene. MeCP2 is a transcription factor that regulates thousands of genes, creating a challenge to distinguish between those that are transcriptional noise and those that are pathogenic and might be targeted therapeutically. RTT is initially diagnosed based on symptoms and then confirmed by mutations in the *MeCP2* gene (typical RTT); however, ~5% of RTT patients are found to be *MeCP2*-mutation-negative (atypical RTT). To reduce transcriptional noise, we identified autopsy samples from five atypical patients and conducted differential RNA sequencing relative to samples from six typical RTT (R255X) and nine matched neurotypical controls. These experiments revealed that pathways associated with Heat-Shock (HS) signaling are dramatically elevated in both populations at baseline, and we have now confirmed this finding in 40 temporal cortex RTT autopsy samples and *Mecp2*$^{+/-}$ mice. To investigate whether increased HS signaling is compensatory or pathogenic, we conducted *in vivo* hyperthermia experiments complemented with cellular stress array analyses. Preliminary data points to altered kinetics and magnitude of induction of cellular stress genes at early time points. This suggests promiscuous HS signaling is pathogenic and amplifies the cellular stress response in the absence of MeCP2. Pharmacological data aligns with the hyperthermia experiments, as induction of the HS-response evokes epileptiform activity in mouse models of RTT. We will present our up-to-date findings on the role of altered HS signaling in RTT.

Disclosures: S. Gonzalez: None. **J. Cikowski:** None.

Poster

PSTR260: Neurodevelopmental Disorders I

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR260.08/A58

Topic: A.07. Developmental Disorders

Support: R01NS112171
IRSF3503

Title: M_1 receptor potentiation rescues phenotypes and neuronal activity in Rett syndrome

Authors: *M. SMITH[1], J. CIKOWSKI[1], G. DODIS[1], C. HOLT[1], A. M. VANDERPLOW[1], S. GONZALEZ[1], Y. RHEE[1], C. M. NISWENDER[2], R. G. GOGLIOTTI[1];
[1]Mol. Pharmacol. and Neurosci., Loyola Univ. Chicago, Maywood, IL; [2]Pharmacol. and Vanderbilt Ctr. for Neurosci. Drug Discovery, Vanderbilt Univ., Nashville, TN

Abstract: Rett syndrome (RTT) is a neurodevelopmental disorder that results from loss of function mutations in the methyl-reader protein known as *Methyl CpG Binding Protein 2* (*MeCP2*). Previously, we established that RTT human autopsy samples have decreased levels of the M_1 receptor and found that acute treatment with an M_1 positive allosteric modulator (PAM, VU0453595) improves social, cognitive, and respiratory phenotypes in a *Mecp2*$^{+/-}$ mouse model. RNA sequencing and Western blot data from *Mecp2*$^{+/-}$ mice treated with VU0453595 suggest that efficacy on respiratory phenotypes may be linked to the assembly and presentation of NMDARs in the brainstem and levels of Gsk3β inhibition. Whole-brain, light-sheet imaging shows that M1 PAM efficacy is linked to normalization of hyperactivity in regions that mediate respiration. A positive correlation was also seen between the degree of apnea reduction induced by VU0453595 and increased inhibitory drive onto hyperactive respiratory nuclei in the medulla. These results further support M_1 as a target for RTT therapeutics. However, *MeCP2* mutations are heterogeneous in RTT patients, and each results in unique changes in gene expression patterns, including M_1 receptor levels. This raises the question of whether target disruption is required for the efficacy of VU0453595. We have now established that mouse models of RTT also have distinct M_1 expression patterns, and that reduced M_1 levels is likely a prerequisite for compound efficacy. These results advocate for personalized medicine approaches for M_1 PAMs as well as other potential RTT targets. Funding: This work is supported by R01NS112171 and IRSF 3503

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Poster

PSTR260: Neurodevelopmental Disorders I

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR260.09/A59

Topic: A.07. Developmental Disorders

Support: International Rett Syndrome Foundation
International Research Center for Neurointelligence
Conte Center at Harvard
Rett Syndrome Angels

Title: Preclinical rescue of Mecp2-deficient cortical circuits by curbing choroid plexus Otx2

Authors: *X. M. VALENCIA[1], A. PATRIZI[2], M. FAGIOLINI[1,3], T. K. HENSCH[4,1,3];
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Abstract: Parvalbumin-positive (PV+) fast-spiking basket cell maturation controls critical periods of brain development and is typically altered across many neurodevelopmental disorders. PV circuits are hyper-connected in Mecp2 knockout (KO) males and heterozygous (HET) female mouse models of Rett Syndrome (RTT). Here, we first confirmed an intensified PV+ 'gate' throughout the brain of Mecp2 mutants and in the post mortem cortex from Rett patients. We then identified potential factors underlying such PV+ hyper-maturation, focusing on the Otx2 homeoprotein, a non-cell autonomous regulator of PV+ cell maturation. Notably, the choroid plexus (ChP) is a major source of Otx2 in the postnatal brain. We found a striking increase in Otx2 production in the ChP of Mecp2 mutants and in the cerebrospinal fluid from Rett patients. We tested whether selective down regulation of Otx2 in the ChP would prevent or delay the onset of RTT phenotypes. Both genetic and viral reduction of Otx2 protein from birth doubled the lifespan, improved physical appearance, motor performance and rescued cortical organization in Mecp2 KO mice. For greater clinical relevance, we then evaluated whether manipulation of Otx2 could also be effective during the active regression phase of the disorder. Mecp2 male and female mutant mice and wild-type control animals were injected at P30-35 or P56-70, respectively. We found that male lifespan increased, accompanied by a significant amelioration of physical appearance, motor performance and overall RTT phenotype. Females additionally improved on key RTT phenotypes, such as hindlimb clasping, gait and spontaneous movement. Our results establish the ChP as an accessible and effective therapeutic target for Rett Syndrome downstream of Mecp2 deficiency.

Disclosures: X.M. Valencia: None. **A. Patrizi:** None. **M. Fagiolini:** None. **T.K. Hensch:** None.

Poster

PSTR260: Neurodevelopmental Disorders I

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR260.10/A60

Topic: A.07. Developmental Disorders

Support: NIH Grant HD111864

Title: Advancing Therapeutic Strategies for Rett Syndrome and MeCP2-Related Disorders: Harnessing the Potential of Antisense Oligonucleotides

Authors: *A. M. VANDERPLOW[1,2], G. E. DODIS[1,2], Y. RHEE[1,2], J. J. CIKOWSKI[1,2], R. G. GOGLIOTTI[1,2];
[1]Mol. Pharmacol. and Neurosci., Loyola Univ. Chicago, Chicago, IL; [2]Stritch School of Medicine, Loyola University Chicago, Chicago, IL

Abstract: Rett syndrome (RTT) is a neurodevelopmental disorder that is caused by loss-of-function mutations in the *Methyl CpG Binding Protein 2* (*MeCP2*) gene. Seminal studies have

showcased the potential reversal of RTT in mouse models through MeCP2 level restoration; however, the optimal mechanism to achieve this clinically is unclear. While initially promising, gene therapy for RTT faces challenges due to MeCP2's dose sensitivity, whereby even a slight increase can lead to adverse effects. This narrow therapeutic window presents a significant challenge, as MeCP2 must be delivered efficiently throughout the entire human brain while ensuring each cell receives a precise and minimal amount of the protein.
The 3' untranslated region (3'UTR) plays a critical role in gene regulation, with microRNAs (miRNAs) binding to mRNA to fine-tune expression. Given that each miRNA's contribution is modest, blocking miRNA binding emerges as a potential therapeutic strategy for diseases like RTT, where the therapeutic window is narrow. Research has shown that miRNAs play a repressive role in MeCP2 expression at multiple sites, and overexpression of these miRNAs has been demonstrated to reverse symptoms associated with excess MeCP2.
To exploit their endogenous regulatory functions, we have developed an approach to de-repress MeCP2 expression using a series of site-blocking antisense oligonucleotides (sbASOs) designed to outcompete miRNAs for binding to the *MeCP2* 3'UTR. We anticipate that this approach will have efficacy in patients with missense or late-truncating mutations, where decreased function can be overcome by increased abundance. Our results demonstrate that sbASOs can incrementally elevate MeCP2 levels in a dose-dependent manner, plateauing at subtoxic thresholds in SH-SY5Y cells, RTT patient fibroblast, and neuronal stem cell lines. Additionally, quantifying downstream effector proteins VGF and BDNF, which are typically reduced in RTT syndrome, suggests that the increase in mutant MeCP2 protein leads to functional improvement. Preliminary *in vivo* experiments have shown efficacy in the brains of wild-type mice, both with regard to increased MeCP2 protein and anxiety-like phenotypes. Future studies will evaluate this approach in RTT mouse models, aiming to increase mutant MeCP2 expression and improve symptoms. These findings underscore the potential of sbASO-based therapies in treating MeCP2-related disorders and highlight the significance of precision medicine in addressing complex neurodevelopmental conditions.

Disclosures: A.M. Vanderplow: None. **G.E. Dodis:** None. **Y. Rhee:** None. **J.J. Cikowski:** None. **R.G. Gogliotti:** None.

Poster

PSTR260: Neurodevelopmental Disorders I

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR260.11/A61

Topic: A.07. Developmental Disorders

Title: Natural History of a female mouse model of Rett Syndrome

Authors: *D. GARCIA AROCENA[1], L. P. BOGDANIK[2], C. M. LUTZ[3];
[1]The Jackson Lab., Sacramento, CA; [2]Preclinical Services, The Jackson Lab., Bar Harbor, ME; [3]Rare Dis. Translational Ctr., The Jackson Lab., Bar Harbor, ME

Abstract: Rett syndrome (RTT) is a neuropsychiatric disorder predominantly caused by mutations in the X-linked gene methyl CpG-binding protein 2 (MECP2). In patients, it causes symptoms including anxiety, tremors, uncoordinated movements, respiratory dysrhythmias and breathing difficulties, and seizures. Males with mutations of their single copy of the gene suffer neonatal encephalopathy and die in infancy, and most surviving patients with RTT are females that are heterozygous for *MECP2* mutations. In these females, random X-chromosome inactivation leads to mosaic wild type MECP2 expression and consequently a syndromic phenotype. Most previous studies in mouse models of RTT were conducted in Mecp2-null male mice, because they exhibit earlier and more severe phenotypes in many assays, avoiding the confounding influence of X chromosome inactivation. Given that RTT primarily affects females, heterozygous Mecp2-KO female mice represent a more translationally-relevant model of RTT than Mecp2-null male mice. Here we characterize the natural history of females heterozygous for the Mecp2-KO mutation. After several months, heterozygous female mice showed behavioral symptoms including abnormal Bird score, increased frequency of apnea, abnormal gait, and decreased electroconvulsive threshold. In conclusion, heterozygous female Mecp2-KO mice, in addition to their hemizygous male counterparts, are a robust platform for the in vivo evaluation of potential therapeutics for various forms of Rett Syndrome.

Disclosures: D. Garcia Arocena: A. Employment/Salary (full or part-time):; The Jackson Laboratory. **L.P. Bogdanik:** A. Employment/Salary (full or part-time):; The Jackson Laboratory. **C.M. Lutz:** A. Employment/Salary (full or part-time):; The Jackson Laboratory.

Poster

PSTR260: Neurodevelopmental Disorders I

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR260.12/A62

Topic: A.07. Developmental Disorders

Support: 101.366 IP-LS

Title: Human-derived RETT syndrome electrophysiology and molecular phenotypic screening platform for the development of new therapies

Authors: *C. NUNES[1], M. PASCUAL-GARCIA[2], A. CHERNOV[2], M. FRANCISCO[2], E. HARDE[3], A. HIERLEMANN[2], M. SCHRÖTER[2];
[1]ETHZ-BSSE, Basel, Switzerland; [2]BSSE, ETH Zurich, Basel, Switzerland; [3]F. Hoffmann-La Roche, Basel, Switzerland

Abstract: Rett syndrome (RETT) is a rare neurodevelopmental disorder with a prevalence of 1 in 10000-15000 individuals and affects mainly females. It is linked to sporadic mutations in the Methyl-CpG-binding Protein 2 (MeCP2) gene, which is essential for normal neuronal development and maintenance of neuronal function. RETT is characterized by an early period of apparently normal development, which is then followed by a sudden loss of acquired psychomotor skills. As of today, there is no cure for RETT and available pharmacological treatments primarily aim to manage symptoms. Therapeutic approaches, currently under development, aim to target the MeCP2 expression levels or their downstream pathways. To better understand how gene expression alterations in MeCP2, as well as MeCP2 protein levels, relate to neuronal dysfunction, and eventually the observed range of neurological symptoms in RETT patients, we set out to develop an integrated multimodal phenotypic screening approach. Here, we present preliminary data, obtained from 2D and 3D human stem cell-derived control/MeCP2-mutant neural cultures, by using a combination of functional and molecular assays. Applying high-throughput high-density microelectrode array (HD-MEA) measurements and immunohistochemical analyses, preliminary data indicate that MeCP2-deficient 2D neural cultures show a higher division rate, and later on altered neuronal firing rates. Co-culturing human neurons with rodent astrocytes resulted in more pronounced neuronal activity and allowed for long-term developmental tracking of cultures. Neuronal networks developed more robustly in 3D-spheroid cultures with higher expression of Map2 (microtubule-associated protein 2) in WT-MeCP2 cultures and the spontaneous appearance of astrocytes along differentiation. Further work is aimed at genetically modulating MeCP2 expression on MeCP2-mutant cultures and assessing its effect on neuronal culture development and electrophysiology.

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Poster

PSTR260: Neurodevelopmental Disorders I

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Topic: A.07. Developmental Disorders

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Simons Collaboration on the Global Brain
National Institute of Neurological Disorders & Stroke (R01NS057819)
Howard Hughes Medical Institute

Title: Analyses of behavioral and neuronal responses during decision-making reveal deficits in Rett syndrome mice

Authors: *Y. KI[1], H. Y. ZOGHBI[2], N. LI[3];
[1]Baylor Col. of Med., Houston, TX; [2]Mol. and Human Genet., Baylor Col. of Medicine/HHMI, Houston, TX; [3]Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Rett syndrome (RTT) is a neurodevelopmental disorder characterized by a wide range of symptoms, with severe apraxia being a notable feature. Apraxia is the inability to perform motor planning and is often associated with basal ganglia dysfunction. However, our knowledge of the circuit alterations in the basal ganglia and how they relate to the behavioral symptoms in RTT is limited. Here we used a novel approach to analyze circuit malfunction underlying behavior in a mouse model of RTT that carries a *methyl-CpG-binding protein 2* (*Mecp2*)-null allele (RTT mice).

In an automated home-cage system (Hao et al, eLife, 2021), self-motivated mice engaged in tactile decision-making tasks for several months without human supervision. In the decision-making task, mice discriminated object location using whiskers and reported object location using directional licking. Parallel testing allowed us to assay two dozen mice at the same time. Instead of cross-sectional analysis, this approach longitudinally tracked the onset and progression of behavior deficits in the RTT mice over time relative to littermate wild-type (WT) mice. We discovered that RTT mice were able to learn the decision-making task similarly to WT mice at 12 to 16 weeks of age. Once the mice achieved proficiency in the decision-making task, we conducted additional assessments of their flexible motor planning by reversing the sensorimotor contingency. The sensorimotor contingency reversals allowed us to examine the mice's ability to adapt to new task rules. RTT mice exhibited slower reversal learning compared to WT mice at 16 to 20 weeks of age, which deteriorated with age.

To examine the underlying changes in neural dynamics, we combined this behavioral paradigm with multi-Neuropixels probe recordings across a frontal cortico-basal-ganglia loop required for the tactile decision-making, including anterior lateral motor cortex (ALM), lateral striatum, and ventromedial thalamus. Preliminary analyses revealed reduced preparatory activity across these brain regions in RTT mice.

Our study outlines a platform to assay motor planning deficits in the Rett mouse model and their underlying neural dynamics that could allow future interrogations of the involved brain regions.

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Poster

PSTR260: Neurodevelopmental Disorders I

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Program #/Poster #: PSTR260.14/A64

Topic: A.07. Developmental Disorders

Support: MH-11856304

Title: Neuronal synchrony in the mPFC during social interactions is altered in Mecp2 knockout mice, which form social hierarchies with more submissive individuals

Authors: *C. ACEVEDO-TRIANA[1], L. POZZO-MILLER[2];
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Abstract: The activity of neurons in the mouse medial prefrontal cortex (mPFC) is linked to interactions with others of the same species, and is thought to be responsible for encoding social memories that help establish social hierarchies. Neurodevelopmental disorders often present with atypical social behaviors, and in a mouse model of Rett syndrome, impaired social memory is caused by heightened activity of the monosynaptic projections from the ventral hippocampus to the mPFC. The classical 'tube' test over 6 consecutive days revealed that *Mecp2* knockout (KO) mice formed social ranks, but displayed more submissive behaviors and a low social engagement compared to wildtype (WT) mice. We confirmed this low engagement of *Mecp2* KO mice during social conflict using a novel 'warm spot' test, where the same 3 age-matched mice of each genotype compete to stand on a single warm spot in a cage with a cooled floor. The 'dominant' WT mouse occupied the warm spot far longer than the other 2 mice ('intermediate' and 'submissive'), while the 3 *Mecp2* KO mice equally shared the warm spot regardless of their social rank and showing more submissive behaviors than WT mice. To record neural activity in the mPFC during the 'warm spot' test, we performed a single surgery to implant a GRIN lens previously coated with a mixture of AAVs expressing CaMKII-driven GCaMP8 and silk fibroin in the mPFC of *Mecp2* and WT mice. After 3 weeks, *in-vivo* Ca2+ imaging from pyramidal neurons in the prelimbic mPFC confirmed the presence of socially sensitive neurons, i.e., neurons that increase or decrease activity during social interactions. mPFC pyramidal neurons in *Mecp2* KO mice showed fewer and smaller Ca2+ transients during baseline, as well as during social interactions in the 'warm spot' test. In addition, the activity of socially sensitive neurons in *Mecp2* KO mice seems to be less synchronous than in WT mice during specific epochs of social interactions in the 'warm spot' test. A different cohort of *Mecp2* KO and WT mice were imaged while head-fixed during brief exposures to cagemate urine or almond-impregnated cotton tip swabs, and confirmed the specificity of socially sensitive neurons that responded mainly to the cagemate urine. Together, these results suggest that the low social engagement during social conflicts displayed by male Rett mice is related to an altered synchrony of socially sensitive pyramidal neurons in the mPFC during social interactions.

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Poster

PSTR260: Neurodevelopmental Disorders I

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Topic: A.07. Developmental Disorders

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Believe in a Cure
R01 MH117405

Title: Investigation of cellular and molecular disruptions in the mammalian brain in neurodevelopmental diseases using spatial transcriptomics

Authors: *R. D'SOUZA, T. LAW, M. NEMERA, R. MOORE, J.-V. BOUA, N. HAMAGAMI, J. EDWARDS, H. GABEL;
Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: The mammalian brain consists of an enormous diversity of neuronal and non-neuronal cells, the identities of which can be determined using their distinct transcriptomic profiles. Spatial transcriptomic studies have contributed immensely in uncovering the heterogeneity and spatial distribution of cell populations in distinct regions of the brain, but the use of this technology in investigating molecular and cellular changes that may occur in neurodevelopmental disease remains limited. Using MERFISH (Multiplexed Error-Robust Fluorescent in situ Hybridization), we have investigated changes in transcriptomic identity and cellular composition in mouse models of neurodevelopmental diseases, including models of Rett Syndrome and Foxg1 Syndrome. The expression levels of 490 genes were assessed in cells from distinct cortical areas and hippocampal regions. While the spatial locations of distinct cell populations were largely preserved in the investigated disease models, we found differences in the relative prevalence of cell ‘subclasses’ and ‘types’ in different brain regions. In heterozygous MeCP2 knockout (MeCP2 KO/+) female mice, a model for Rett Syndrome in which wild-type (WT) and MeCP2 KO cells form a mosaic, a significant positive correlation was observed between the level of non-CpG methylation and the upregulation of MeCP2-repressed genes at cellular and subregional levels. We investigated the role of MeCP2 in regulating type-specific gene expression programs in excitatory neurons residing in sublayers of layer (L) 2/3 of primary visual cortex (V1). In superficial L2/3, we find that MeCP2 maintains the suppression of genes normally preferentially expressed in the deep sublayer of L2/3. In MeCP2 KO excitatory neurons in superficial L2/3, we observed a reduction in the expression of genes that are normally preferentially expressed in this sublayer, suggesting a role of MeCP2 in the appropriate expression level of these genes. Together, the data suggests a critical role of MeCP2 in maintaining the fine-scale transcriptomic identity of highly related cell types in V1. Using heterozygous Foxg1 mutant mice, a model for Foxg1 Syndrome, MERFISH allowed for the detection of changes in the relative composition of neuronal and non-neuronal cell types in several cortical areas, particularly for a number of glutamatergic neuron types in L2/3, L4, L5, and L6. Together, the findings demonstrate the utility of spatial transcriptomic technology in investigating cellular and molecular disruptions in neurodevelopmental diseases.

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Poster

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The George Washington University 2018-2019 Cross-Disciplinary Research Fund

Title: Mecp2 deficiency alters the response selectivity of prefrontal cortical neurons to different social stimuli

Authors: *N. BOYLE[1], Y. LI[1], X. SUN[2], P. XU[1,4], C.-H. LAI[1], S. BETTS[1], D. GUO[1], R. SIMHA[2], C. ZENG[3], J. DU[5,6], H. LU[1];
[1]Pharmacol. and Physiol., [2]Computer Sci., [3]Physics, George Washington Univ., Washington, DC; [4]Med. Sci. and Technol. Innovation Ctr., Shandong First Med. Univ. & Shandong Acad. of Med. Sci., Jinan, Shandong, China; [5]Anat. and Neurobio., [6]Neurosci. Inst., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Rett syndrome, a severe neurodevelopmental disorder caused by mutations in the MeCP2-gene, is characterized by cognitive and social deficits. Previous research has identified hypoactivity in the mPFC pyramidal neurons of MeCP2-deficient mice in response to both social and nonsocial stimuli. In order to further explore the neural circuitry behind social deficits in Rett syndrome, we employed the miniaturized in-vivo fluorescence microscope to observe the activity of discrete mPFC neural ensembles in female wild-type and MeCP2-deficient mice during the 3-Chamber test of sociability. Our findings have identified six mPFC neural ensembles that were selectively tuned to different stimuli. Importantly, RTT mice recruited fewer neurons to ensembles responding to social interaction and consistently produced lower stimulus-ON ensemble transient rates. Despite lower transient rates, RTT mice displayed an increase in the percentage of social-ON neurons in later sessions, indicating a potential compensatory mechanism for the decreased firing rate. These results highlight the limited plasticity that remains in the RTT mPFC and provides further insight into the neural circuit dynamics of social encoding.

Disclosures: N. Boyle: None. **Y. Li:** None. **X. Sun:** None. **P. Xu:** None. **C. Lai:** None. **S. Betts:** None. **D. Guo:** None. **R. Simha:** None. **C. Zeng:** None. **J. Du:** None. **H. Lu:** None.

Poster

PSTR260: Neurodevelopmental Disorders I

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR260.17/A67

Topic: A.07. Developmental Disorders

Support: NINDS, NIH, R01NS113140

Title: Dendrimer-conjugated Ketamine as a glia targeted therapy for Rett Syndrome

Authors: *K. LAC[1], P. VYAS[2], W. LIYANAGE[3,4], J. ALLENDE LABASTIDA[5], J. LIU[6], M. P. AVALOS[7], K. M. RANGARAMANUJAM[8], S. KANNAN[6];
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Abstract: Rett Syndrome (RTT) is a neurodevelopmental disorder linked to a mutation of the X-linkedgene, methyl-CpG-binding protein-2 (MeCP2). Although many off label medications are used tomanage the symptoms of RTT, there is currently a lack of therapies that halt diseaseprogression. Recent preclinical and clinical data demonstrated the therapeutic potential ofketamine, an N-methyl-D-aspartate receptor (NMDAR) antagonist, in Rett syndrome. Eventhough ketamine shows significant efficacy in RTT, it is associated with major adverse effects likerespiratory depression, psychomimetic effects and abuse potential. Glial dysfunction has beenimplicated in the pathogenesis and worsening of symptoms in Rett syndrome, but specificallytargeting glial cells with low dose of ketamine in the brain is challenging. For this purpose, wehave used PAMAM hydroxyl dendrimer (HD, ~4 nm, non-toxic) that specifically targetsdysregulated glia in Mecp2-deficient mice but not in the WT mice upon systemic administrationthereby reducing the off-target effects of free ketamine. Here, we first determined if targetedmonotherapy with dendrimer conjugated ketamine (HD-ketamine) improves the survival andneurobehavioral phenotype in Mecp2-null (KO) mice. We observed increased survival with HD-ketamine versus free ketamine-treated or saline-treated Mecp2 KO littermates. Systemicallyinjected HD-ketamine also improved the motor function in Mecp2 KO mice in open field test.We next treated the WT and 4 weeks old pre-symptomatic Mecp2-/+ Het mice

biweekly for 8-weeks with saline or 2.5 mg/Kg IP of free ketamine or HD-ketamine and their neurobehavioralphenotype was examined pre- and post-treatment. The pre-symptomatic het mice treated withHD-ketamine showed delayed symptoms of RTT, improvement in neurobehavioral scores, andexploratory behavior. Our neurobehavioral studies showed improvement in the compositedisease phenotype. Further studies are ongoing to test the behavioral and histopathologicaleffects of HD-ketamine versus free ketamine in Mecp2 Het model. Our mechanistic studies willalso elucidate how the microglial targeting with HD-ketamine impacts the Rett phenotypethrough an increased BDNF and anti-inflammatory (non-NMDAR) pathways in vivo. Overall, ourdata provide preclinical proof of concept for the ability of dendrimer conjugated ketamine toameliorate neurological dysfunction and reverse at least some circuit-level defects caused byloss of MeCP2 in Rett syndrome.

Disclosures: K. Lac: None. **P. Vyas:** A. Employment/Salary (full or part-time):; The Johns Hopkins. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Samata Therapeutics. **W. Liyanage:** A. Employment/Salary (full or part-time):; The Johns Hopkins. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Samata Therapeutics. **J. Allende Labastida:** None. **J. Liu:** None. **M.P. Avalos:** None. **K.M. Rangaramanujam:** A. Employment/Salary (full or part-time):; Johns Hopkins. 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.; Samata Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Samata Therapeutics. **S. Kannan:** A. Employment/Salary (full or part-time):; Johns Hopkins. 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.; Samata Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Samata Therapeutics.

Poster

PSTR260: Neurodevelopmental Disorders I

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR260.18/A68

Topic: A.07. Developmental Disorders

Support: HIH Grant 1R01HD100607-01A1, Office Of The Director, National Institutes Of Health (OD)

Title: Pharmacological Normalization of Enhanced GABAergic Signaling in Neonatal Ts65Dn Mice, a Genetic Model of Down Syndrome

Authors: J. DOAN[1], J. JIN[1], C. FERNANDEZ[1], S. NGUYEN[1], C. SPENCER[1], ***A. KLESCHEVNIKOV**[2];
[1]Univ. of California San Diego, LA JOLLA, CA; [2]Neurosciences, Univ. of California San Diego, LA JOLLA, CA

Abstract: Introduction: Down syndrome (DS) is characterized by developmental delays and alterations in neural circuit formation, particularly affecting the GABAergic system. In Ts65Dn mice, a genetic model of DS, these alterations manifest as enhanced efficiency of GABAergic signaling in the hippocampus. This study characterizes GABAB/Girk2 signaling in the dentate gyrus (DG) of neonatal Ts65Dn mice, assessing protein expression levels, signaling efficiency, and electrophysiological properties. Importantly, the impact of the GABAB receptor antagonist CGP35348 on the inhibitory-to-excitatory (I/E) ratio in the dentate gyrus was then evaluated through pharmacological intervention from postnatal day 2 to 16.
Results: Enhanced GABAB/Girk2 signaling was observed in Ts65Dn mice, correlating with increased Girk2 protein levels and altered electrophysiological properties, including increased efficiency of postsynaptic GABAB receptor signaling and reduced intrinsic excitability. Treatment with CGP35348 effectively normalized the increased I/E ratio in Ts65Dn mice, without affecting normosomic littermates, suggesting a restoration of neural circuit dynamics.
Conclusion: The study highlights the critical role of GABAB/Girk2 signaling in the abnormal neural development associated with DS and demonstrates the potential of early pharmacological intervention to correct these abnormalities. Modulating GABAB/Girk2 signaling during key developmental windows may offer a therapeutic strategy to mitigate cognitive deficits in Down syndrome.

Disclosures: J. Doan: None. **J. jin:** None. **C. Fernandez:** None. **S. Nguyen:** None. **C. Spencer:** None. **A. Kleschevnikov:** None.

Poster

PSTR260: Neurodevelopmental Disorders I

Location: MCP Hall A

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Program #/Poster #: PSTR260.19/A69

Topic: A.07. Developmental Disorders

Support: NIH/NICHD grant R61HD109748
GLOBAL Down Syndrome Foundation

Title: Interim analysis results of an open-label trial for Down Syndrome Regression Disorder

Authors: *R. IDATE[1], A. RACHUBINSKI[1,2], R. KAMMEYER[2,6,3], N. BOYD[7], B. VOGEL[7], R. M. SHROPSHIRE[1], L. NGUYEN[7], L. K. ROAN[1], L. PATEL[1,6], M. GALBRAITH[1,6], E. SANNAR[4,6], J. D. SANTORO[7,8], J. ESPINOSA[1,5];
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Abstract: Down syndrome (DS), caused by a triplication of chromosome 21 (T21), results in intellectual and developmental disability, immune system dysregulation, and an increase in some immune mediated co-occurring conditions. One of these conditions is Down Syndrome Regression Disorder (DSRD), which is a rare but devastating condition in adolescents and young adults with symptoms that can include mutism, catatonia, and the loss of previously acquired daily living skills. This study compares three current therapy options: the benzodiazepine, lorazepam, and the immune modulating treatments of tofacitinib and intravenous immunoglobulin (IVIg). In this phase 2 open-label clinical trial, individuals with a confirmed diagnosis of DSRD ages 8-30 were randomized into one of three arms: 1. lorazepam (2 mg three times daily), 2. Intravenous immunoglobulin (IVIg) (2g/kg initial, then 1g/kg monthly); or 3. tofacitinib (5 mg twice daily). After screening, participants have five study visits over a 12-week treatment period and optional follow-up visit at 16 weeks. Participants underwent assessments to measure neurological health, activities of daily living, catatonia, movement and motor function, and speech at the Baseline and 12-week visits. Sixteen participants were enrolled in the first phase of the study across two sites (lorazepam n=5, IVIg n=5, tofacitinib n=6). A qualitative interim analysis indicates that all treatments were well-tolerated, with most adverse events reported as mild, regardless of the treatment arm. There was a total of 59 adverse events (AEs) possibly and definitely related to the study drug, and all were mild except one related to IVIG and four related to tofacitinib. While not all participants showed a global improvement on their assigned therapy, participants showed improved scores across multiple measurements of neurological health including catatonia (Bush-Francis Catatonia Rating Scale) with a median improvement of 6 points for the lorazepam arm, 12 points for the IVIg arm and 6.5 points in the tofacitinib arm. There were improvements in movement and motor function (Timed 25-foot Walk) with a median improvement of 1.24 seconds for the IVIg arm, and 3.61 seconds in the tofacitinib arm. All three treatments were associated with improvement in general cognition (Neuropsychiatric Inventory and Down Syndrome Mental Status Exam). The interim findings suggest that each of the three treatment arms could be safe interventions for DSRD. The study will continue to enrollment towards a total of 66 participants to investigate differences between therapy arms.

Disclosures: R. Idate: None. **A. Rachubinski:** None. **R. Kammeyer:** None. **B. Vogel:** None. **R.M. Shropshire:** None. **L. Nguyen:** None. **L. Patel:** None. **J.D. Santoro:** None.

Poster

PSTR260: Neurodevelopmental Disorders I

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR260.20/A70

Topic: A.07. Developmental Disorders

Support: NIH Grant R01NS102382
NIH Grant R01NS122108
NIH Grant R01AG073779
NIH Grant R01AG064579

Title: A Trisomy 21-linked Hematopoietic Gene Variant Confers Human Microglia Resilience to Alzheimer's Disease

Authors: *M. JIN[1], Z. MA[1], R. DANG[1], S. FINKBEINER[2], E. HEAD[3], P. JIANG[1];
[1]Rutgers Univ., Piscataway, NJ; [2]Ctr. for Systems and Therapeut., Gladstone Inst., San Francisco, CA; [3]Pathology & Lab. Med., Univ. of California, Irvine, Irvine, CA

Abstract: While challenging, identifying individuals displaying resilience to Alzheimer's disease (AD) and understanding the underlying mechanism holds great promise for the development of new therapeutic interventions to effectively treat AD. Down syndrome (DS), or trisomy 21, is the most common genetic cause of AD. Interestingly, some people with DS, despite developing AD neuropathology, show resilience to cognitive decline. Furthermore, DS individuals are at an increased risk of myeloid leukemia due to somatic mutations in hematopoietic cells. Recent studies indicate that somatic mutations in hematopoietic cells may lead to resilience to neurodegeneration. Microglia, derived from hematopoietic lineages, play a central role in AD etiology. We therefore hypothesize that microglia carrying the somatic mutations associated with DS myeloid leukemia may impart resilience to AD. Using CRISPR-Cas9 gene editing, we introduce a trisomy 21-linked hotspot CSF2RB A455D mutation into human pluripotent stem cell (hPSC) lines derived from both DS and healthy individuals. Employing hPSC-based in vitro microglia culture and in vivo human microglia chimeric mouse brain models, we show that in response to pathological tau, the CSF2RB A455D mutation suppresses microglial type-1 interferon signaling, independent of trisomy 21 genetic background. This mutation reduces neuroinflammation and enhances phagocytic and autophagic functions, thereby ameliorating senescent and dystrophic phenotypes in human microglia. Moreover, the CSF2RB A455D mutation promotes the development of a unique microglia subcluster with tissue repair properties. Importantly, human microglia carrying CSF2RB A455D provide protection to neuronal function, such as neurogenesis and synaptic plasticity in chimeric mouse brains where human microglia largely repopulate the hippocampus. When co-transplanted into the same mouse brains, human microglia with CSF2RB A455D mutation phagocytize and replace human microglia carrying the wildtype CSF2RB gene following pathological tau treatment. Our findings suggest that hPSC-derived CSF2RB A455D microglia could be employed to develop effective microglial replacement therapy for AD and other age-related neurodegenerative diseases, even without the need to deplete endogenous diseased microglia prior to cell transplantation.

Disclosures: M. Jin: None. **Z. Ma:** None. **R. Dang:** None. **S. Finkbeiner:** None. **E. Head:** None. **P. Jiang:** None.

Poster

PSTR260: Neurodevelopmental Disorders I

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR260.21/A71

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Rett Syndrome Research Trust Grant 20190460 (to M.E., M.F.)
NIH Pioneer DP1OD025535 (to V.G.)
Merkin Translational Research Grant (to M.E., V.G., A.H.M., M.F.)
The Donna and Benjamin M. Rosen Bioengineering Center
Howard Hughes Medical Institute (to M.E.)

Title: Neurophysiological Abnormalities and Rescue in Rett Syndrome Model Mice using miRNA regulated AAV.CAP-B22.MeCP2 Gene Replacement

Authors: *K. MAHE, S. GUDAVALLI, A. HORI, R. DU, M. FLYNN, M. B. ELOWITZ, V. GRADINARU;
Biol. and Biol. Engin., Caltech, Pasadena, CA

Abstract: Rett syndrome (RTT) is characterized by severe neurodevelopmental impairments due to loss-of-function mutations in the MeCP2 gene and requires precise gene dosage throughout the whole brain for effective therapeutic strategies. A recently developed miRNA-based synthetic incoherent feed-forward loop (IFFL) system maintains ectopic MeCP2 expression within a therapeutic window, which is essential for avoiding overexpression toxicity (Flynn, et al., 2024; Du et al., 2024). This circuit can be incorporated within advanced viral vectors for targeted, noninvasive systemic delivery to the brain. In a female RTT mouse model, it achieved gene dosage compensation and reduced behavioral symptoms associated with neurological dysfunction. However, it remains unclear how different levels of MeCP2 expression reshape neural activity in key brain areas and lead to motor and cognitive phenotypes such as those seen in RTT. To address this question, we analyzed how IFFL-MeCP2 circuits modify excitatory neurons in two relevant areas in RTT female mice: motor cortex and hippocampus. We first used 2-photon imaging of GCaMP labeled neurons at single-cell resolution in layer 2/3 of the motor cortex during spontaneous locomotion in head-fixed RTT mice. These longitudinal recordings revealed bursting-like activity and sustained elevation in calcium signaling during locomotion, indicating an imbalance towards excitatory neurotransmission in motor coordination. Systemic delivery of a tightly regulated MeCP2 cassette reduced this bias and aligned this excitatory activity closer to physiological conditions at 13 weeks of age. We are also investigating the effects of MeCP2 overexpression on neuronal function in RTT mice treated with either a loosely

regulated or unregulated MeCP2 cassette. In parallel, we examined the hippocampus, focusing on the dentate gyrus to assess the therapeutic impact on memory-related neuronal activity. During a trace fear conditioning task using fiber photometry, we observed prolonged excitatory activity following footshock and disrupted freezing behavior in aged RTT mice, suggesting an imbalance characterized by over-excitation that disrupts normal memory encoding. We are currently investigating whether tightly or unregulated MeCP2 delivery can reshape this elevated excitatory activity to either improve or reduce the encoding and retrieval of fear memory. Together, these findings underscore the role of precision gene therapy using novel systemically delivered IFFL circuits and AAV capsids that cross the blood-brain barrier, offering a refined and dosage compensated approach to addressing neurophysiological dysfunctions in RTT.

Disclosures: K. Mahe: None. **S. Gudavalli:** None. **A. Hori:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.I.T. filed IP for methods with A.H. as inventor. **R. Du:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.I.T. filed IP for methods with R.D. as inventor. **M. Flynn:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.I.T. filed IP for methods with M.F. as inventor. **M.B. Elowitz:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.I.T. filed IP for methods with M.E. as inventor. **V. Gradinaru:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.I.T. filed IP for methods with V.G. as inventor. Capsida Biotherapeutics Cofounder and BoD member.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR261.01/A72

Topic: A.07. Developmental Disorders

Support: PEW Biomed Innovation-2021-A-18047
Postdoctorado ANID n°3230704

Title: Acute alcohol exposure alters gene expression of splicing factors in the developmental rat brain

Authors: M. OLIVARES COSTA[1], A. A. HIDALGO[1], F. FAUNES[2], A. KRAL[3], A. R. KRAINER[3], M. E. ANDRES[4], ***P. A. HAEGER**[1];
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Abstract: Prenatal alcohol consumption leads to alterations in neurodevelopment that manifest as fetal alcohol spectrum disorder (FASD). Within the FASD phenotype, we can observe facial abnormalities and changes in mental function that persist into adulthood. Alternative splicing increases the complexity of the neuronal proteome and consequently regulates the establishment and maintenance of neuronal development and synaptic plasticity. Bioinformatics studies conducted in our laboratory reveal that alcohol exposure alters the expression of splicing variants of synaptic and post-transcriptional regulatory functions, both in humans and animal models. These molecular events influenced by alcohol are observable in the short term rather than in the long term. We evaluated mRNA expression of splicing factors in the hippocampus and prefrontal cortex, 6 and 24 hours after alcohol exposure on of 7 days old rats. We found a time- and tissue-dependent expression specifically of the splicing factors (SF) SRRM4 and SRRM3. Currently, we are analyzing how these changes affect exon retention pattern in splicing variants, targets of these SFs, and their subsequent impact on cognitive alterations in FASD.

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Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR261.02/A73

Topic: A.07. Developmental Disorders

Support: NIG Grant U01AA029965
NIH Grant R01AA029486
NIH GRant P60AA010760
VA Merit I01BX001819

Title: Effects of neonatal ethanol exposure and withdrawal on hippocampal astrocyte gene expression in vivo

Authors: N. GORHAM, J. G. HASHIMOTO, *M. GUIZZETTI;
Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Astrocytes have been shown to play a critical role in the developmental effects of ethanol. We have previously characterized the effects of ethanol on the astrocyte-produced extracellular matrix, which alters hippocampal pyramidal neuron neurite outgrowth and dendritic arborization. Using the Translating Ribosome Affinity Purification (TRAP) procedure followed by RNA-Seq, we are able to study the changes caused by neonatal ethanol exposure on astrocytes *in vivo*. Here we show the differences in hippocampal astrocyte gene translation during exposure and after withdrawal of ethanol. Litters of Aldh1l1-EGFP-Rpl10a mice were

exposed to ethanol vapor or control conditions for 4 hours a day from postnatal day 2 (PD2) to PD7. Pups were either euthanized immediately following the ethanol or control exposure on PD7 or on PD8, after a 20-hour withdrawal period. Following the TRAP procedure, astrocyte translating RNA was analyzed by RNAseq at both time-points; total hippocampal RNA from the input fractions was also sequenced. At PD7, when alcohol was still present in the pups' blood, we identified 1576 genes regulated in hippocampal astrocytes and 932 genes regulated in the bulk hippocampus RNA. At PD8, we identified 1912 ethanol regulated genes in the astrocyte translating RNA and 373 ethanol regulated genes in the whole hippocampus. Comparing ethanol regulated genes between the astrocyte and input fractions showed 399 genes regulated in both fractions at PD7 and 174 genes at PD8. When comparing exposure and withdrawal time points, there is only modest overlap between ethanol regulated genes in astrocytes. Of the 1576 genes regulated in exposure group and 1912 genes regulated in withdrawal group, only 182 genes showed persistent regulation between the two time points. In addition, 65 genes switch the direction of regulation between the two time-points. Gene Ontology enrichment analysis identified peroxisome related categories in genes that were enriched in astrocytes and regulated by ethanol at PD8. Analysis of astrocyte enriched genes that were down-regulated by ethanol at PD7 showed enrichment in genes involved in chondroitin sulfate biosynthesis. Confirmation of these findings in two genes, *Chpf2* and *Chsy1*, were conducted by qRT-PCR and RNA-FISH. These findings highlight the response of astrocytes to ethanol during a critical period of brain development. In addition, the different responses seen in astrocytes following ethanol exposure and withdrawal highlights the dynamic responses of astrocytes to ethanol.

Disclosures: N. Gorham: None. **J.G. Hashimoto:** None. **M. Guizzetti:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR261.03/A74

Topic: A.07. Developmental Disorders

Support: NINDS Grant R01NS123163-01

Title: Using BioID as a tool to understand PACS1 Syndrome pathogenesis

Authors: *A. L. SCHRODER, A. D. GUEMEZ-GAMBOA;
Neurosci., Northwestern Univ., Chicago, IL

Abstract: PACS1 Syndrome is a NDD hallmarked by craniofacial dysmorphisms and intellectual disability. Patients with PACS1 Syndrome have a single *de novo* missense mutation at c.607C>T of the Phosphofurin Acidic Cluster Sorting 1 (*Pacs1*) gene, which causes an Arginine to Tryptophan substitution in the peptide (p.R203W). PACS1 is a multifunctional

sorting protein, with key roles in regulating trafficking of target proteins to and from the trans-Golgi Network (tGN). The pathogenic variant is in the Furin Binding Region of PACS1, which is the region responsible for many known interactions with target proteins via acidic cluster motif recognition. We have previously shown that PACS1 (+/R203W) forebrain organoids develop mature glutamatergic neurons with impaired expression of synaptic signaling genes when compared to isogenic controls. Additionally, PACS1 (+/R203W) neurons have prolonged network bursts, which has implications for circuit formation. While these results highlight the impact that p.R203W may play in the broader context of neural development, it remains unknown how this pathogenic variant alters the function of PACS1 in the neuron. To decipher this question, I am using BioID technology to address how the p.R203W interactome differs from that of wildtype PACS1. I hypothesize that p.R203W operates as a neomorphic gain-of-function allele and acquires new interactions with proteins implicated in synaptic transmission. Results from these experiments will be informative and clarify potential molecular mechanisms of PACS1 Syndrome pathogenesis.

Disclosures: A.L. Schroder: None. **A.D. Guemez-Gamboa:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR261.04/A75

Topic: A.07. Developmental Disorders

Support: NIH Grant NS137490

Title: The Role of Nedd4-2 Variants in PVNH Pathology

Authors: *Y. WANG[1], N.-P. TSAI[2];
[1]Mol. and Integrative Physiol., Univ. of Illinois at Urbana-Champaign, Urbana, IL; [2]Univ. of Illinois, Urbana-Champaign, Champaign, IL

Abstract: Periventricular nodular heterotopia (PVNH or PNH) is the most common type of brain-malformation due to the dysfunction of neuronal migration during early development. Dueto the limited understanding of PVNH pathology and the lack of well-known biomarkersfor PVNH, this disease cannot be diagnosed until patients show up with PVNH-relatedsymptoms such as seizures. Furthermore, patients with PVNH usually suffer fromseizures, learning disabilities, and cardiovascular diseases, which generate life-longissues and make PVNH a life-threatening disease. Currently, the pathology of PVNH isnot well understood, and anti-seizure medications used to treat PVNH patients are stillfacing drug resistance. Genetic mutations of the neural precursor cell expresseddevelopmentally downregulated 4-like (Nedd4-2 or Nedd4L) gene have been identified inmany PVNH patients. As an E3 ubiquitin ligase, Nedd4-2 plays a

critical role in regulatingion channel activities, protein synthesis under endoplasmic reticulum (ER) stress, andseizure susceptibility in the brain. However, the function of Nedd4-2 during neuronalmigration remains unknown. Therefore, we aim to uncover the role of Nedd4-2 inregulating neuronal migration, by which the Nedd4-2 variants could lead to PVNH. Ourdata showed that conditional depletion of Nedd4-2 (Nedd4-2 cKO) in neurons caused thereduced migration. Moreover, PVNH-associated Nedd4-2 mutations led to a reducedactin polymerization rate in HEK 293T cells. The results suggest that Nedd4-2 mightmediate neuronal migration by regulating actin polymerization. Although we previouslyshowed that Nedd4-2 can regulate the activity of actin depolymerization protein cofilin,manipulating cofilin activity did not enhance neuronal migration caused by Nedd4-2depletion. It suggests that Nedd4-2 regulated actin polymerization as well as neuronalmigration through a cofilin-independent mechanism. In summary, our data indicates therole of Nedd4-2 in regulating neuronal migration, whose failure is the key reason for PVNH.The underlying mechanism still requires further investigation.

Disclosures: Y. Wang: None. **N. Tsai:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR261.05/A76

Topic: A.07. Developmental Disorders

Title: The 14-3-3γ affects neuronal development, morphogenesis, and synapse formation in the cerebral cortex

Authors: *E. CHO[1], J. LEE[2], E. HWANG[3], J.-Y. PARK[4];
[1]Korea Basic Sci. Inst., Seoul, Korea, Republic of; [2]Univ. of Wisconsin-Madison, Madison, WI; [3]Brain Sci. Inst., Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [4]Sch. of Biosystem and Biomed. Science, Col. of Hlth. Science, Korea Univ., Seoul.

Abstract: Neurodevelopmental disorders are caused by abnormalities during brain development that lead to functional deficits in memory formation, emotion, and behavioral regulation. Therefore, studying mechanisms for neurodevelopment is essential for recovery from neurodevelopmental diseases. 14-3-3 proteins regulate several intracellular processes by binding to numerous target proteins, and although seven isoforms have been reported in mammals, individual isoforms are still insufficiently studied. Recent genetic studies have reported that 14-3-3γ has been implicated in several neurodevelopmental disorders. However, the detailed function 14-3-3γ in neural development and differentiation remains unknown. In this study, we investigated the effects 14-3-3γ on neuronal morphogenesis and cerebral cortex development using 14-3-3γ knockout mice. In this study, 14-3-3γ knockout homozygous mice die before birth, and heterozygous mice have smaller brain sizes than wild-type mice. Moreover, 14-3-3γ was

abundantly expressed in the early postnatal cortex and influenced cortical layer formation. Particularly, the most significant effects of 14-3-3γ deficiency were observed on specific cortical layers consisting of pyramidal neurons, and changes in the levels of specific transcription factors involved in the development of the neuronal projection were identified. Additionally, 14-3-3γ was the most expressed isoform in cortical neurons, with higher expression in cortical neurons than in glia. These results suggest the importance of 14-3-3γ in cortical neurodevelopment. Furthermore, in vitro studies of primary cortical neurons have shown that 14-3-3γ deficiency causes dendritic and excitatory synaptic defects. In vivo, sparse labeling of cortical neurons from 14-3-3γ heterozygous knockout mice reconfirmed these results. Finally, biochemical analyses confirmed changes in glutamate receptor expression in the cortex of heterozygous knockout mice. This study demonstrates the importance of 14-3-3γ in identifying the pathogenesis and therapeutic approaches of neurodevelopmental disorders by identifying the role of 14-3-3γ in neuronal morphogenesis and development of cortical layers.

Disclosures: E. Cho: None. **J. Lee:** None. **E. Hwang:** None. **J. Park:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR261.06/A77

Topic: A.07. Developmental Disorders

Support: Glenn Foundation and the American Federation for Aging Research (grant ID: BIG21042)

Title: The G-quadruplex helicase DDX5 regulates ASXL3 expression in primary human astrocytes

Authors: *V. M J[1], N. TANDON[2], A. S. TSVETKOV[3];
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Abstract: Guanine (G)-rich nucleic acid sequences in the human genome and transcriptome can fold into non-canonical secondary structures known as G-quadruplexes (G4s or G4-DNA and G4-RNA). G4-DNA plays important roles in replication, transcription, DNA recombination, and telomere maintenance, while G4-RNA regulates various RNA functions including translation and splicing. However, overly stable G4-DNA induces genomic instability, whereas abnormally stabilized G4-RNA disrupts RNA-dependent processes. Many G4-binding transcription factors, G4-binding proteins (G4BPs), and G4 helicases bind to the G4 structures and modulate their landscapes in cells. Among the helicases, we focused on the DEAD-box protein 5 (DDX5), an ATP-dependent G4 helicase that resolves G4-DNA and G4-RNA and regulates notably

transcription by unfolding promoter G4s.
We indeed discovered that G4 homeostasis is altered in primary astrocytes cultured from the brain of young and aged patients who underwent epilepsy surgeries, with more G4s and lower expression of DDX5 in aged cells compared to young ones. To elucidate the role of DDX5 in regulating transcription, we conducted a genome-wide expression analysis (RNA-seq) and identified a total of 460 genes differentially regulated by DDX5 out of 14,821 genes with measured expression, 214 upregulated and 246 downregulated genes, belonging to networks involved in cell cycle, p53 signaling, senescence, and longevity. One of our top hit genes was *ASXL3*, the corresponding ASXL3 protein being a chromatin modifier and epigenetic regulator. Mutations in *ASXL3* lead to Bainbridge-Ropers Syndrome, a neurodevelopmental disorder characterized by intellectual disability, behavioral abnormalities, seizures, and microcephaly. We discovered that ASXL3 expression levels decrease with age and showed that ectopically elevating DDX5 expression in astrocytes upregulates the levels of the ASXL3 protein. We also unveiled putative G4-DNA motifs in *ASXL3*'s promoter and gene sequence, indicating that DDX5 may positively modulate *ASXL3* transcription by unfolding G4s in the *ASXL3* promoter and gene.
In conclusion, we discovered that *ASXL3* gene expression is age-dependent and could be modulated by novel epigenetic changes governed by G4-DNA structures in primary human astrocytes. Our data revealed a novel mechanism of DDX5-dependent *ASXL3* transcription, shedding light on the molecular mechanisms in age-related neurological disorders.

Disclosures: V. M j: None. **N. Tandon:** None. **A.S. Tsvetkov:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR261.07/A78

Topic: A.07. Developmental Disorders

Support: NIH Grant HD042182

Title: Disrupted neurogenesis and transcriptional regulation of basal progenitors in the developing cortex of 22q11DS model

Authors: *S. RUKH[1], D. W. MEECHAN[2], T. M. MAYNARD[3], Z. ERWIN[4], A.-S. LAMANTIA[5];
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Abstract: 22q11 DiGeorge Syndrome (22q11DS), which is caused by heterozygous deletion of a minimum of 32 contiguous genes on human chromosome 22, is associated with multiple clinically defined neurodevelopmental disorders (NDDs). We previously reported that diminished bP proliferation in the LgDel mouse model of 22q11DS leads to altered frequency of layer 2/3 projection neurons (PNs), prefiguring aberrant cortical circuit connectivity and behavioral deficits similar to 22q11DS patients. Nevertheless, the precise nature of divergent cortical progenitor progression that results in diminished neurogenesis due to 22q11 gene deletion, remains uncertain. Using a combination of in vivo and in vitro cell biological assays, we have found selective disruption of cortical neural progenitor progression in the developing LgDel cortex. We have found that premature neurogenesis and parallel transcriptional changes in subventricular zone-located basal progenitors (bPs) diminish layer 2/3 PN frequency in LgDel. This disruption begins as layer 2/3 PN genesis begins and continues throughout the period of layer 2/3 PN neurogenesis (E12.5/E14.5/E16.5) based upon frequency of birthdated layer 2/3 PN cohorts in LgDel vs. wild type (WT) postnatal day 5 mouse pups. We are currently determining whether divergent bP proliferative capacity is cell-class autonomous, or reflects altered apical progenitor generation of bPs due to 22q11 gene deletion. Transcriptomic analysis reveals a transcriptional shift in LgDel bPs, characterized by overexpression of neurogenesis-promoting genes and a coordinated up-regulation of at least one epigenetically regulated multigene locus. Moreover, we identify alterations in the dynamic expression range of 22q11 and candidate genes within LgDel bPs, suggesting a complex interplay between genetic and epigenetic factors in neurogenesis regulation. Single cell transcriptome analysis is underway to determine whether these changes occur across the entire bP population or are targeted to a subclass of bPs. Together, our analysis shows cellular, transcriptional and epigenetic changes in the LgDel cortical neurogenesis, resulting in neurodevelopmental deficits associated with 22q11DS.

Disclosures: S. Rukh: None. **D.W. Meechan:** None. **T.M. Maynard:** None. **Z. Erwin:** None. **A. LaMantia:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

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Program #/Poster #: PSTR261.08/A79

Topic: A.07. Developmental Disorders

Support: NIMH Grant MH107182
NCI P30CA060553

Title: Long-term lithium treatment restores decreased myelin basic protein levels caused by Ank3 deletion in adult forebrain excitatory neurons

Authors: *S. YOON[1], M. DOS SANTOS[1], N. KHALATYAN[2], J. N. SAVAS[2], P. PENZES[1];
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Abstract: Rare genetic variants in *ANK3*, which encodes ankyrin-G, are associated with neuropsychiatric disorders, however, their pathogenesis is poorly understood. We find that mice with both prenatal deletion in cortical excitatory neurons and oligodendrocytes (*Ank3*$^{-/-}$:Emx1-Cre), and adolescent deletion in forebrain excitatory neurons (*Ank3*$^{-/-}$:CaMKIIα-Cre), display hyperactivity, hypoanxiety, and hypodepression like behaviors. Knockdown of *Ank3* in primary cultured cortical neurons decreased GCaMP6f-mediated average fluorescence peak amplitude. Moreover, calcium imaging of cortical slices from *Ank3*$^{-/-}$:CaMKIIα-Cre mice also shows decreased neuronal calcium peak amplitude. Quantitative proteomic analysis of cortical synaptic membranes reveals upregulation of Taok2, Serine/threonine-protein kinase TAO2, and downregulation of Mbp, Myelin basic protein, significantly. Long-term treatment of lithium, a well-known mood stabilizer for bipolar disorder, restores the expression level of Mbp in *Ank3*$^{-/-}$:CaMKIIα-Cre mice. Our results indicate that deleting *Ank3* in adults leads to changes in the synaptic proteome, which disrupts neuronal network activity and results in neuropsychiatric behavioral impairments.

Disclosures: S. Yoon: None. **M. Dos Santos:** None. **N. Khalatyan:** None. **J.N. Savas:** None. **P. Penzes:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

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Support: Lo Kwee-Seong Biomedical Research Fund (J.I)
Faculty Innovation Award (FIA2020/A/04) from the Faculty of Medicine, CUHK (J.I.)
UGC/RGC Research Matching Grant Scheme (J.I.)
Hong Kong PhD Fellowship (PF20-43681; Y.Z.)

Title: Characterizing neurodevelopmental alterations in CDKL5 deficiency using cerebral organoids

Authors: *Y. ZHU[1], Z. ZHENG[1], H. W. TSANG[2], P. IP[1];
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Abstract: CDKL5 deficiency disorder (CDD) is an X-linked neurodevelopmental disorder which is characterized by early-onset epilepsy, global developmental delay, intellectual disability, autistic features, visual impairment, and motor impairment. The prevalence of CDD is estimated to be 2.36 per 100 000 live births. Currently, there is no cure for CDD patients. To develop effective therapeutic strategies for CDD, it is crucial to gain a better understanding of the molecular and cellular functions of CDKL5 in brain development. However, the exact roles of CDKL5 in causing CDD pathophysiology are still unclear. Therefore, there is a need to develop a disease-relevant CDD human models to understand the functional roles of CDKL5 in CDD. It is also important to identify its bona fide substrates and dissect its function in clinically relevant cell types and models. The utilization of three-dimensional brain organoids generated from patients-derived induced pluripotent stem cells (iPSCs) offers a powerful model for unraveling mechanisms underlying CDD and provides a unique opportunity to advance our understanding of CDD pathology and potentially facilitate the development of therapeutic strategies. By combining single-cell RNA sequencing and immunostaining techniques, this study identified a previously uncharacterized deficits among neural progenitor cells, the major stem cells in developing human cortex, in CDD organoids. Given CDKL5 is a serine/threonine kinase, we explored several potential downstream substrates of CDKL5. The outcome of this study proposed a novel mechanism underlying CDD pathology and will provide important insights into exploration of novel treatment strategies for CDD.

Disclosures: Y. Zhu: None. **Z. Zheng:** None. **H.W. Tsang:** None. **P. Ip:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

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Program #/Poster #: PSTR261.10/B2

Topic: A.07. Developmental Disorders

Support: NIH NIMH K00 MH133250

Title: Investigating cholinergic function in a neurodevelopmental mouse model of TRIO

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Abstract: Cholinergic synapses are ubiquitous in the developing mammalian brain and actively neuromodulate brain functions including attention, learning, and memory. The cholinergic system is consistently perturbed in neurodevelopmental disorders (NDDs). Here, we address cholinergic alterations following forebrain excitatory neuron-specific deletion of *TRIO*, a high-risk gene for autism, schizophrenia, and related developmental disorders. The integrity of the cholinergic system depends critically on proper synthesis, release, and hydrolysis of

acetylcholine. Our comparative proteomic analyses revealed that several key regulators of cholinergic transmission (e.g. acetylcholinesterase, choline acetyltransferase, acetylcholine receptors) are significantly altered in *TRIO*-deficient mice. We are investigating whether these cholinergic deficits contribute to altered cholinergic tone in the prefrontal cortex by measuring ACh-evoked currents. In the cortex, *TRIO*-deficient mice demonstrate excitatory-inhibitory imbalance, which we plan to rescue with cholinergic-specific pharmacological targets. We also are evaluating if cholinergic alterations differentially impact attention, learning, and memory in *TRIO*-deficient mice compared to wild type littermates. This project will determine that altered cholinergic tone contributes to circuit dysfunction and behavioral deficits in the *TRIO*-deficient mouse model of NDDs.

Disclosures: N. Certain: None. **S. Myers:** None. **A.J. Koleske:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

Support: DOD Grant PR211767

Title: Behavioral Investigation and Neuronal Network Analysis of Transgenic Mice Carrying Causative Genetic Mutations of Congenital Heart Disease.

Authors: *D. WEST, A. PATEL, N. COULSON, D. CORTES, A. ZHANG, K. SCHWAB, T. BECKER-SZURSZEWSKI, S. HARTWICK, G. GABRIEL, C. LO, Y. WU;
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Abstract: **Introduction:** Congenital heart disease (CHD) is the most common birth defect affecting about 1% of all live births in the US. Neurodevelopmental deficits (NDDs) are often found in conjunction with CHD, even after successful surgical palliation. The origin of neuronal development remains unidentified. The prior consensus for NDDs appearing in CHD patients points to compromised hemodynamics caused by defects in the heart. The defective heart being responsible for creating a deficiency in substrate delivery and causing a hypoxic injury on the developing brain. Our study aims to show that the NDDs are a result from a change of genes in addition to compromised hemodynamics, rather than hemodynamics alone. Human patients carrying *PCDHA9* gene mutations exhibited varied degrees of CHD, including hypoplastic aorta, coarctation of the aorta (CoArc), and bicuspid aortic valve (BAV), which are commonly associated with the hypoplastic left heart syndrome (HLHS). *Pcdha9*$^{m/m}$ mice showed incomplete penetrance of the heart phenotype: ~11% had BAV whereas the rest appeared to have normal hearts. To test the hypothesis that the intrinsic causative mutation for CHD causes the NDD, we

characterized the brain network architecture and neurocognitive behaviors on *Pcdha9*$^{m/m}$ mice that had normal hearts. **Methods:** *Pcdha9*$^{m/m}$ mice were subjected to series of neurocognitive testing, including an Open Field Assay, Three-Chamber Sociability Assay (three trials consisting of habituation and sociability), and the Sociability Trial. 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 *Pcdha9*$^{m/m}$ mice. **Results/Discussion:** We show that *Pcdha*$^{m/m}$ mice exhibit NDDs. Our Behavior testing demonstrates that *Pcdha*$^{m/m}$ mice behave differently than wild type mice. Using the behavior patterns demonstrated from the behavior testing, we can see areas such as the Hippocampus, Cortical Amygdala, Isocortex, and a few other areas of interest have some underlying
differences. DTI networking further demonstrates that point by showing a significant difference between *Pcdha*$^{m/m}$ mice and wild type mice. These areas' differences in results showcase that NDD cannot solely be attributed to a change in hemodynamics and should include genomic changes. **Conclusion:** Our study found that the causative genes for CHD caused neurodevelopmental deficits in mice.

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Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

Support: NIH NINDS R01NS107428

Title: Biallelic and de novo variants in LLGL2 cause global neurodevelopmental delay and microcephaly in humans and zebrafish

Authors: *A. A. ZAIB[1,2], Z. M. AHMED[3], J. YAPING[3], M. C. KRUER[4], D. WEIS[5], S. ZEIDLER[6], J. A. MAYR[7], S. RIAZUDDIN[8], S. RIAZUDDIN[9];
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Abstract: *LLGL2* (MIM: 618483) encodes a polarity protein complex component 2 (Lethal giant larvae 2) that establishes basolateral polarity, asymmetric cell division, and cell migration. Hereby, we report six individuals from 4 unrelated families harboring splicing and three missense variants in the *LLGL2* segregating with neurodevelopmental delay (NDD) together with moderate to severe intellectual disability (ID) and microcephaly. This study aims to characterize the functional consequences of NDD-associated variants in *LLGL2*, identified using a whole exome sequencing approach. *In silico* analysis on splicing variant c.2901+G>A was predicted to decrease splicing donor site recognition confidence from 0.92 to 0.48. Exon trapping assay showed partial retention of the intron 22 sequence in the final transcript, predicted to lead to a frameshift due to aberrant splicing. To get a deeper insight into cellular mechanisms impacted by the variants, we generated a knock-in cell line for a variant c.1456G>A; p.Gln486Lys using HDR-mediated CRISPR-Cas9 genome. We observed dysregulated mRNA levels of *LLGL2* and its predicted interactors. Subcellular localization of LLGL2 at plasma membrane protrusions in mutants was compromised. During cell division, increased frequency of multinucleated cells and abnormal cytokinesis in mutants were observed along with mitotic spindle disorganization. Chromosome segregation defects in knock-in cells accompanied normal mitotic spindles; thus, we observed the potential functional influence concerning DNA damage. Gamma-H2AX staining in mutant cells showed a significantly increased number of gamma-H2AX foci compared to control cells. All observed findings in the knock-in variant were recapitulated by all missense variants through the overexpression system. Furthermore, we studied *llgl2* role in early development using morpholino-mediated knockdown in zebrafish and observed significant developmental deficits in the *llgl2* morphants. Upon behavioral assessment, morphants showed significantly reduced movement patterns via spontaneous motility assay compared to control siblings. Moreover, human wild-type mRNA but none of the mutant mRNA's co-injections with llgl2 MO rescued the observed deficits, suggesting the evolutionary role of protein in early development. In our ongoing studies, we characterize the neurological impact imposed by the knockdown of *llgl2* and recapitulate the effect of all four NDD-associated variants in yeast complementation studies. In summary, our *in vitro* and *in vivo* findings suggest LLGL2 as a potential regulator of early development in humans and zebrafish.

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Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

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Program #/Poster #: PSTR261.13/B5

Topic: A.07. Developmental Disorders

Support: CIHR Grant PJT-159586
NSERC CGS-D

Title: Neurodevelopmental effects of prenatal cannabinoid exposure on schizophrenia susceptibility: exploring lipidomic alterations in patient-derived cerebral organoids

Authors: *K. ZHAKSYLYK[1], M. H. SARIKAHYA[1], S. L. COUSINEAU[2], H. MAHMOOD[3], A. DEMBLA[3], S. R. VANIN[3], M. RODRIGUEZ-RUIZ[1], K. YEUNG[2], D. B. HARDY[3], W. J. RUSHLOW[1], S. R. LAVIOLETTE[1];
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Abstract: Prenatal cannabinoid exposure (PCE) is implicated in disrupting fetal brain development and increasing susceptibility to neuropsychiatric disorders, including schizophrenia (SCZ). However, the underlying mechanisms behind this link remain poorly understood. Our research aims to elucidate these mechanisms using cerebral organoids derived from human induced pluripotent stem cells (iPSCs). Disturbances to the neurolipidome, representing alterations in the fatty acid and phospholipid composition of neural cells, are a hallmark of SCZ. Recent research on prenatal Δ9-tetrahydrocannabinol (THC) exposure has revealed persistent neurolipidomic abnormalities in rodents, alongside cognitive and emotional deficits resembling prodromal SCZ stages. As SCZ involves complex human-specific factors, we utilized patient-derived cerebral organoids to characterize lipidomic anomalies within SCZ and explore how prenatal cannabinoid exposure alters the neurolipidomic landscape. Organoids from healthy controls (CTRL, n=4) and SCZ patients (n=4) were exposed to THC (100 ng/ml), cannabidiol (CBD; 500 ng/ml), or THC-CBD combination (100 ng THC/500 ng CBD) for 15 days, until organoids reached 1 month of development, a period resembling early fetal cortical growth. Techniques included lipidomic analyses using mass spectrometry imaging, gene expression assays via immunofluorescence, western blotting, quantitative PCR (qPCR), and RNA-sequencing. Preliminary characterization revealed expected neuronal markers in the vehicle-treated CTRL and SCZ organoids at day 30 and day 180 developmental stages. THC-CBD and THC exposure showed distinct lipidomic (mass spectrometry imaging) and transcriptomic (RNA-seq) profiles compared to CBD and vehicle in control cell lines; this was far more pronounced in SCZ lines. SCZ organoids exposed to THC exhibited severe alterations in all assessed metrics. These changes resembled rodent model findings of prenatal THC exposure and lipidomic and molecular anomalies. qPCR showed differential expressions of neuronal markers, particularly in SCZ THC organoids. Comprehensive RNA sequencing data comparing treatment groups will be presented, focusing on lipidomic pathways. Western blotting and immunofluorescence assays are ongoing. This study utilizing human-derived cerebral organoids has begun to unravel cellular-level effects of gestational exposure to THC on human brain development and its association with uniquely human neuropsychiatric disorders.

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Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

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Title: Interactive influence of selenium and methylmercury on in vitro maturation of parvalbumin-expressing interneurons

Authors: M. WATANABE[1], A. SASUCLARK[2], K. ROSHTO[3], ***M. W. PITTS**[4];
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Abstract: Methylmercury (MeHg) is a neurotoxin of great concern to public health that is ubiquitously present in marine food chains. At the molecular level, MeHg irreversibly binds to catalytic selenium (Se) moieties present in antioxidant selenoproteins, leading to redox dysregulation and mitochondrial dysfunction. Growing evidence indicates that oxidative stress impedes maturation of GABAergic circuitry, resulting in a permanent imbalance between excitatory and inhibitory neurotransmission. Moreover, among GABAergic cell types, fast-spiking, parvalbumin-expressing interneurons (PVIs) are most acutely impacted by redox imbalance. During development, PVIs are preferentially encapsulated by specialized extracellular matrix structures, termed perineuronal nets (PNNs), which stabilize perisomatic synaptic input and act as protective barriers against redox insults. Consequently, alterations in PVIs and PNNs are well chronicled in neuropsychiatric disease, and evidence from animal models indicates that redox imbalance during adolescence impedes their maturation. Herein, we examined the interactive effects of MeHg and Se on PVI maturation in primary cortical cultures. Parallel studies were conducted to monitor longitudinal progression of *in vitro* electrophysiological activity using microelectrode arrays (MEA). Cultures were raised in media containing optimal levels of Se (100 nM) until DIV14, followed by challenge with a subtoxic dosage of MeHg (200 nM) from DIV14 - DIV28, which represents a critical period where PNNs mature and neural networks stabilize. Relative to controls, MeHg treatment reduced antioxidant activity and impaired PNN formation at 28 days *in vitro*. MeHg also affected the electrophysiological profile of developing cultures, as MeHg-treated cultures exhibited impairments in long-term potentiation in conjunction with reduced spike counts for both network bursts and in response to stimulation. Further studies showed that co-administration of additional Se (400 nM) from DIV14 - DIV28 counteracted many of the deleterious effects caused by 200

nM MeHg exposure. In sum, these findings demonstrate the interactive influence of Se and MeHg on redox homeostasis and GABAergic maturation.

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Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

Support: NIH NS105680
Internal Discretionary Funds

Title: Tmem184b human variants cause neurodevelopmental disruptions and seizures via alteration of metabolic signaling

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Abstract: Transmembrane protein 184B (TMEM184B) is an endosomal seven-pass transmembrane protein with evolutionarily conserved roles in synaptic structure and axon degeneration. However, whether these roles extend to neuronal growth processes during development is unclear. Human variation in the *TMEM184B* gene has not previously been reported, and therefore the contribution of TMEM184B to human neurological development and/or function is unknown. Here we report a group of pediatric patients who have *de novo* heterozygous variants in *TMEM184B*. All patients harbor missense or nonsense changes and have neurodevelopmental disruptions including intellectual disability, corpus callosum hypoplasia, seizures, and/or microcephaly. To understand the contribution of TMEM184B to neural development *in vivo*, we modeled its reduction in zebrafish using morpholinos to target endogenous TMEM184B. We found this causes dose-dependent microcephaly phenotypes, aligning with patient syndromes. Using over-expression of patient variants to model dominant effects seen in the clinic, we constructed a variant allelic series and identified the K184E and G162R variants as likely dominant negative contributors. To assess if these patient variants compromise cell viability, we quantified their effects on apoptosis and their expression levels. K184E increased apoptosis and was expressed at lower levels, while G162R exhibited no differences for both. Although apoptosis and expression levels are different between our

dominant negative variants, we found they cause the most significant increase in nuclear localization of transcription factor EB (TFEB), a master regulator of lysosomal biogenesis, suggesting nutrient signaling pathways are disrupted. Based on our data, we propose a model where TMEM184B structural disruptions decreases cell viability and alters lysosomal biogenesis, leading to neurodevelopmental disorders such as microcephaly.

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Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

Title: The Impact of TaVNS on Recovery and Neural Plasticity after HIE in Neonatal Rats.

Authors: *M. AKIRTAVA[1], A. HONG[1], M. R. YOUSSEF[2], K. CAMBURN[2], M. G. WILEY[3], C. S. ROBINSON[4], S.-K. SIMS[5];
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Abstract: INTRODUCTION: Neonatal hypoxic-ischemia (HI) results may result in deficits in cognition, language, sensory, and motor function. Few neuroprotective options exist to combat these adverse outcomes. While treatments for adults with stroke include anticoagulants, motor and cognitive rehabilitation, neonatal stroke care is limited to supportive care, such as hyperthermia and hyperbaric oxygen therapy. A potential treatment option, Transcutaneous Vagus nerve stimulation (taVNS), has significant anti-inflammatory effects in adult ischemic brain injury and may decrease HI-induced brain injury and improve outcomes. In addition, preclinical and clinical research over the last decade has identified a role for the protein Brain-Derived Neurotrophic Factor (BDNF) in brain plasticity within the intact brain and following central nervous system damage. Recent studies have also explored the relationship between taVNS and BDNF levels in other disordered states noting significantly increased average BDNF levels in the taVNS group. Hence, we explored the benefit of taVNS on functional recovery, BDNF, and plasticity after HI.
METHODS: In post-natal day 7 (PND7) Sprague-Dawley rats, HI was induced using the Vannucci Model. For our model of hypoxic ischemic, a ligation of the right carotid artery was induced which is followed by a two-hour exposure to an 8% oxygen/ 92% nitrogen in an enclosed chamber. Male and female rat pups were subjected to a 2 h hypothermia in a

temperature-controlled chamber as a standard of care. Rat pups were then subject to 30-minute taVNS treatments for 7 days. TaVNS was administered at an intensity of 1.0mA below the perceptual threshold. Each 30-minute treatment session included 6 trials of a 40sec train, 0.5msec duration, 20Hz frequency, with 4.5 minute breaks between trials. To evaluate post-stroke recovery, rats were assessed at PND 7 through 10 on the righting reflex, cliff avoidance, 2-arm wire suspension, left arm wire suspension, negative geotaxis. Immunohistochemistry for synaptophysin and MAP2, which are reliable indicators of synaptic and dendritic plasticity, were also quantified. We also performed enzyme-linked immunosorbent assay (ELISA) analysis on serum samples taken from rat pups for mature BDNF.
RESULTS: The objective of these studies is to address these gaps in knowledge and evaluate the role and therapeutic potential taVNS in neonatal HI recovery Preliminary data suggests a predictive relationship between taVNS treatment and recovery after HI. Our results imply that greater functional recovery correlates with higher levels of synaptophysin and MAP2 which suggests greater neuroplasticity with taVNS treatment.

Disclosures: M. Akirtava: None. **A. Hong:** None. **M.R. Youssef:** None. **K. Camburn:** None. **M.G. Wiley:** None. **C.S. Robinson:** None. **S. Sims:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR261.17/B9

Topic: A.07. Developmental Disorders

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Title: Comparative Primary Cilia Directionality Analysis in Cortical Regions of Newts, Turtles, Mice, and Monkeys: Indications of Reverse Neuronal Movement for Postnatal Positioning

Authors: *S. MIRHOSSEINI, J. YANG, L. QIU, X. CHEN;
Univ. of New Hampshire, Durham, NH

Abstract: Primary cilia serve as cell antennae in most vertebrate cells including neurons. It is well-known that primary cilia regulate embryonic neurodevelopment, but little is known about their roles in postnatal neurodevelopment. We have previously shown that neuronal primary cilia in loosely layered cortical regions, such as the mouse neocortex, point in the same direction.

However, primary cilia of principal neurons in the condensed laminated regions, such as the mouse hippocampal CA1 and piriform cortex, manifest opposite directionality. In contrast, astrocytes and interneurons in the hippocampus, and neurons in nucleated brain regions do not display specific cilia directionality. We further discovered that the cell bodies of principal neurons in the mouse hippocampal CA1 superficial sublayer, the subiculum, cingulate cortex, and neocortex undergo a previously unnoticed, slow but substantial "reverse movement" for postnatal positioning and lamina refinement. The 6-layered neocortex in the mammalian brain is homologous to the dorsal cortex in reptiles, while the 3-layered allocortex includes the olfactory cortex and hippocampus in mammals, which are equivalent to the lateral and medial cortex in reptiles, respectively. To evaluate the generality of reverse movement for postnatal positioning, we determine primary cilia orientations in different cortical regions in newts (amphibians), turtles (reptiles), mice (mammals), and monkeys (mammals and primates) and investigate the roles of neuronal primary cilia in postnatal cell positioning. This comparative cilia directionality analysis will help reveal how principal neurons in various cortices complete neuronal positioning and advance our understanding of how the allocortices evolve into the neocortices.

Disclosures: S. Mirhosseini: None. **J. Yang:** None. **L. Qiu:** None. **X. Chen:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

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Program #/Poster #: PSTR261.18/B10

Topic: A.07. Developmental Disorders

Support: NIH NINDS R01NS107428

Title: Hemizygous rare variants in the coiled-coil domain containing protein 120 (CCDC120) cause a distinct neurodevelopmental disorder

Authors: *T. AKHTER[1,2], Z. M. AHMED[1,3,4], D. WEGNER[5], T. S. BARAKAT[6], A. FERNANDEZ-JAEN[7], E. RAHIKKALA[8], A. SHILLINGTON[9], R. RABIN[10], J. R. FRIEDMAN[11], T. BIERHALS[12], S. MAHIDA[13], K. LARACY[13], L. URPA[14], S. RIAZUDDIN[15], S. RIAZUDDIN[1,3];
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Oulu, Finland; [9]Neurobehavioral Psychiatric Div. of Genetics, Cincinnati Children's Hosp., Cincinnati, OH; [10]NYU Grossman Sch. of Med., New York, NY; [11]Dept. of Neurosciences and Pediatrics at the Univ. of California, San Diego, CA; [12]Ctr. for Obstetrics and Pediatrics, Inst. of Human Genetics, Med. Ctr. Hamburg-Eppendorf (UKE), Hamburg, Germany; [13]Dept. of Neurology, Boston Children's Hosp., Boston, MA; [14]Inst. for Mol. Med. Finland (FIMM), Univ. of Helsinki, Helsinki, Finland; [15]Jinnah Burn and Reconstructive Surgery Centre, Allama Iqbal Med. Res. Ctr., Univ. of Hlth. Sci., Baltimore, Pakistan

Abstract: *CCDC120* (MIM:300947) encodes a coiled coil domain containing protein 120, which aids in the assembly of centriolar subdistal appendages, cytoskeletal rearrangement, and neurite outgrowth. Here, we report a frameshift and thirteen hemizygous missense variants in *CCDC120* segregating with phenotypes of neurodevelopmental disorder (NDD) in seventeen affected individuals from fourteen ethnically diverse families. All individuals exhibit NDD features, including mild to severe intellectual disability, epilepsy, and microcephaly, along with other congenital manifestations. This study aims to elucidate the functional consequences of NDD-associated *CCDC120* variants. We employ a multidisciplinary approach combining cellular and animal models using CRISPR/Cas9 genome editing to understand the molecular mechanism underlying *CCDC120*-related NDD phenotypes. *In-silico* analysis and 3D protein modeling simulation predicted alterations in protein folding and interaction with other molecules for the missense variants. *In vitro*, overexpression studies in mouse neuroblastoma Neuro2a cells showed an accumulation of protein around the cell body in the variant's overexpressing cells instead of migrating within the vesicular structures in neurite shaft and to the growth cone relative to the wild type protein. Moreover, we observed significantly reduced CCDC120 expression levels in overexpressed variants. Knocking down *ccdc120* in *neurod1-GFP* transgenic zebrafish resulted in severe developmental deficits, including craniofacial skeletal deformities and microcephaly complementing the patient's phenotype. Light and sound induced visual and acoustic locomotion analyses of morphants showed reduced to no movements, suggesting compromised motor abilities. Craniofacial cartilage staining of morphants revealed mandibular hypoplasia. Whole-mount brain imaging depicted significantly reduced brain size, neuronal population, and disrupted parallel fibers in the cerebellum of morphants, the motor coordinating center of the brain, giving compelling evidence of direct involvement of *ccdc120* in neurodevelopment. Intriguingly, all observed morphant phenotypes were rescued by co-injection of human $CCDC120^{WT}$ mRNA but not by transcripts encoding NDD variants, emphasizing their loss of function impact. In preliminary studies, we are investigating the role of *Ccdc120* in mouse neurodevelopment by employing *in-utero* electroporation and a CRISPR genome editing approach. Taken together, our investigations in cellular and animal models implicate *CCDC120* as a significant player in neurodevelopment and that its variants contribute to NDD phenotypes.

Disclosures: T. Akhter: None. **Z.M. Ahmed:** None. **D. Wegner:** None. **T.S. Barakat:** None. **A. Fernandez-Jaen:** None. **E. Rahikkala:** None. **A. Shillington:** None. **R. Rabin:** None. **J.R. Friedman:** None. **T. Bierhals:** None. **S. Mahida:** None. **K. Laracy:** None. **L. Urpa:** None. **S. Riazuddin:** None. **S. Riazuddin:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

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Program #/Poster #: PSTR261.19/B11

Topic: A.07. Developmental Disorders

Support: NIH Grant HD103360
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Title: Stimulus-driven protein synthesis in iPSC derived Human Cortical Neurons with EEF1A2 mutations

Authors: *L. COLE, M. S. MOHAMED, E. KLANN;
New York Univ., New York, NY

Abstract: Translation of mRNA to protein is a highly complex and regulated process that is essential for cells to function normally. eEF1A2, a neuronal and muscle specific translation factor, plays an important role in the elongation step of translation. Along with other factors, eEF1A2 controls translation in response to neuronal activity. Mutations in EEF1A2, the gene encoding eEF1A2, are associated with neurodevelopmental disabilities, including severe intractable epilepsy and autism, but the molecular mechanisms that result in neuron dysfunction caused by these mutations are not well understood. We have been determining how mutations in EEF1A2 associated with disease phenotypes affects translation. Under normal conditions, neurons increase protein synthesis in response to neuronal stimulation, but in mouse neurons, mutations in EEF1A2 cause decrease de novo protein synthesis. To determine whether induced pluripotent stem cell-derived human cortical neurons with mutations of the EEF1A2 gene properly upregulate translation in response to neuronal activity, we have stimulated these mutant neurons with BDNF and forskolin. Using SUnSET to measure translation, IHC to visualize, and western blotting to measure activity markers, we will compare activity-dependent translation in wild type and EEF1A2 mutant neurons. Preliminary results show that in response to forskolin, mutant neurons exhibit significant decreases in translation, whereas wild-type neurons exhibit increases in translation. In contrast, in response to BDNF, both mutant and wild-type neurons exhibit significantly increased translation. Understanding how translation is impacted by disease causing-EEF1A2 mutations can provide insight into the progression of this disorder and possibilities for treatment.

Disclosures: L. Cole: None. **M.S. Mohamed:** None. **E. Klann:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

Location: MCP Hall A

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Program #/Poster #: PSTR261.20/B12

Topic: A.07. Developmental Disorders

Support: NIH U01MH115747
U01MH116487

Title: A foundational atlas of autism protein interactions reveals molecular convergence

Authors: *R. VARTAK[1], B. WANG[2], Z. NAING[1], F. ZALTSMAN[2], B. POLACCO[1], K. HENNICK[2], M. LASSER[2], H. WILLSEY[2], K. OBERNIER[1], R. HUTTENHAIN[1], T. JAN NOWAKOWSKI[2], M. STATE[3], A. WILLSEY[4], N. KROGAN[1];
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Abstract: Despite identification of high-confidence autism spectrum disorder (ASD) risk genes, translation to viable treatment targets remains elusive. Using affinity purification-mass spectrometry, we constructed a foundational protein-protein interaction (PPI) network involving 100 ASD risk genes in HEK293 cells, revealing over 1,700 PPIs, 87% of which have not been previously described. Importantly, we found that the interactors were enriched for expression in the human brain, including neural progenitor cells, and for ASD but not Schizophrenia genetic risk. The network showed coalescence on protein complexes and specific processes, including neurogenesis, transcription and chromatin modification. Artificial intelligence (AI) using AlphaFold2 predicted putative PPI interfaces and overlay with ASD patient-derived mutations revealed altered PPIs that allowed for prioritization for interrogation in Xenopus tropicalis, human induced pluripotent stem cells and brain organoids. One such ASD mutation in the transcription factor Foxp1, which disrupted interaction with Foxp4, led to reconfiguration of DNA binding sites and developmental pathologies such as increased deep cortical layer neurons, a phenotype linked to ASD. This work provides insights into the molecular mechanisms of ASD, serving as a platform for therapeutics development for autism, other neuropsychiatric disorders as well as any genetically defined disease

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Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR261.21/B13

Topic: A.07. Developmental Disorders

Title: Metabolic Signatures of Brain Injury in a Neonatal Hypoxic-Ischemic Encephalopathy Model

Authors: N. WOLFF[1], ***M. TRIANTAFYLLOU**[1], P. KRATIMENOS[1,2], D. GRAHAM[3], S. M. AJA[3], A. EVERETT[3], E. GOLDSTEIN[4], G. SANIDAS[1], C. BYRD[1], J. GHAEMMAGHAMI[1], G. SIMONTI[1], I. KOUTROULIS[1,2], F. J. NORTHINGTON[3,5];
[1]Children's Natl. Med. Ctr., Washington, DC; [2]George Washington Univ. Sch. of Med. and Hlth. Sci., Washington, DC; [3]Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [4]Neurosci. and Regenerative Med., Univ. of Augusta, Augusta, GA; [5]Johns Hopkins All Children's Hosp., Baltimore, MD

Abstract: The association between metabolic dysregulation and poor neurological outcomes in hypoxic-ischemic encephalopathy (HIE) has been established. However, there are few studies investigating metabolomic signatures in HIE or translational neonatal hypoxia-ischemia (HI) models and their therapeutic implications.P10 mice were categorized into groups (n=9/group) based on induced insults: HI with unilateral carotid artery ligation and exposure to FiO2=8%x 45 min (modified Rice-Vannucci model), providing HI-ipsilateral and hypoxia-contralateral, and naive samples. Brain tissue was frozen 24 hours after HI. LC-MS was utilized to measure hemisphere metabolites, analyzed via MetaboAnalyst and Ingenuity Pathway Analysis. Altered pathway activity was classified as increased, decreased, or of unknown directionality. Differential pathway activity was observed comparing HI to hypoxia or HI to naive hemispheres, implicating specific metabolic alterations due to the combination of hypoxia and ischemia. Dysregulation of arginine and carnitine metabolism, along with decreased taurine and N-acetyl aspartic acid levels, in HI suggests increased risk for epileptiform activity, susceptibility to excitotoxicity, loss of neuroprotective mechanisms, and impaired neurodevelopment. Furthermore, decreased NAD+ signaling, increased cADP-ribose levels, and inhibited tRNA charging observed in HI may correlate with axonal degeneration. Notably, dysregulation of NAD+ metabolism establishes a link between affected pathways, including sirtuin signaling crucial for axonal growth and dendritic arborization.Overall, these metabolic changes are associated with mitochondrial dysfunction, energy depletion, oxidative stress, impaired autoregulation and neuronal apoptosis. HIE induces metabolic injury leading to axonal degeneration and neuronal death. Our findings highlight potential therapeutic targets to mitigate HIE-induced metabolic dysregulation and prevent long-term neurodevelopmental deficits.

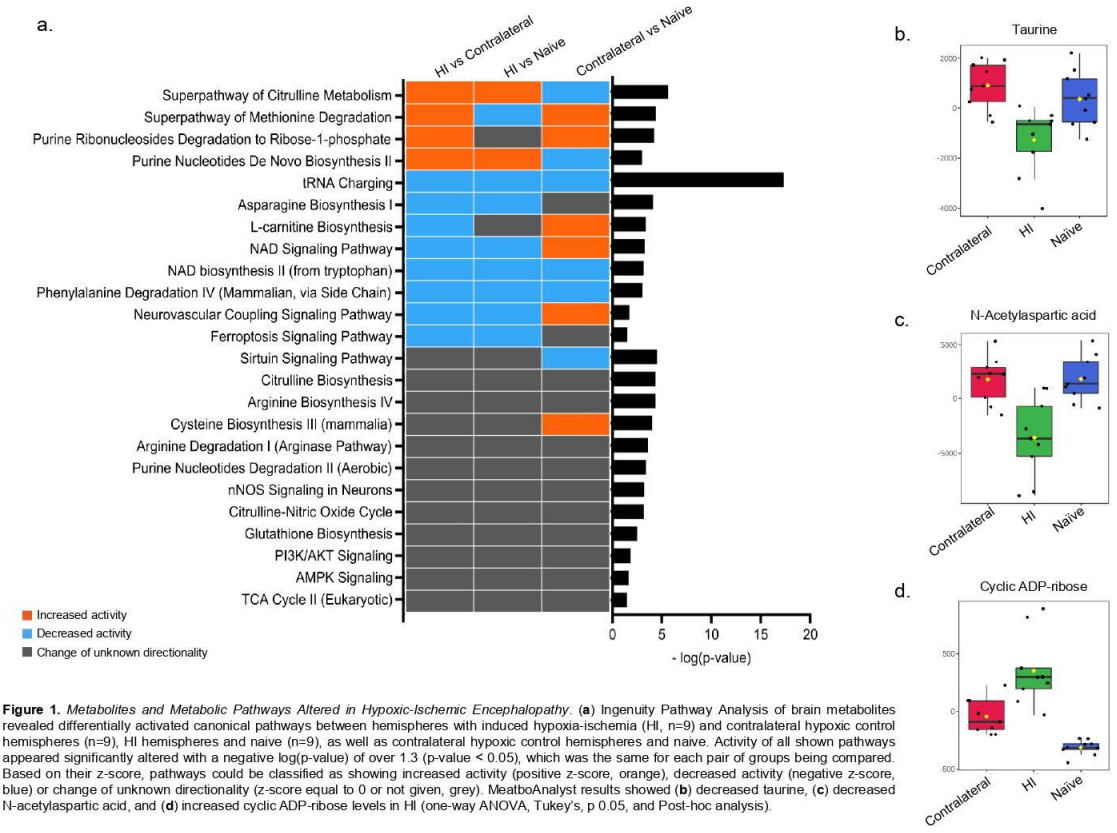

Figure 1. *Metabolites and Metabolic Pathways Altered in Hypoxic-Ischemic Encephalopathy.* (**a**) Ingenuity Pathway Analysis of brain metabolites revealed differentially activated canonical pathways between hemispheres with induced hypoxia-ischemia (HI, n=9) and contralateral hypoxic control hemispheres (n=9), HI hemispheres and naive (n=9), as well as contralateral hypoxic control hemispheres and naive. Activity of all shown pathways appeared significantly altered with a negative log(p-value) of over 1.3 (p-value < 0.05), which was the same for each pair of groups being compared. Based on their z-score, pathways could be classified as showing increased activity (positive z-score, orange), decreased activity (negative z-score, blue) or change of unknown directionality (z-score equal to 0 or not given, grey). MeatboAnalyst results showed (**b**) decreased taurine, (**c**) decreased N-acetylaspartic acid, and (**d**) increased cyclic ADP-ribose levels in HI (one-way ANOVA, Tukey's, p 0.05, and Post-hoc analysis).

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Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

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Burroughs Wellcome Fund

Title: Germline mutation in ANKRD11 identified in a cohort of individuals with refractory epilepsy and focal cortical dysplasia

Authors: *A. E. BERGLIND[1], M. GADE[1], A. FREEMAN[2], M. R. WINAWER[2], M. HEGDE[3], C. R. CADWELL[3], E. L. HEINZEN[1];
[1]Univ. of North Carolina, Chapel Hill, Chapel Hill, NC; [2]Columbia Univ., New York, NY; [3]Univ. of California, San Francisco, San Francisco, CA

Abstract: Advances in sequencing technology and availability of resected tissue have allowed for the study of the contribution of genetic variation in epilepsy disorders with various underlying genetic etiologies and clinical manifestations, including focal cortical dysplasia (FCD). Different subtypes of FCD may show disorganized or absent cortical lamination, prominent radial architecture, blurring of the grey-white matter boundary, increased heterotopic neurons in the subcortical white matter, dysmorphic neurons, and balloon cells. In this study, we sequenced individuals with intractable focal epilepsy who have undergone brain resection, including individuals with FCD, to identify pathogenic somatic and germline variants that may contribute to disease risk on a subset of genetically unexplained cases. DNA isolated from resected tissue was analyzed in a cohort of 278 patients with radiographically lesional and nonlesional focal epilepsy, including hemimegalencephaly (n = 32), FCD type I and related phenotypes (n = 126), FCD type II (n = 98), or FCD type III (n = 22). High depth exome sequencing from a patient with FCD type I revealed a pathogenic germline mutation in *ANKDR11* c.2197C>T (p.R733X). The mutation R733X has been previously reported in individuals with inborn genetic diseases and KBG syndrome. KBG syndrome is a rare autosomal dominant genetic disorder for which loss-of-function is a known mechanism of disease, characterized by short stature, distinctive craniofacial features, skeletal malformations, developmental delay, seizures, and intellectual disabilities. Neuropathological review of resected brain tissue from the R733X case revealed disorganization of neurons in the deep cortical layers. Two additional *ANKRD11* variants [c.4604A>G (p.K1535R) and c.4147G>T (p.G1383C)] of unknown significance were identified in two additional cases with radiographically nonlesional focal epilepsy (neuropathologic assessments pending). The *ANKRD11* locus encodes an ankyrin repeat-containing protein that inhibits ligand-dependent transactivation by recruiting histone deacetylases to the coactivator/nuclear receptor complex. *ANKRD11* has been shown previously to regulate neuronal migration and positioning during cortical development in mice but this has not been shown in human tissue as these patients rarely undergo brain resections. These results support that *ANKRD11* could potentially be a novel FCD gene. Additional functional studies are required to evaluate the role of *ANKRD11* on neuronal activity and development.

Disclosures: A.E. Berglind: None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

Support: NIH R21HD113311-01
New Jersey DOH CAUT24BRP009

Title: Cytokine regulation of neurodevelopment in a novel human iPSC-based neuroimmune organoid model

Authors: *A. PAPETTI, P. JIANG;
Rutgers Univ., Piscataway, NJ

Abstract: Maternal immune activation (MIA) has been associated with enhanced risk of offspring developing neurodevelopmental disorders, such as autism spectrum disorder (ASD), schizophrenia (SZ), and mood disorders. The precise molecular mechanisms connecting maternal inflammation and altered human brain development in the offspring remain elusive, hindering the development of therapeutic interventions. Several pro-inflammatory cytokines have been identified as being significantly upregulated during immune activation, including interleukin-6 (IL-6). IL-6 is of particular interest in the context of MIA, as it has consistently been reported to be elevated during the ensuing immune response, can cross the blood-brain barrier in the developing fetus, and has been found to be elevated in the brains of individuals with autism. However, the specific impact of increased IL-6 levels on human neural development has yet to be uncovered. To study the cell-type specific effects of elevated IL-6 exposure, we have established a novel human induced pluripotent stem cell (iPSC)-based organoid model comprising excitatory and inhibitory neurons, macroglia, and microglia. We hypothesize that IL-6 stimulation disrupts the fate of neural progenitors toward different types of macroglial cells (i.e., astrocytes and oligodendrocytes) and neurons, particularly specific interneuron subclasses. Furthermore, IL-6 alters microglial functions, disturbing synaptic development and refinement through their neurotrophic and pruning functions. We have validated the neural and glial cell type composition of our organoid model, as well as their responsiveness to the cytokine IL-6 using a combination of immunohistochemistry and flow cytometry. Additionally, using this model we have revealed a significant increase in the calretinin interneuron subpopulation following elevated IL-6 exposure. Our work demonstrates the cytokine regulation of neurodevelopment, and our model can support the study of additional cytokines associated with physiological and pathological brain development.

Disclosures: A. Papetti: None. **P. Jiang:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR261.24/B16

Topic: A.07. Developmental Disorders

Support: SUNDRY LAB STARTUP FUNDS

Title: Transcriptomic atlas of ASD risk genes in idiopathic autism reveals molecular convergence

Authors: *M. MAMUN[1], J. RAMESH[1], S. CHEN[2], A. RAJAN[3], S. CHETTY[4];
[1]Massachusetts Gen. Hosp., Boston, MA; [2]Ctr. for Regenerative Med., Massachusetts Gen. Hosp., Boston, MA; [3]Massachusetts Gen. Hosp., brookline, MA; [4]Psychiatry, Massachusetts Gen. Hospital/Harvard Med. Sch., Boston, MA

Abstract: Autism spectrum disorder (ASD) characterized by impairment in social communication and interaction, and repetitive or restricted patterns of behaviors or interests has typically been classified into syndromic and non-syndromic/idiopathic autism based on clinical criteria and disease etiology. The idiopathic ASD (iASD) is marked by the presence of these primary autistic features, however, the syndromic ASD involves presence of additional complications or broader neurological syndrome(s). Due to super-complexities of phenotypic heterogeneity and genetic architecture, 85% cases are of iASD with unknown disease etiology while only 10-15% are of syndromic ASD with known genetic etiology. Despite etiological heterogeneity in iASD, studies in molecular profiling revealed consistent patterns of transcriptomic dysregulation and enrichment of ASD risk genes in postmortem cortex of iASD. The genetic risk factors for syndromic ASD, by definition, can be traced and assessed at genomic levels, which could serve as strong candidates for study in iASD. To facilitate the exploration of transcriptomic signatures of ASD risk genes in the postmortem cerebral cortex of iASD and neurotypical controls, here we build up a publicly accessible database named ‘TARGAN’ (Transcriptomic Atlas of ASD Risk Genes in Non-syndromic Autism). Additionally, we analyzed the transcriptomics of ASD risk genes in postmortem brain tissues of ASD and neurotypical individuals as well as induced neuronal models of iASD-N (iASD with normal brain size) and iASD-DM (iASD with disproportionate megalencephaly). We show transcriptomic dysregulations of the ASD risk genes across the cortex with greatest differences found in primary visual and auditory cortex of iASD and 15qDup syndrome, and incremental transcriptomic perturbations are observed following disease severity in ASD. Co-expression network analysis reveals downregulation of neuronal and synaptic genes and alterations in cell-type-specific gene expression affecting inhibitory and excitatory neurons in the both ASD groups including ASD-N and ASD-DM. Upregulated DE ASD risk genes enriches for astrocytes and microglial cells of prefrontal cortex in the iASD. Enrichment analysis by expression of transcription factors (TFs) ranks SOX5 as a top enriched perturbed TF across the cortex of iASD and 15qDup syndrome as well as induced neuronal models of ASD-N and ASD-DM. Thus, our findings collectively highlight how transcriptomic perturbations of ASD risk genes converge into specific molecular pathologies across the cerebral cortex in iASD with an invaluable resource ‘TARGAN’ to advance disease biology of iASD.

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Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

Support: T32HD108079

Title: Molecular regulation of intellectual disability genes in the developing cerebral cortex

Authors: *T. S. FINN;
MCD Biol., Univ. of California Santa Cruz, Santa Cruz, CA

Abstract: Intellectual disability (ID) is a DSM-5 defined mental disorder affecting 2-3% of the global population. It is characterized by defects in intellectual functioning and behavior which arise during critical developmental periods. ID has been linked to some environmental factors but the predominant underlying causes are genetic in origin. It is estimated that up to 10% of human genes may play a role in ID but a common feature has yet to be elucidated. Due to the role of the cerebral cortex in higher order information processing, it is likely the genetic origins of ID arise during cortical development. Interestingly, a large portion of ID genes are regulated by Satb2, which serves as a master regulator of cortical cell fate specification. Additionally, *Satb2* haploinsufficiency results in a neurodevelopmental disorder called SATB2-Associated Syndrome which is characterized by developmental delay, severe intellectual deficiency, limited or absent speech, behavioral problems, and craniofacial abnormalities. Therefore, it is likely Satb2 plays a large role in ID gene regulation. Understanding the molecular mechanisms of Satb2 is critical for elucidating a common genetic origin of ID. We use Satb2 deficient mice as a model for ID to investigate the molecular mechanisms underlying chromatin structure, and gene expression, and how changes result in defective cortical architecture, excitatory neuronal projections, and electrophysiology. These findings suggest a larger role for Satb2 than initially thought and provide the foundation for further study in genetic factors of ID.

Disclosures: T.S. Finn: None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

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National Institute of Dental and Craniofacial Research Award 1R21DE032806-01A1
National Center for Advancing Translational Sciences UL1TR002014
ational Institutes of Health 2UL1TR002014-05A1
PSU IEE SEED grant
MRI/Huck Convergence SEED funding

Title: Behavioral Phenotyping and Transcriptomic Insights into Astrocytic RBM8A Haploinsufficiency in Neurodevelopmental Disorders

Authors: *T. PURI[1], J. MOTT[3], Y. MAO[2];
[1]Penn State Univ., State College, PA; [2]Biol., Penn State Univ., University Park, PA;
[3]Pennsylvania State Univ., University Park, PA

Abstract: Neurodevelopmental disorders pose significant challenges to central nervous system (CNS) function and motor skills, encompassing a spectrum of conditions with diverse etiologies. Among the molecular players implicated in these disorders, RNA Binding Motif Protein 8a (RBM8A) has emerged as a crucial regulator of gene expression. RBM8A orchestrates various post-transcriptional processes, including mRNA splicing, translation, and degradation, exerting profound effects on neurodevelopmental pathways. Astrocytes, a predominant glial cell type in the CNS, play critical roles in supporting neuronal function and regulating brain homeostasis. Despite their importance, the specific contributions of astrocytic RBM8A to neurodevelopment and behavior remain poorly understood. To address this gap, we generated a conditional heterozygous knockout (cKO) mouse model targeting Rbm8a, specifically in astrocytes. Molecular analyses confirmed decreased RBM8A expression in astrocytes of cKO mice, utilizing multiple techniques, including RT-PCR, Sanger sequencing, qRT-PCR, immunohistochemistry, and Western blot. Behavioral assessments encompassing a range of locomotor and anxiety-related paradigms were conducted to characterize the phenotypic consequences of astrocytic RBM8A haploinsufficiency. The locomotor activity of the mice was assessed using open-field tests, revealing hyperactive behaviors in both male and female cKO mice compared to controls. Furthermore, sex-specific changes in anxiety-related behaviors were observed, with cKO mice displaying alterations in anxiety levels in the elevated plus maze test relative to controls. Interestingly, other behavioral tests did not reveal significant differences in obsessive, repetitive, or depressive behaviors between experimental and control groups. Furthermore, to elucidate the molecular mechanisms underlying RBM8A's function in astrocytes, we employed RNA Immunoprecipitation Sequencing (RIP-Seq) on adult WT C57/B6 mouse brains, revealing1370 RBM8A-bound genes, identified through stringent criteria (fold change > 5, Q value < 0.05). Integration of behavioral phenotyping with molecular and cellular analyses offers insights into the role of RBM8A in astrocytes during neurodevelopment.

Disclosures: T. Puri: None. **J. Mott:** None. **Y. Mao:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR261.27/B18

Topic: A.07. Developmental Disorders

Support: NIH: 1R01NS129823-01
NINDS Diversity Supplement: 3R0NS129823-02S1
AHA: 23IPA1051611

Title: Caught Red Handed: A methodological advancement of two-photon imaging to quantify macrophage behavior in mouse embryos with IVH

Authors: *J. BENSON[1], M. ZAWADZKI[2,3], M. LEHTINEN[4];
[1]Boston Children's Hosp., Longwood, MA; [2]Harvard Med. Sch., Boston, MA; [3]Pathology, Boston Children's Hospital, Boston, MA; [4]Pathology, Boston Children's Hosp., Boston, MA

Abstract: A leading cause of morbidity in premature infants, post-hemorrhagic hydrocephalus (PHH) is a condition in which accumulation of cerebrospinal fluid (CSF) has been triggered by rupturing of capillaries in the germinal matrix that releases blood into the lateral ventricle. Permanent neurological deficits are caused not only by compression of brain tissue but also by red blood cell (RBC) products that may linger in the developing ventricles for days to weeks. Our histological examinations of embryonic mice 24 hours following intraventricular blood injections suggest that macrophages are likely to influence the severity of PHH by scavenging RBCs. This project's goal is to introduce a novel imaging methodology to visualize the behavior of Kolmer cells, macrophages that line the ventricles and the surface of the choroid plexus (ChP) in our established model of PHH in mice. Our preliminary data have confirmed that we can reliably dissect the cerebral cortex with minimal blood loss to visualize the choroid plexus and the associated macrophages at E15.5, and that embryos remain viable for at least one hour. We will use real time two-photon recordings to quantify changes to the morphology, mobility, and motility of choroid plexus macrophages in saline and RBC injected embryos. By analyzing the difference in macrophage activity in the absence and presence of RBCs we will be able to elucidate the contribution of macrophages to the pathophysiology of PHH in a mouse model analogous to the human condition. We expect to find evidence for scavenging RBCs, suggesting a novel treatment target.

Disclosures: J. Benson: A. Employment/Salary (full or part-time):; Boston Children's Hospital. **M. Zawadzki:** None. **M. Lehtinen:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR261.28/B19

Topic: A.07. Developmental Disorders

Support: R01MH129589
OSU Start-Up funds

Title: Deciphering the Impact of Maternal Stress on Fetal Brain Development and Placental Function in Mice Through Single-Cell RNA Sequencing

Authors: *B. VEROSKY[1], H. CHEN[1], T. L. GUR[2];
[2]Psychiatry and Behavioral Hlth., [1]The Ohio State Univ., Columbus, OH

Abstract: Maternal stress and related psychiatric disorders during pregnancy are associated with an elevated risk of psychopathology in the offspring. Utilizing a mouse model of maternal restraint stress, we have previously shown that prenatal stress causes deficits in social behavior and anxiety-like behavior in the offspring, which are partly mediated through immune disruptions in the fetus. However, the specific disruptions in the fetal brain induced by prenatal stress, as well as the underlying transmission mechanisms from the mother, remain unclear. Given that the placenta plays a critical role in shielding the fetus from perturbations in the maternal environment, its dysfunction likely has a significant role in facilitating stress-induced developmental changes. To investigate these effects, eleven-week-old pregnant C57BL/6 mice underwent daily restraint stress for two hours from gestational day (GD) 10.5 to GD16.5 or were left undisturbed (n = 3/group). One male and one female fetal brain per dam were collected immediately after the final stress exposure on GD16.5 for single cell RNA-sequencing (scRNAseq). Additionally, we collected fetal brains and placentas (n = 3 dams/group; 1 female and 1 male included from each dam) on GD17.5 for scRNAseq analysis to evaluate sustained transcriptional changes after stress exposure. Our findings indicate that prenatal stress significantly reduces the proportion of maternal macrophages relative to fetal macrophage populations in the placenta ($p < 0.05$). Gene set enrichment analysis of each cell cluster shows a significant decrease in interferon signaling across several immune cell types ($padj < 0.05$). Further, this analysis in both placental and fetal brain cells exhibited reduced oxidative phosphorylation in most neural and placental cell populations ($padj < 0.05$), suggesting perturbed energy utilization by the fetus. The transcriptomic profile of fetal brains shows an immediate stress response on GD16.5, which transitions to lasting changes by GD17.5 across multiple neural cell types. This study suggests that prenatal stress causes a transient response to stress with disruptions in interferon signaling and oxidative phosphorylation continuing after stress exposure, which may indicate these pathways as potential targets for mitigating adverse effects on fetal brain development and subsequent behavioral outcomes.

Disclosures: B. Verosky: None. **H. Chen:** None. **T.L. Gur:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR261.29/B20

Topic: A.07. Developmental Disorders

Title: Regulation of Neuronal Differentiation by the Lysine Methyltransferase Activity of NDD-associated Enzyme ASH1L

Authors: *J. HANQUIER, E. M CORNETT;
Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Aberration in neuronal differentiation has emerged as a major convergence point for neurodevelopmental disorders (NDDs). Neuronal differentiation is regulated in part by lysine methylation on histone and non-histone proteins; in fact, our quantitative mass spectrometry (TMT LC-MS/MS) analysis reveals lysine methylation events on non-histone proteins across distinct stages of neuronal differentiation. Moreover, lysine methyltransferases (KMTs) and demethylases (KDMs) are critical for proper brain development, though the mechanisms are not defined. Dysregulation of KMTs/KDMs has critical developmental and cellular consequences, and ~30% of these enzymes are associated with NDDs. The KMT ASH1L is associated with NDDs in human patients and mouse models, and haploinsufficiency of ASH1L results in developmental and differentiation defects. Studies have shown that ASH1L regulates neuronal differentiation, arborization, and synaptic pruning; however, mechanistic understanding of how ASH1L regulates these processes is not known. While a role for ASH1L KMT activity has been proposed, it has not been directly tested, and there are conflicting reports in the literature on the physiologically relevant substrates of ASH1L.
To address this gap in knowledge, we characterized differentiation of Lund human mesencephalic (LUHMES) neural progenitor cells (isolated from the mesencephalon of an 8-week old female; immortalized via v-myc overexpression) into dopaminergic-like neurons while inhibiting ASH1L KMT activity with a small molecule compound, AS-99. Disruption of ASH1L KMT activity resulted in a decrease in neurite branching, supporting a direct role for ASH1L KMT activity in the regulation of neuronal differentiation. To gain mechanistic insight into ASH1L regulation of differentiation, we have performed an exhaustive biochemical characterization of ASH1L methyltransferase activity using *in vitro* peptide arrays, revealing its substrate selectivity. From this selectivity profile, we have identified putative non-histone substrates of ASH1L, and future work will be geared towards delineating ASH1L substrates and determining the consequences of histone and/or non-histone substrate methylation on neuronal differentiation progression.

Disclosures: J. Hanquier: None. **E. M Cornett:** None.

Poster

PSTR261: Molecular Mechanisms of Neurodevelopmental Disorders

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR261.30/B21

Topic: A.07. Developmental Disorders

Support: R00NS112604

Title: Na+/k+ atpase shapes neurophysiology in the developing neocortex

Authors: A. C. BRIEGEL[1], ***K. SORIANO**[1], S. GOLINSKI[1], R. S. SMITH[2];
[2]Pharmacol., [1]Northwestern Univ., Chicago, IL

Abstract: During prenatal brain development, maintenance of sodium and potassium currents in neurons begins prenatally through expression of the Na+/K+ ATPase α-3 subunit (ATP1A3). Modern gene discovery approaches have identified ATP1A3 mutations resulting in human disease phenotypes spanning various stages of childhood development, including malformations of cortical development (MCDs); rapid-onset dystonia-parkinsonism; alternating hemiplegia of childhood (AHC); and childhood onset schizophrenia, among others. MCDs are the earliest presenting ATP1A3 neurological disease and reflect abnormalities generated during the mid-gestational stages of cortical development, a period with several overlapping developmental processes such as neural proliferation, migration, and differentiation. Interestingly, in children with ATP1A3 variants associated with postnatal disorders without MCDs (i.e. AHC), α-3 dysfunction also exists prenatally. To explore α-3's role in brain development, our study employed patch clamp and calcium imaging methods to investigate the influence of modulating Na+/K+ ATPase on the resting membrane potential (Vm) and neuronal activity patterns during normal cortical development. Our in vitro findings reveal that ATP1A3 function underlies both simple and complex neurophysiological patterns in the prenatal brain and provides a potential physiological basis for the broad spectrum of neurodevelopmental diseases associated with varying degrees of pump dysfunction and Vm regulation.

Disclosures: A.C. Briegel: None. **K. Soriano:** None. **S. Golinski:** None. **R.S. Smith:** None.

Poster

PSTR262: Acetylcholine: Ligand-Gated Receptors and Ion Channels

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR262.01/B22

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

Support: NIH (NS031744)

Title: Assembly of salt bridges and lipid membrane contacts control ionic current through single nicotinic receptor channels

Authors: *L. ALHALHOOLY, S. M. SINE;
Dept. of Physiol. and Biomed. Engin., Mayo Clin., Rochester, MN

Abstract: Ion channels rapidly transport small inorganic ions along their electrochemical gradients. For most ion channels, charged residues within the ion translocation pathway determine the rate and selectivity of ion transport. By contrast, we find that a conserved intramembrane salt bridge and an adjacent lipid interacting residue set the unitary current amplitude of the homomeric α7 nicotinic receptor. Combining single channel electrophysiology and mutagenesis we find that disrupting either structure individually has little effect on the current amplitude, but that disrupting both structures markedly reduces the current amplitude. By combining wild type and double-mutant subunits, we find that each double-mutant subunit contributes equally to the decrease in current amplitude. Moreover, to assess effects on pore diameter, studies of monovalent cations of varying size reveal marked changes in the rank order of ionic permeability in receptors with double-mutant subunits. The results reveal a system of interdependent salt bridges and contacts with the lipid membrane that establishes a pore diameter appropriate for physiologically permeant cations.

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

Poster

PSTR262: Acetylcholine: Ligand-Gated Receptors and Ion Channels

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR262.02/B23

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

Support: NIH Grant NS129676

Title: Identifying the binding site of a substituted carbamate positive allosteric modulator on the α4β2 nicotinic acetylcholine receptors

Authors: *A. D. ROLLING, N. SANCHEZ, J. GAONA, M. BERMUDEZ, A. K. HAMOUDA;
Univ. of Texas at Tyler, Tyler, TX

Abstract: The α4β2 nicotinic acetylcholine receptors play important role in higher cognitive functions such as learning and memory and are implicated in many neuropsychiatric diseases and

nicotine addiction. The pathophysiological importance of these brain receptors has encouraged efforts to develop pharmacophores that selectively potentiate α4β2 nAChRs (positive allosteric modulators, PAMs). A chemically synthesized carbamate (C9M; (R)-1-(3,5-diisopropyl-1H-pyrazol-1-yl)-3-methylbutan-2-yl 4-ethoxyphenylcarbamate; Bioorg Med Chem Lett. 2008 Oct 15;18(20):5643-7) has been identified as a PAM that preferentially potentiates the α4β2 nAChRs, showing promising pharmacological effects on both (α4)2(β2)3 and (α4)3(β2)2 nAChR isoforms. This kindled our efforts to identify its binding site in the α4β2 nAChRs using mutational analyses coupled with in vitro electrophysiological recordings from Xenopus laevis oocytes. So far, the effects of amino acid substitutions at 19 positions within the α4 subunit were tested. Point mutation α4C254S, α4S258M, α4C259F, or α4L291V almost completely inhibited C9M potentiation. These results predict C9M binding site within the transmembrane domain in close proximity to the α4 subunit M1 helix. The effect of additional amino acid substitutions on C9M potentiation are currently being used to precisely determine the mode of C9M binding and its interactions with nearby amino acid residues. This information will aid in the development of ligands with higher affinity and selectivity for the α4β2 nAChRs.

Disclosures: A.D. Rolling: None. **N. Sanchez:** None. **J. Gaona:** None. **M. Bermudez:** None. **A.K. Hamouda:** None.

Poster

PSTR262: Acetylcholine: Ligand-Gated Receptors and Ion Channels

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR262.03/B24

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

Support: NINDS: 1R16NS129676-01

Title: Initial evaluation of a positive allosteric modulator series for the(α4)3(β2)2Nicotinic acetylcholine receptor.

Authors: *J. D. JONES[1], J. GAONA[2], M. BERMUDEZ[3], A. SANCHEZ[2], R. BEAUDOIN[4], W. FELIX[5], G. THAKUR[5], B. BILL[2], A. K. HAMOUDA[6];
[1]Biol., The Univ. of Texas at Tyler, Tyler, TX; [2]Univ. of Texas at Tyler, Tyler, TX; [3]Univ. of Texas, Tyler, Tyler, TX; [4]Univ. of Texas at Tyler, Lindale, TX; [5]Northeastern Univ., Boston, MA; [6]Pharmaceut. Sci. and Hlth. Outcomes, Univ. of Texas At Tyler, Tyler, TX

Abstract: The (α4)3(β2)2 nicotinic acetylcholine receptor (nAChR) is the major heteromeric nAChR isoform expressed in the cortex that is believed to play a role in memory, cognition, and neuronal survival during aging. As such, selective potentiators of the (α4)3(β2)2 nAChR have potential therapeutic benefits in conditions associated with decline in the output of nAChR in the brain. In previous work, we have studied the pharmacology of the allosteric agonist CMPI (3-(2-

chlorophenyl)-5-(5-methyl-1-(piperidin-4-yl)-1H-pyrazol-4-yl) isoxazole) at the (α4)3(β2)2 nAChR and identified its binding site at the α4:α4 subunit extracellular interface. As part of our ongoing efforts to define structural features that confer CMPI binding selectivity at the α4:α4 interface, we synthesized and characterized the in vitro pharmacology of a series of CMPI analogs using whole-cell current recording from Xenopus laevis oocytes expressing (α4)3(β2)2 nAChR. Of the analogs synthesized so far, all have maintained selectivity for (α4)3(β2)2 over (α4)2(β2)4 nAChR. They did not potentiate (α4)2(β2)3 nAChR when co-applied with either 1 μM or 10 μM ACh, while analogs such as A12a, A12c, and A12e potentiated current induced by 10 μM ACh at the (α4)3(β2)2 nAChR with a fold potentiation greater than 4 at 1 μM. Additionally, we found that amino acid substitutions in the α4 subunit extracellular domain that decrease CMPI potentiation (e.g., G67M, E90I, Q150F) also abolish/reduce A12a and A12e potentiation of (α4)3(β2)2 nAChR. These results establish that the binding pocket of A12a and A12e is located at the α4:α4 extracellular interface of (α4)3(β2)2 nAChR. Furthermore, we found that substitutions at the pyrazole ring of CMPI (e.g., to a thiazole in A12a or imidazole in A12c and A12e) are tolerated within the α4:α4 binding pocket and maintain selectivity for (α4)3(β2)2 over (α4)2(β2)3 nAChR. Finally, we assessed the toxicology of the three derivatives *in vivo* (Danio rerio) and *in vitro* demonstrating that they are well tolerated. These results indicate that the CMPI binding pocket can accommodate ligand structural changes, allowing for the development of ligands that bind with higher affinity and selectivity at this positive allosteric modulator site. Ongoing experiments aim to characterize the pharmacology, application, and determine the concentration dependent effects of additional CMPI analogs.

Disclosures: J.D. Jones: None. **J. Gaona:** None. **M. Bermudez:** None. **A. Sanchez:** None. **R. Beaudoin:** None. **W. Felix:** None. **G. Thakur:** None. **B. Bill:** None. **A.K. Hamouda:** None.

Poster

PSTR262: Acetylcholine: Ligand-Gated Receptors and Ion Channels

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR262.04/B25

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

Title: The DNA of addiction: exploring the genetic factors in nicotine dependence and cessation

Authors: *D. NOTHAFT[1], A. BRASCH[2], R. BRISTOL[3], S. E. EATON[3];
[1]Arizona State Univ., Tempe, AZ; [2]Arizona State Univ., Norfolk, NE; [3]Dept. of Psychology, Arizona State Univ., Tempe, AZ

Abstract: The decline in cigarette smoking from 20.9% to 11.5% between 2005 and 2021 reflects a positive public health trend. However, there is an alarming surge in e-cigarette and nicotine pouch use among youth, with a 1,733% increase among high school students from 2011 to 2019. Nicotine, a highly addictive substance, is associated with increased risks of

cardiovascular, respiratory, and gastrointestinal disorders. Research into nicotine cessation is crucial for addressing the potential future health crises related to morbidity and mortality. Nicotine dependence can affect nearly anyone, but certain individuals struggle more with cessation, possibly due to upregulation of nicotinic acetylcholine receptors (nAChRs) in the brain stem and pre-frontal cortex (PFC). Exploring the genetic factors, particularly the CHRNA4 and CHRNB2 genes, could provide insights into nicotine use that is resistant to cessation. Utilizing resources like the *Allen Brain Cell Atlas*, we co-localized these genes to the brain stem and PFC. Further, a review of the literature and analysis of single-nucleotide polymorphisms (SNPs), behavioral survey, and electronic health record (EHR) data from the *All of Us* research study suggests that specific SNPs, such as rs2236196, may influence treatment-resistant nicotine dependence, while the SNPs rs1044396 and rs1044397 appear to be particularly responsive to Varenicline (Chantix) therapy. Additionally, the rs2072661 SNP appears consistently across all cohorts, which suggests it may play a role in nicotine dependence in general. While our findings are preliminary, they underscore the need for more comprehensive research on the genetic and epigenetic factors contributing to challenges in nicotine cessation.

Disclosures: D. Nothaft: None. **A. Brasch:** None. **R. Bristol:** None. **S.E. Eaton:** None.

Poster

PSTR262: Acetylcholine: Ligand-Gated Receptors and Ion Channels

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR262.05/B26

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

Support: NIH R01DA043567
NIH 5T32DA007027-48

Title: Site and outcomes of lynx1 allosteric modulation of α3β4-nicotinic receptors with relevance to nicotine use disorder

Authors: *D. KNEISLEY[1], H. OH[1], Y. CAO[3], W. IM[3], J. M. MIWA[4], P. WHITEAKER[2]; [1]Pharmacol. & Toxicology, [2]Dept. of Pharmacol. and Toxicology, Virginia Commonwealth Univ., Richmond, VA; [3]Biol. Sci. & Bioengineering, [4]Biol. Sci., Lehigh Univ., Bethlehem, PA

Abstract: Smoking, maintained by nicotine-seeking behaviors, is the leading cause of preventable death worldwide. In the brain, nicotine acts on nicotinic acetylcholine receptors (nAChR). The α3β4-nAChR subtype mediates aspects of nicotine withdrawal by modulating activity of GABAergic neurons of the interpeduncular nucleus. The prototoxin lynx1 is also highly expressed in these neurons, and lynx1 allosterically diminishes α3β4-nAChR response (differentially depending on receptor subunit ratio). In order to determine the interactions through which lynx1 exerts its effects, molecular dynamics simulations were used to identify

residues of α3β4 where lynx1 may interact. Two-electrode voltage clamp electrophysiology (TEVC) experiments were used to assess the effects of mutating the putative lynx1 allosteric binding site (at the α3/α3 subunit interface) on macroscopic receptor function. These mutations were expected to decrease receptor sensitivity to lynx1 effects and several did, confirming that lynx1 effects are mediated by this site. However, in some cases we observed increased sensitivity instead, and mutations resulted in diminished ACh-induced function in the absence of lynx1; combined, these observations may indicate competition between lynx1 and ACh at the α3/α3 site. To understand how these mutations alter function of individual receptors, we used cell-attached single-channel patch clamp electrophysiology to compare the properties of selected mutants to wild-type (WT) receptors. Lynx1 effects on the WT receptor include decreased single-channel conductance, shorter open bursts, and increased closed dwell times. Accordingly, mutants with diminished macroscopic function are expected to demonstrate less frequent and/or shorter bursting activity, decreased single-channel conductance, or longer closed times for their baseline function (in the absence of lynx1), contributing to their observed decrease in macroscopic function. With both our mutations and lynx1 diminishing receptor activity, possibly through related alterations in single-channel properties, addition of lynx1 to the mutated receptors is expected to further decrease measures of single-channel activity. The results of these experiments will help to elucidate the mechanisms by which the mutations and lynx1 interact to alter receptor function and lynx1 sensitivity at the α3/α3 subunit interface.

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

Poster

PSTR262: Acetylcholine: Ligand-Gated Receptors and Ion Channels

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR262.06/B27

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

Support: NIH intramural grant NS003135

Title: Nicotinic receptor-mediated high frequency bursting in dopaminergic axons produces refractory inhibition.

Authors: *P. F. KRAMER[1], F. CLEVER[2], A. YANEZ[3], Z. M. KHALIQ[4];
[1]Univ. of Michigan, Ann Arbor, Ann Arbor, MI; [2]HWNI Neurosci., UC Berkeley, Berkeley, CA; [3]NIH, Bethesda, MD; [4]NIH/ NINDS, Bethesda, MD

Abstract: Dopamine release from terminal axons of dopaminergic neurons in the striatum is crucial for movement, motivation, and proper function of the basal ganglia network. Dopamine transmission is characteristically signaled through alternating tonic and burst modes, as well as

transient reductions below baseline tonic levels that follows a transient increase (burst-pause). These fluctuations in dopamine release are regulated by both somatodendritic and axonal mechanisms. While much is known about the mechanisms in the somatodendritic region regulating release, much less is understood about mechanisms intrinsic to the axon. The nicotinic acetylcholine receptor (nAChR) can excite dopaminergic axons, sometimes to the point at which they fire an action potential in the axon to elicit dopamine release. Interestingly, nicotinic signaling has also been implicated in a frequency-dependent inhibition of dopamine release at action potential burst frequencies over ~25 Hz. Nicotinic receptors have therefore been hypothesized to act as a molecular "low-pass filter". Here we used calcium imaging and direct recordings of axonal voltage to examine that hypothesis. We find that, contrary to its role as a low-pass filter, acetylcholine activation of axonal nicotinic receptors can induce a high frequency burst of action potentials (over 100 Hz). This high frequency burst also puts dopaminergic axons into a brief refractory period that limits axonal excitability. In addition to firing action potentials, nAChRs also mediate a subthreshold depolarization in the axon. To test the effect of this subthreshold depolarization on excitability in isolation we locally pressure ejected ACh while recording axonal voltage. Simultaneously we injected current into the axon to produce an input/output curve in the presence and absence of nAChR activation. We find that the subthreshold depolarization has a minimal effect on excitability, slightly increasing the chance of firing an action potential. We therefore report a novel burst-pause mechanism of dopamine release that is intrinsic to the axonal compartment, and we confirm that subthreshold axonal nicotinic EPSPs are excitatory.

Disclosures: P.F. Kramer: None. **F. Clever:** None. **A. Yanez:** None. **Z.M. Khaliq:** None.

Poster

PSTR262: Acetylcholine: Ligand-Gated Receptors and Ion Channels

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR262.07/B28

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

Support: NIH/NIEHS 5K22ES011639
BYU internal funding

Title: Quantitative single-cell analysis of neuronal nicotinic receptor subunit mRNA expression in rat CA1 hippocampal interneurons

Authors: *S. SUDWEEKS;
Cell Biol. & Physiol., Brigham Young Univ., Provo, UT

Abstract: Hippocampal interneurons are a sparse, but very diverse population of cells. These GABAergic interneurons may be few in number, but they play an important role in regulating the

synchronous firing of the much more numerous hippocampal pyramidal neurons. Using single-cell quantitative PCR to analyze 93 individual rat CA1 hippocampal interneurons, we quantified neuronal nicotinic acetylcholine receptor (nAChR) mRNA subunit expression and detailed possible nAChR subtype combinations for the α2, α3, α4, α5, α7, β2, β3, and β4 subunits. We show that the majority of interneurons in the CA1 of the rat hippocampus contain detectable levels of nAChR subunit mRNA. Our results highlight the complexity of the CA1 nAChR population. Interestingly, the α3 nAChR subunit is one of the highest expressed subunit mRNAs in this population, while the α4 is one of the least likely subunit mRNAs to be detected in CA1 interneurons. The β2 nAChR subunit is the highest expressed beta subunit mRNA in these cells. Statistical analysis indicates that there are likely over 100 different nAChR subunit mRNA combinations expressed in rat CA1 interneurons. These results provide a valid avenue for identifying nAChR subtypes that may be effective hippocampus-specific pharmacological targets.

Disclosures: S. Sudweeks: None.

Poster

PSTR262: Acetylcholine: Ligand-Gated Receptors and Ion Channels

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR262.08/B29

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

Support: INBRE
IDSA

Title: 3-o-ethyl l-ascorbic acid is a potent and selective positive allosteric modulator of alpha9/alpha10, nicotinic acetylcholine receptors

Authors: *S. AKABERI[1,2], P. SAPKOTA[2], S. YEASMIN[2], J. OMAN[2], S. PASHIKANTI[2], M. K. SCHULTE[2];
[1]Biomed. and Pharmaceut. Sci., Idaho State Univ., boise, ID; [2]Biomed. and Pharmaceut. Sci., Idaho State Univ., Pocatello, ID

Abstract: This study investigated the functional effects of the Ascorbic acid analog, 3-O-Ethyl L-Ascorbic Acid (EA) on nicotinic acetylcholine receptors (nAChRs). L-Ascorbic acid has been identified as a positive allosteric modulator (PAM) on α9/α10 nAChRs (Boffi JC et al., 2013). This is of interest due to the potential for PAMs of this receptor in the treatment of hidden and age related hearing loss. In order to better understand the molecular interactions of ascorbate, we have initiated SAR studies of ascorbic acid potentiation. During this study, we evaluated one commercially available analog, 3-O-Ethyl L-Ascorbic Acid as a potential α9/α10 nAChR PAM. Using two electrode voltage clamp and nAChRs expressed in Xenopus laevis oocytes we

evaluated the effects of EA on α9/α10, α7, and α4/β;2 nAChRs. EA was found to selectively potentiate α9/α10 nicotinic receptors stimulated by acetylcholine in a concentration-dependent manner (EC50 = 0.18 μM, ΔImax (acetylcholine) at 1μM EA =236%). This potency is over 1600X greater than L-Ascorbic acid (EC50 = >300 μM). EA thus emerges as a novel candidate for development of α9/α10 PAMS. Further research into EA's mechanisms, pharmacokinetics, and clinical efficacy is necessary to fully uncover its potential. We are also currently evaluating EA for its antioxidant properties and its interaction with ascorbate transporters.

Disclosures: S. Akaberi: None. **P. Sapkota:** None. **S. Yeasmin:** None. **J. Oman:** None. **S. Pashikanti:** None. **M.K. Schulte:** None.

Poster

PSTR262: Acetylcholine: Ligand-Gated Receptors and Ion Channels

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR262.09/B30

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

Support: NIH Intramural Grant 1ZIANS003137

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

Authors: *J. ROSENTHAL[1], Q. YUAN[1], J. LI[2], G. YANG[1], J. YIN[1], D. ZHANG[1,3], Y. LI[1,3]; [1]Natl. Inst. of Neurolog. Disorders and Stroke, NIH, Bethesda, MD; [2]Janelia Res. Campus, HHMI, Ashburn, VA, ; [3]Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: Synaptic diversity which arose during evolution of the central nervous system (CNS) has led to different synapse types utilizing both shared and distinct proteins at their respective postsynaptic compartments. Here, we have employed proximity labeling in the *Drosophila* CNS to probe the local protein network at cholinergic postsynaptic sites containing the nicotinic acetylcholine receptor (nAchR) subunits Dα1 and Dα6. Our results support the existence of a context-independent “core” proteome as well as a large suite of proteins which are preferentially or exclusively enriched in either the larval or adult stage. We also demonstrate that the proteome remains mostly intact in nAchR mutants, *via* subunit compensation, whereas mutants of the RhoGEF *Sif* lack this compensation and possess a highly disrupted nAchR interactome. Together, these findings support a unique molecular identity for the central cholinergic synapse and will inform future analyses regarding synaptic evolution and organization.

Disclosures: J. Rosenthal: None. **Q. Yuan:** None.

Poster

PSTR262: Acetylcholine: Ligand-Gated Receptors and Ion Channels

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR262.10/B31

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

Support: CIHR MOP 89825
CIHR PRJ 178372

Title: Chrna5 nicotinic receptors in the mouse interpeduncular nucleus, an optogenetic, electrophysiological, and pharmacological interrogation

Authors: *C. L. RICHTER GOREY[1], S. SIVAKUMARAN[1], Y. LIU[3], E. K. LAMBE[2]; [2]Physiol., [1]Univ. of Toronto, Toronto, ON, Canada; [3]Univ. of Toronto, Dept. of Physiol., Toronto, ON, Canada

Abstract: The α5 subunit of nicotinic acetylcholine receptors (nAChRs) plays a key role in cholinergic signaling relevant to executive function, mood regulation, and addictive behaviour. Previous studies underscore its impact in maintaining a rapid and desensitization-resistant cholinergic response in the cortex. The expression of the α5 gene, Chrna5, is strongest in the interpeduncular nucleus, but its local contributions to endogenous cholinergic signalling are not well understood. The interpeduncular nucleus contains GABAergic neurons that innervate targets in the serotonergic raphe and the cholinergic lateral dorsal tegmental nucleus. A key excitatory input to the interpeduncular nucleus is from the medial habenula via combined cholinergic and glutamatergic co-transmission. The interplay between these co-transmitters and the role of α5 nicotinic receptors are not well understood. While exogenous acetylcholine and nicotinic agonists directly depolarize interpeduncular neurons in a Chrna5 dependent manner, the electrophysiological impact of endogenously activated nicotinic receptors is less straightforward. Here, we utilize electrical and optophysiological methods to probe interpeduncular cholinergic modulation and to assess the impact of specific cholinergic manipulations (e.g. nicotinic allosteric potentiation and acetylcholinesterase inhibition). These manipulations in brain slices from compound transgenic wild-type and Chrna5 knockout mice are an important next step to clarify the impact of endogenous nicotinic signalling in the interpeduncular nucleus and its dependence on Chrna5.

Disclosures: C.L. Richter Gorey: None. **S. Sivakumaran:** None. **Y. Liu:** None. **E.K. Lambe:** None.

Poster

PSTR262: Acetylcholine: Ligand-Gated Receptors and Ion Channels

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR262.11/B32

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

Support: NIH P20GM103395
NIH R16GM150455

Title: Visinin-like protein-1 modulates α4β2 nicotinic acetylcholine receptor function and isoform expression

Authors: *S. M. SUAREZ[1], H. DANIELSON[2], M. M. WELTZIN[3];
[1]Chem. and Biochem., Univ. of Alaska, Fairbanks, AK; [2]Brown Univ., Providence, RI; [3]Chem. and Biochem., Univ. of Alaska Fairbanks, Fairbanks, AK

Abstract: Neurological conditions, like nicotine use disorder (NUD), are associated with disruptions in brain cholinergic tone, partly due to dysregulation of α4β2 nicotinic acetylcholine receptors (nAChRs). The predominant α4β2 nAChR subtype occurs in two isoforms with high and low sensitivity to agonists (HS and LS, respectively). Nicotine upregulates α4β2 nAChRs and enhances plasma membrane expression of the HS-isoform, contributing to overexcitation of brain reward pathways. Developing an efficacious NUD therapy may depend on reducing over activation of these α4β2nAChRs-expressing reward pathways. Visinin-like protein-1 (VILIP-1) is an endogenous calcium sensor that modulates α4β2 nAChR expression by interacting with the α4subunit. We hypothesize VILIP-1 modulates α4β2 nAChR function and isoform expression. *Xenopus laevis* oocytes were injected with concatenated (linked) α4 and β2 subunits to independently express either α4β2 nAChR isoform. Functional interaction of VILIP-1 was assessed using two-electrode voltage clamp electrophysiology. Acetylcholine (ACh) potency was measured by generating concentration-response curves, and receptor function was measured using the maximum receptor response. Our results show VILIP-1 does not affect HS or LS isoform ACh potency. However, VILIP-1 significantly attenuates LS isoform function, while having minimal effects on the HS isoform. VILIP-1 did not alter α7 nAChR ACh-driven function or potency, suggesting VILIP-1 is selective for the LS α4β2 nAChR isoform. As VILIP-1 enhances plasma membrane expression of α4β2 nAChRs, it may also modulate expressed α4β2 nAChR isoforms. Using individual α4 and β2 subunits to generate mixed populations of isoformexpression, and the α4(+)/β2(-) interface selective agonist Sazetidine-A, we evaluated the impact of VILIP-1 on α4β2 nAChR proportional isoform expression. Six days post c-RNA injection, oocytes exhibited approximately 20% HS-isoform expression. Interestingly, VILIP-1 was able to restore LS-isoform proportional expression. These results suggest VILIP-1 functional modulation may occur at the α4/α4 interface which is unique to the LS-isoform. Mutating the VILIP-1 myristoylation site (VILIP-1(G2A)) had minimal effect on either isoform, suggesting myristoylation may not be needed to drive functional modulation. In a live cell culture model using neuronal-like mammalian N2a cells, groups containing VILIP-1 enhanced α4 subunit expression at the plasma membrane. Our findings advance the understanding of intrinsic control mechanisms regulating nAChR function and expression and may contribute to the advancement of nicotine use cessation therapies.

Disclosures: S.M. Suarez: None. **H. Danielson:** None. **M.M. Weltzin:** None.

Poster

PSTR262: Acetylcholine: Ligand-Gated Receptors and Ion Channels

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR262.12/B33

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

Support: The Infectious Diseases Society of America
Idaho INBRE/COBRE

Title: Herpes virus 1 glycoprotein D interacts with α7 nicotinic acetylcholine receptors

Authors: *S. YEASMIN[1], K. SHARMA[2], C. NICOLET[2], A. RANJIT[2], D. XU[2], A. HABASHI[2], M. K. SCHULTE[2];
[1]Idaho State Univ., Pocatello, ID; [2]Biomed. and Pharmaceut. Sci., Idaho State Univ., Pocatello, ID

Abstract: This project investigates the hypothesis that part of the neurotrophic effect of Herpes virus1 (HSV1) may be linked to an interaction of herpes virus 1 glycoprotein D (HSV1 gD) with nicotinic acetylcholine receptors (nAChRs). nAChRs are ligand-gated ion channel receptors with important roles in both the central and peripheral nervous systems. Rabies virus and SARS-CoV-2 have been shown to interact with nAChRs, potentially through similarities in structure to the LY6 family of proteins, a similarity shared with alpha bungarotoxin (***α***;-Bgtx). HSV1 is another endemic neurotropic virus that has been linked to neurological diseases including encephalitis, and Alzheimer's disease. An In-silico structural homology search using the known α7 receptor antagonist, ***α***;-Bgtx identified structural homology between HSV1 gD and ***α***;-Bgtx, a homology shared with LY6 proteins. We used a combination of binding and functional assays to investigate the ability of HSV1gD to interact with nicotinic receptors and to determine if the homologous loop region is involved in this interaction. Surface Plasmon Resonance (SPR)studies showed a clear interaction between the HSV1 gD ectodomain and the acetylcholine binding protein from *Lymnaea stagnalis* (*L*- AChBP) in a 1:1 binding model (Kd = 2.12 x 10^{-6} M). A smaller fragment of the HSV1 gD ectodomain containing the homologous loop region (92AA) also showed an interaction with the *L*-AChBP with a similar affinity (Kd = 1.07 x 10-7 M). A synthetic peptide containing the α-Bgtx homologous loop region of HSV1 gD (24AA) also showed an interaction, albeit with lower affinity (Kd = 4.32 x 10-4 M). Two electrode voltage clamp studies of nAChRs expressed in *Xenopus laevis* oocytes were used to determine the functional effects of binding. The whole ectodomain of HSV1 gD inhibited agonist induced currents at sub micromolar concentrations (Ki = 0.243 µM), the 92AA fragment containing the homologous loop inhibited responses at slightly lower potency (Ki = 2.05 µM), and the short 24AA peptide containing this loop inhibited with even lower potency (Ki = 86.2 µM). These data support the hypothesis that

the neurotoxin-like loop region of HSV1 gD is capable of interacting with α7 nAChRs. Molecular docking studies of the HSV1 gD ectodomain suggest a putative binding site for HSV1 gD near the critical C and F loops of the nAChR at a location similar to ***α***;-Bgtx. These findings may help to understand the mechanism of action of HSV1 on the nAChRs and explore how HSV1 plays a role in Alzheimer's disease. Potentially similar actions of HSV1 and SARS-Cov2 may help explain the possible synergistic effects of these two viruses in the CNS.

Disclosures: S. Yeasmin: None. **K. Sharma:** None. **C. Nicolet:** None. **A. Ranjit:** None. **D. Xu:** None. **A. Habashi:** None. **M.K. Schulte:** None.

Poster

PSTR263: Astrocytes in Animal Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR263.01/B34

Topic: B.09. Glial Mechanisms

Title: Senescent astrocytes derived from iPSCs generate inflammation prone environment and they are more sensitive to cytokines inducing a neurotoxic state

Authors: *H. KOBAYASHI, T. WAKUI, H. KATO, S. ENDOH-YAMAGAMI;
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Abstract: Aging is the largest risk factor for the development and progression of neurodegenerative diseases, with cellular senescence being known as a hallmark of aging. Astrocytes have also been identified as contributors to neuroinflammation and neurodegenerative diseases through cellular senescence. However, acquiring human senescent astrocytes is challenging, leading to restrictions in investigating their role in neurodegenerative diseases. The invention of iPSCs has simplified the process of obtaining human brain cells, yet obstacles arise concerning aging as iPSCs undergo reprogramming, which rejuvenates the cells. In our study, we have successfully established iPSC-derived senescent astrocytes and evaluated the impact on neuroinflammation and neurodegenerative diseases. The iPSC-derived senescent astrocytes showed elevated expression of senescent markers, including p16, γH2AX, and SA-β-Gal. Furthermore, we established a tri-culture system of neurons/astrocytes/microglia, utilizing the senescent astrocytes, and evaluated the morphology of microglia. Notably, microglia on the senescent astrocytes showed reduced processes, suggesting a shift towards an inflammatory state. We subsequently evaluated the involvement of the senescence astrocytes in neurodegenerative diseases. It has been reported that neurotoxic A1 astrocytes are increased in neurodegenerative diseases. Hence, we compared the sensitivity of the senescent astrocytes and normal astrocytes to stimuli known to induce neurotoxic astrocytes, observing distinct differences between the two groups. Additionally, we are currently evaluating the impact of the senescent astrocytes on neuronal function by calcium imaging. In this study, we have

successfully generated the human iPSC-derived astrocytes expressing senescent markers. Furthermore, we observed that the senescent astrocytes made the environment tend towards an inflammatory state and they were more prone to transition into a neurotoxic state. Our senescent astrocytes will be useful for studying the mechanism of age-related neurodegenerative diseases and neuroinflammation.

Disclosures: H. Kobayashi: A. Employment/Salary (full or part-time):; FUJIFILM Corporation. **T. Wakui:** A. Employment/Salary (full or part-time):; FUJIFILM Corporation. **H. Kato:** A. Employment/Salary (full or part-time):; FUJIFILM Corporation. **S. Endoh-Yamagami:** A. Employment/Salary (full or part-time):; FUJIFILM Corporation.

Poster

PSTR263: Astrocytes in Animal Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR263.02/B35

Topic: B.09. Glial Mechanisms

Support: SENC Travel Grant 2024

Title: Astrocytic ensembles control cue-motivated behavior

Authors: *I. SERRA HUETO, J. QUINTANILLA, J. GARCIA-MARQUES, M. NAVARRETE;
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Abstract: To fully understand how information is processed in complex cell circuits, we need techniques able to specifically target and control the activity of the elements involved, including astrocytes. Currently available tools like optogenetics and chemogenetics affect the entire astrocytic population, making it difficult to target specific subsets. This lack of specificity hinders our understanding of the role of astrocytes in behavior.
We present a tool to translate the activity-mediated calcium signals of astrocytes into gene expression in a light-dependent manner, i.e. AstroLight. Using AstroLight in parallel with electrophysiology, pharmacology, fiber photometry and behavioral techniques, we demonstrate mice astrocytic involvement in motivated behaviors of the Nucleus Accumbens (NAc). First, we designed AstroLight vectors, validating their expression after viral infection. Then, using fiber photometry we monitored *in vivo* the astrocyte calcium dynamics in the NAc, demonstrating their involvement during a goal-directed behavioral task. Afterward, we used AstroLight to express channelrhodopsin (ChR2) in the active astrocytes related to reward consumption or exploratory behaviors. Three-dimensional quantification of the ChR2/AstroLight ratio across the NAc revealed astrocytic ensembles related to these different behavioral features. Finally, we modulated those ensembles through optogenetic stimulation, showing that activating the

astrocyte ensemble related to a specific reward recalls the direction of behavior towards that precise option.
Our results show AstroLight as a powerful tool to study astrocyte-neuron interaction's function with precise spatial and temporal control and reveal that astrocytic ensembles can impact neuronal activity and shape behavioral responses positioning them as relevant component of the circuit.

Disclosures: I. Serra Hueto: None. **J. Quintanilla:** None. **J. Garcia-Marques:** None. **M. Navarrete:** None.

Poster

PSTR263: Astrocytes in Animal Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR263.03/B36

Topic: B.09. Glial Mechanisms

Support: NIH Grant R01DA041208
NIH Grant MH083728
NIH Grant MH094268
Korea Brain Research Institute basic research program 24-BR-04-04

Title: NPAS3 regulates astrocyte bioenergetics: implication for cognitive dysfunction

Authors: *K. MURLANOVA[1], O. PLETNIKOVA[2], K. NOVOTOTSKAYA-VLASOVA[1], S. HUSEYNOV[1], J. KIM[3], M. PLETNIKOV[1];
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Abstract: Abnormal astrocyte bioenergetics has been implicated in cognitive impairment. Despite its name, the basic helix-loop-helix transcription factor neuronal PAS3 (NPAS3) is highly expressed in astrocytes, but its functions in astrocytes are still not well understood, including transcriptional regulation of metabolic pathways. Here, we assessed the contribution of NPAS3 to the astrocyte energy metabolism, neuronal functions, and behavior. Our unbiased transcriptional analysis identified NPAS3-dependent regulation of expression of *Slc25a18* that encodes glutamate carrier 2 (GC2) to transport glutamate over the mitochondrial inner membrane for oxidation in the Krebs cycle. Constitutive knockout of *Npas3* gene decreased RNA and protein levels of GC2 and was associated with reduced oxidative phosphorylation and decreased levels of lactate production and release in primary astrocytes. Over-expression of *Slc25a18* in the *Npas3*-deficient primary astrocytes restored the decreased lactate level. Astrocyte-specific knockout of the *Npas3* gene in the brain showed the same decrease in GC2 expression and

oxidative phosphorylation in astrocytes directly isolated from the brain as well as reduced lactate levels in brain tissues and cerebrospinal fluid. Astrocyte-restricted knockout of *Npas3* decreased neuronal excitability and reduced dendritic spine density of cortical pyramidal neurons as assessed in ex vivo. Both conditional transgenic and viral knockout of *Npas3* in frontal cortical astrocytes of adult mice produced impaired trace fear conditioning without altering their locomotor activity in open field or anxiety in elevated plus maze test. Single intraperitoneal injection of L- but not D-lactate (1mg/kg) rescued deficient trace fear conditioning. Our findings provide mechanistic insights in the regulation of astrocyte bioenergetics by NPAS3 to contribute to neuronal function and cognition.

Disclosures: K. Murlanova: None. **O. Pletnikova:** None. **K. Novototskaya-Vlasova:** None. **S. Huseynov:** None. **J. Kim:** None. **M. Pletnikov:** None.

Poster

PSTR263: Astrocytes in Animal Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR263.04/B37

Topic: B.09. Glial Mechanisms

Support: NIH R01NS115809

Title: Circadian-related sex differences in astrocyte morphology within the mouse substantia nigra pars compacta and its implications for Parkinson's disease

Authors: *D. DAS[1], C. RODRIGUEZ[1], K. LINARES[1], E. BANCROFT[1], R. SRINIVASAN[1,2]; [1]Dept. of Neurosci. & Exptl. Therapeut., Texas A&M Univ. Hlth. Sci. Ctr., Bryan, TX; [2]Texas A&M Institute for Neuroscience (TAMIN), College Station, TX

Abstract: Astrocytes, which are the most abundant glial cells in the central nervous system, have rapidly emerged as critical regulators of neuronal function during health and disease. The ability of astrocytes to govern neuronal function via their secretome, neurotransmitter re-uptake, calcium signals, and the release of ATP as well as cytokines is now well established. Importantly, these processes are highly dependent on brain region-specific morphological relationships between astrocytes and neurons. In this regard, our lab has previously shown that in the mouse substantia nigra pars compacta (SNc), S100B labeled astrocytic processes completely envelope the somata of tyrosine hydroxylase (TH) expressing dopaminergic (DA) neurons. In this study, we sought to assess if this unique morphological relationship between astrocytic processes and DA somata in the SNc changes in a circadian and sex-dependent fashion. Three to four-month-old male and female C57BL/6 mice, exposed to a 12:12 h light-dark cycle, were transcardially perfused at 9 AM (subjective day and "inactive period") and 9 PM (subjective night and "active period"), and midbrain sections were obtained and stained with TH and S100B.

We discovered that when compared to the 9 AM timepoint, at 9 PM, wrapping of S100B-containing astrocytic processes around SNc DA somata was significantly decreased by ~50 % only in male and not in female mice. This corresponded with a similar time-dependent decrease in S100B astrocytic process density across entire fields of view only in male mice. Surprisingly, at 9 PM, female, but not male mice demonstrated a significant ~30 % decrease in the intensity of S100B containing astrocytic processes across entire fields of view, as well as on SNc DA neuron somata. To elucidate the molecular mechanisms underlying these circadian-dependent structural changes in astrocytes, we focused on the 5-hydroxytryptamine (5-HT)-6 Receptors (5-HT6Rs) based on data from the Astrocyte RNASeq Explorer database that show high expression of 5-HT6Rs in striatal astrocytes. We found a ~ 50% higher number of 5-HT6Rs in astrocytes from male mice at 9 PM compared to 9 AM. In mouse primary midbrain neuron-astrocyte co-cultures expressing 5-HT6Rs, serotonin administration changed actin dynamics *in vitro*, which could be inhibited by pre-treatment with the selective 5-HT6R antagonist SB399885. Taken together, our results have important implications for understanding the role of circadian rhythms and sex-differences during the pathogenesis of Parkinson's disease.

Disclosures: D. Das: None. **C. Rodriguez:** None. **K. Linares:** None. **E. Bancroft:** None. **R. Srinivasan:** None.

Poster

PSTR263: Astrocytes in Animal Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

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Topic: B.09. Glial Mechanisms

Support: HKRGC-CRF-4012-22G
HKRGC-GRF 14115821
HKRGC-GRF 14112423
HKRGC-GRF 14102221

Title: Structural and Functional Plasticity of Cortical Astrocytes in Motor Learning

Authors: *Y. ZHENG[1,2], S. WANG[1,2], Y. KE[2], W. YUNG[1];
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Abstract: Motor learning is the process of acquiring and adapting to novel motor skills through repetitive practice. While previous studies on the mechanisms of motor learning mostly centered on neuronal plasticity, empirical evidence has shown that astrocytes are also potentially important in the regulation of motor learning due to their active roles in modulating synaptic transmission and neuronal communication. In this project, we investigated the role of astrocytes

in terms of their structural and functional plasticity during motor learning. Our results first demonstrate increased astrocytic volume in the peri-synaptic astrocytic processes (PAPs) and newly-formed tripartite synapses upon motor learning, together with a reorganized profile of glutamate transporters and mitochondrial morphologies in PAPs. We also monitored the calcium dynamics in M1 astrocytes using two-photon imaging and dissected the spatiotemporal distribution of Ca^{2+} signals in M1 astrocytes throughout the task training. Our results showed that abundant astrocytic Ca^{2+} activities were involved in the motor learning process and that a clear temporal pattern more concentrated around the movement window was gradually shaped with the progress of learning. These reorganized Ca^{2+} activities are more likely to occur within microdomains rather than somata, and are more likely to possess a propagating nature rather than being static. Together, we have provided strong evidence that astrocytes play an active role in the process of motor learning by revealing the substantial plasticity of astrocytes in terms of morphologies and Ca^{2+} activities.

Disclosures: Y. Zheng: None. **S. Wang:** None. **Y. Ke:** None. **W. Yung:** None.

Poster

PSTR263: Astrocytes in Animal Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR263.06/B39

Topic: B.09. Glial Mechanisms

Title: The AMPK dynamics in hypothalamic astrocytes determines circadian behavior in mice

Authors: *M. LUENGO MATEOS[1], A. GONZÁLEZ VILA[1,2], M. SILVEIRA LOUREIRO[3,1], Á. ESTÉVEZ SALGUERO[3], P. FERNÁNDEZ-SANMARTÍN[3], A. MOHAMMAD ALASOUFI[1,2], M. LÓPEZ[3], O. BARCA-MAYO[1];
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Abstract: The intricate interplay between metabolism and circadian physiology involves the molecular clock, a ubiquitous circuit present in every cell of an organism. This clock comprises rhythmic and self-sustained transcriptional-translational feedback loops, governing genes involved in rhythmic processes such as feeding behavior or sleep-wake cycles. Two pacemakers control circadian behavior and outputs: the suprachiasmatic nucleus (SCN) and the food-entrainable oscillator (FEO), the location of which remains unknown, synchronize with light and feeding signals, respectively. Astrocytes, serving as sensors of peripheral metabolic signals, act as competent oscillators crucial for generating and coordinating physiological and behavioral

circadian outputs. In this study, we investigate the role of astrocytic AMPK and the connection between metabolic homeostasis and the circadian clock.
We observed that rhythmic AMPK phosphorylation is endogenously controlled by the circadian clock in primary hypothalamic astrocytes and in the hypothalamus of mice in the absence of entrainment signals (light and food). This suggests that phosphoAMPK demonstrates an intrinsic, energy-independent rhythmicity. To evaluate the impact of rhythmic AMPK activation in astrocytes, we generated mouse models with deletion of the AMPKg2 or AMPKa1 subunits specifically in GLAST-positive astrocytes, resulting in constant activation or inactivation of AMPK during the circadian cycle, respectively. Notably, constitutive activation of AMPK in astrocytes impairs food anticipatory activity (FAA), while deletion of AMPK enhances FAA, indicating that modulation of AMPK signaling can regulate entrainment by feeding signals. Moreover, constitutive activation of AMPK in astrocytes in male mice increases the free-running period and decreases intercellular coupling within the SCN, whereas deletion of AMPK has the opposite effects. Intriguingly, both models result in a positive energy balance. Furthermore, employing virogenetic approaches and mouse models with deletion of the g2 or a1 subunits in SF1 neurons of the ventromedial hypothalamus, we found that ventromedial astrocytes, but not neurons, are integral to the FEO.
In summary, these findings underscore the crucial role of AMPK oscillation in astrocytes, highlighting its necessity in synchronizing circadian rhythms to both light and feeding cues.

Disclosures: M. Luengo Mateos: None. **A. González Vila:** None. **M. Silveira Loureiro:** None. **Á. Estévez Salguero:** None. **P. Fernández-Sanmartín:** None. **A. Mohammad Alasoufi:** None. **M. López:** None. **O. Barca-Mayo:** None.

Poster

PSTR263: Astrocytes in Animal Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR263.07/B40

Topic: B.09. Glial Mechanisms

Title: Astrocytic involvement in valence processing within the Dorsal Raphe Nucleus (DRN)

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Abstract: The Dorsal Raphe Nucleus (DRN) not only is the largest of the serotonergic nuclei containing approximately a third of all serotonergic neurons ($DRN_{5\text{-}HT}$), but it comprises also a wide variety of neuronal subpopulations, including dopaminergic cells (DRN_{DA}) and astrocytes (DRN_{ASTRO}), which may represent a source of local DRN modulation. The DRN participates in a wide range of behavioral responses related to the processing of aversive stimuli, leading to

anxiety state. While the role of $DRN_{5\text{-}HT}$ and DRN_{DA} in valence processing is emerging, the contribution of DRN_{ASTRO}, as well as the underpinning cellular mechanisms, remain elusive. Our unpublished findings suggest that aversive stimuli prompt increased astrocytic Ca^{2+} activity and noradrenergic signaling in the DRN, with optogenetic stimulation of Locus Coeruleus (LC) projections inducing freezing-like behavior in mice. In this study, we aimed to investigate how DRN_{ASTRO} and regulation of their activity by LC-NE projections influence stimulus valence processing within the DRN circuitry. To this purpose, we combined intersectional viral strategies, *ex-vivo* two-photon Ca^{2+} imaging and *in vivo* fiber-photometry to investigate Pavlovian learning by presenting stimuli with diverse valence. These findings may offer insights into linking aberrant modulation within the DRN to the pathophysiology of comorbid affective symptoms in neurological disorders such as depression, anxiety, and neurodegenerative diseases, all of which entail astrocytic involvement.

Disclosures: S. Barile: None. **C. Rais:** None. **L. Nava:** None. **A. Caltabiano:** None. **R. Tonini:** None.

Poster

PSTR263: Astrocytes in Animal Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR263.08/B41

Topic: B.09. Glial Mechanisms

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Picower Institute Innovation Fund
Japan Society for the Promotion of Science

Title: The role of norepinephrine astrocyte signaling in reinforcement learning

Authors: *A. NATESAN[1], G. DRUMMOND[2], J. SHIH[2], M. CELOTTO[3,4], Y. OSAKO[2,5], S. PANZERI[3], M. SUR[2];
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Abstract: The locus coeruleus (LC) is a small brainstem nucleus that serves as the primary source of the neuromodulator norepinephrine (NE) in the brain. While LC neurons release NE

throughout the brain to regulate arousal and attention, recent work from our lab has also shown two distinct functions of LC-NE in reinforcement learning. In an instrumentally conditioned go/no-go task with graded auditory stimulus detection, phasic LC-NE activity is crucial for task execution under high uncertainty conditions and for optimizing task performance after surprising outcomes. LC-NE silencing during this task reduces the amount of information about the stimulus and the choice in individual neurons in the prefrontal cortex (PFC) and motor cortex (MC). Large LC-NE release following a false alarm trial affects neuronal population dynamics to improve discrimination between go and no-go stimuli on the next trial. While the effect of a previous trial's outcome on the following trial requires LC-NE signals to persist for several seconds, phasic LC-NE activity only lasts for tens of milliseconds. LC-NE has been shown to act on astrocytes, which can integrate and alter neuron signals over diverse timescales, suggesting a means by which information from phasic LC-NE signals can be sustained through the subsequent trial. Here, we explored the effects of LC-NE on astrocytes in the PFC and MC of mice performing our reinforcement learning task. Using 2-photon calcium imaging to record astrocyte and neuronal calcium dynamics in the MC, we find that astrocytes show long-lasting increases in calcium activity following a false alarm. Chemogenetic manipulation of astrocyte calcium using astrocyte-specific GqDREADD-CNO blocks the improvement in performance following a false alarm, suggesting that astrocyte signaling influences this behavioral outcome. To determine how astrocyte manipulations affect neuronal responses during the task, we used high-density neuropixels recordings to analyze PFC and MC neuronal activity while chemogenetically manipulating astrocyte calcium. Disrupting astrocyte calcium activity decreases neuronal encoding during the task. Finally, by knocking down alpha-1a-adrenergic receptors in PFC and MC astrocytes while imaging astrocyte and neuronal responses during the task, we are exploring the role of NE specifically on astrocytes during reinforcement learning. Taken together, our findings indicate a critical role for cortical astrocyte signaling in reinforcement learning.

Disclosures: A. Natesan: None. **G. Drummond:** None. **J. Shih:** None. **M. Celotto:** None. **Y. Osako:** None. **S. Panzeri:** None. **M. Sur:** None.

Poster

PSTR263: Astrocytes in Animal Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR263.09/B42

Topic: B.09. Glial Mechanisms

Support: NIH Grant R21NS097913
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NIH Grant R01NS121542

Title: Cerebellar astrocytes encode and regulate reward-associated behavior

Authors: *C. LI[1], C. SONG[2], W. LI[3];
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Abstract: The cerebellum, traditionally linked to movement, has recently surprised scientists with its involvement in cognitive processes. Astrocytes, previously considered supportive cells, are now recognized for their contributions to higher-order brain functions. We investigated their role in reward processing using fiber photometry in mice genetically modified to express the Ca^{2+} sensor GCaMP6f specifically in astrocytes. For the behavior task, mice entered the trigger and then reward zones to receive water rewards. Our preliminary data revealed a fascinating link between astrocytic Ca^{2+} dynamics and reward processing. Within each recording region of the cerebellar cortex, astrocytes displayed a combination of two distinct Ca^{2+} signaling patterns:
1. Slow Ramping Signal: This signal exhibited a complex relationship with reward processing and varied depending on the specific cerebellar region. In some regions, it showed a gradual rise in Ca^{2+} as mice approached the reward zone, followed by a decrease and a subsequent rise during licking. However, other regions exhibited the opposite trend. Interestingly, the timing of these alterations might be influenced by the learning process;
2. Fast Signal: This category encompasses two subtypes:
I. Spatiotemporal Signal: It likely reflected spatial or visual cues, with rapid Ca^{2+} transients upon entering the reward zone or leaving the trigger zone, suggesting a response to zone transitions;
II. Auditory Cue Signal: It showed rapid Ca^{2+} transients specifically when mice entered the trigger zone with a cued tone, potentially linking them to auditory cues or the task initiation.
Importantly, a single recording region exhibited only one type of fast signal occurring together with one slow ramping signal. Recordings from the norepinephrine (NE) sensor (GRAB_NE1m) mirrored the patterns observed in astrocytic Ca^{2+} signals, suggesting a potential causal link between NE release and these dynamics. Additionally, conditional knockout of D1 dopamine receptors specifically in cerebellar cortex astrocytes resulted in a significant decrease in the amplitude of the slow ramping signal, hinting at a role for D1 receptors in this process.
Finally, we employed chemogenetic tools to manipulate astrocytic activity in specific regions.This manipulation led to observable changes in reward behaviors, including fewer activated trials and reduced licking time.
Overall, our study provides compelling evidence that cerebellar cortex astrocytes actively contribute to reward processing by displaying distinct combinations of Ca^{2+} signaling patterns, highlighting the regional diversity of their roles in the reward circuitry.

Disclosures: C. Li: None. **C. Song:** None. **W. Li:** None.

Poster

PSTR263: Astrocytes in Animal Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR263.10/B43

Topic: B.09. Glial Mechanisms

Title: Contributions of mediodorsal thalamic astrocytes to reward learning

Authors: *K. MARSCHALKO[1,3], N. DAMIANO[4], A. HARTLE[5], K. RUNYON[2], W. M. HOWE[6];
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Abstract: The mediodorsal thalamus (MD) is a key relay between limbic circuits and the medial prefrontal cortex (mPFC). Output from the MD to mPFC is important for reward learning as well as decision making, and disruptions in these connections have been linked to disease states such as schizophrenia and depression. Previous studies on the function of the MD have focused on dynamic fluctuations in neuronal activity within input and output pathways. However, a growing literature emphasizes a key role for astrocytes in the control of complex behaviors through the regulation of synaptic communication and release of gliotransmitters. Astrocytes exhibit task-related fluctuations in intracellular calcium dynamics that may allow them to contribute to this circuitry on the synaptic and behavioral levels. To gain insight on the functional role of MD astrocytes, we designed a set of experiments to investigate astrocytic calcium dynamics during reward learning, paired with causal manipulations of their excitability, and their integration into local MD networks. In preliminary experiments, MD astrocytic calcium dynamics were directly measured in mice (n=7) using an astrocyte specific GcAMP and fiber photometry as mice were engaged in a Pavlovian cue-reward learning task. Analysis of task data revealed a transient increase in astrocytic calcium evoked by a reward-predicting light cue early in training. As mice learned the association between cue and reward, this astrocyte calcium response shifted to the time of reward delivery. Interestingly, this response was dependent on reward delivery and diminished during a single extinction session. We hypothesize that these learning-related responses in astrocytes are a key component of mediodorsal thalamic modulation of reward learning. Ongoing experiments will investigate the chemogenetic manipulations of astrocytes to determine their necessity during reward learning tasks as well as morphological analysis of astrocytes to see how they change over the course of the reward learning task.

Disclosures: K. Marschalko: None. **N. Damiano:** None. **A. Hartle:** None. **K. Runyon:** None. **W.M. Howe:** None.

Poster

PSTR263: Astrocytes in Animal Behaviors

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Program #/Poster #: PSTR263.11/B44

Topic: B.09. Glial Mechanisms

Support: ERC Grant 101087731
ISF Grant 2060/23

Title: Astrocytic role in spatially independent reward encoding

Authors: *Y. MOROSE, A. DORON, I. GOSHEN;
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Abstract: Beyond their homeostatic activities, astrocytes interact with their neighboring neurons and take part in information processing. Interestingly, it was recently found that hippocampal astrocytes exhibited persistent ramping activity when mice approached a reward in a familiar virtual reality environment. This effect was gone after a reward location shift or in a novel environment, and reestablished after training. These results indicate that astrocytes can encode a reward in an expected location, but it is unknown whether they can also do that in a non-spatial environment, for example an auditory one. To address this question, we trained mice to run on a treadmill in a linear track while hearing a gradually elevating pitch that was location independent, and to obtain water rewards coupled with the highest tone. Here we show that trained mice run slower on average as they approach the reward, and immediately after they receive it. The mice get better at performing the task as the training proceeds. We also refined the training method and the technical requirements needed on the experimental set during a session, to enable future real-time Ca^{2+} transients imaging in astrocyte somata and main processes. We hypothesize that astrocytes can encode the reward even when it, and the cues leading to it, are completely location independent. This further exploration will drive a considerable progress in our current understanding of the role of the hippocampal astrocytes in reward encoding.

Disclosures: Y. Morose: None. **A. Doron:** None. **I. Goshen:** None.

Poster

PSTR263: Astrocytes in Animal Behaviors

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Title: A multi-omic single-cell atlas of astrocyte diversity in the healthy and inflamed brain

Authors: *M. R. O'DEA, S. A. LIDDELOW;
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Abstract: Astrocytes are increasingly recognized as a diverse cell type composed of many distinct molecular subtypes. Several recent studies have defined these astrocyte subtypes by gene expression and found that many subtypes are localized in discrete areas of the brain; however, existing studies have reached only limited consensus as to what astrocyte populations exist across the mammalian brain. This may be because prior single-cell RNA sequencing studies typically exhibit poor astrocyte capture efficiency compared to the abundance of these cells in the brain and subsequently produce low numbers of astrocytes per sample. Together with the generally lower RNA content of astrocytes compared to other neural cell types, these factors greatly limit the power of single-cell sequencing experiments and amplify the artifactual effects of technical variation between datasets. Additionally, given the difficulty of purifying large numbers of astrocytes for sequencing, prior studies have been limited to single modality methods. Multi-modal single-cell profiling methods have the potential to improve the identification of astrocyte subtypes by providing orthogonal validation of discrete subpopulations and additional biological context for the gene expression observed in a single cell. Using paired single-nucleus RNA and assay for transposase-accessible chromatin (ATAC) sequencing, we profiled the transcriptomes and epigenomes of over 100,000 astrocytes across the adult mouse brain, both in healthy animals and in animals experiencing an acute inflammatory insult (intraperitoneal lipopolysaccharide injection), creating a multi-omic single-cell atlas of astrocyte diversity in the healthy and inflamed brain. This approach revealed both previously described and novel astrocyte subtypes, which we then mapped to distinct brain regions using MERFISH spatial transcriptomics. We further present a deep generative model to integrate our multi-omic astrocyte atlas with prior single-cell datasets, demonstrating our atlas can be used to improve identification of astrocyte subtypes in underpowered datasets. This integration method can also be used to impute missing gene expression or chromatin accessibility information from single-modality datasets, allowing other studies to leverage our atlas to predict additional genomic information about the cells in their own data. This work provides a new resource describing astrocyte diversity in both the healthy and inflamed brain and develops a model for dataset integration which will empower future studies of the roles of heterogeneous astrocyte populations in nervous system function and dysfunction.

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Poster

PSTR263: Astrocytes in Animal Behaviors

Location: MCP Hall A

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PAPIIT-DGAPA IA208120
PAPIIT-DGAPA IA208022
CONAHCYT PhD scholarship 788790

Title: Astrocytes activity in the prefrontal cortex determine the duration of working memory

Authors: *A. RIVERA VILLASEÑOR[1,2], R. FALCON MOYA[3], O. PEREZ[4], A. ARAQUE[5], M. LOPEZ-HIDALGO[2];
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Abstract: Introduction: Astrocytes integrate, process, and store information of the underlying neural circuitry into calcium activity (Mu et al., 2019) acting as a memory of the synaptic activity (López-Hidalgo and Schummers., 2014; Deemyad et al., 2018). This allows astrocytes to regulate neuronal activity on different time scales participating in cognitive functions such as working memory (WM). **Aim**: To evaluate the role of astrocytes in the prefrontal cortex in the duration of working memory. **Methods:** Male C57BL6/J water-restricted mice (3 months old) were injected into the mPFC with AAV5-GFAP-GCaMP6f and pAAV-GFAP-hM3D(Gq)-mCherry. Mice were trained to discriminate between two vibrotactile frequencies (20/80Hz) and to choose between the left or right nose poke, respectively, to get a reward (4μL of water). Once mice reached a performance of ~80% of correct trials, working memory was evaluated by modifying the delay time in the door opening (1, 3, 6, 9, 12, 15, 18, 25, 40, 60, and 90s). We used chemogenetic (DREADDs) to increase calcium levels in astrocytes of the prefrontal cortex. All the subjects were evaluated under three conditions: Control, saline, and in the presence of the DREADDs ligand, CNO. **Results**: In control conditions, working memory decreased as the delay time in the door opening increased, with random responses starting at 15s. Calcium activity in astrocytes induced by CNO administration significantly increased mice performance in the working memory task at delays of 6, 9, 12, 15, 18, and 25s (repeated measures ANOVA, *$p<0.05$). **Conclusion**: Increased calcium activity in astrocytes increases the duration of working memory in a vibrotactile discrimination paradigm. Keywords: Astrocytes, working memory,

calcium. **Acknowledgments:** work funded by CONAHCYT Ciencia de Frontera 171874, PAPIIT-DGAPA IA208120, IA208022, CONAHCYT PhD scholarship 788790 (A.R.V.).

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Poster

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Topic: B.09. Glial Mechanisms

Support: NIH Grant R01DA041208

Title: Loss of Mu Opioid Receptor in Hippocampal Astrocytes Leads to Neuroinflammatory, Neuronal and Behavioral Changes

Authors: *S. THOMPSON[1], S. HUSEYNOV[2], O. PLETNIKOVA[3], M. PLETNIKOV[4]; [1]Physiol. and Biophysics, SUNY Univ. at Buffalo JSMBS, Lancaster, NY; [2]Physiol. and Biophysics, State Univ. of New York at Buffalo, Buffalo, NY; [3]Pathology and Anatom. Sci., State Univ. of New York at Buffalo, Buffalo, NY; [4]Physiol. and Biophysics, State Univ. of New York, Univ. at Buffalo, Buffalo, NY

Abstract: Mu opioid receptor (MOR) coded by the *Oprm1* gene is the most abundantly expressed opioid signaling GPCR in the brain. Neuronal MORs have been implicated in the mechanisms of reward and affective states. Much less is known about the functions of MOR expressed on glial cells. We found that decreased expression of astrocytic MOR was associated with elevated oxidative phosphorylation and pro-inflammatory changes in astrocytes and microglia. These findings led us to hypothesize that pro-inflammatory changes in MOR-deficient astrocytes of the dorsal hippocampus could potentially affect activity of hippocampal pyramidal neurons and mouse behavior. Using AAV approach, we knockdown (KD) *Oprm1* in astrocytes of the dorsal hippocampus (dHip) and evaluated activity of dHip pyramidal neurons during social interaction in mice. KD decreased expression of MOR in astrocyte up to 60-70%. Using fiber photometry, we found that decreased expression of MOR in dHip astrocytes was associated with increased activity (Ca transients) of dHip pyramidal neurons in mice. During social approach, control mice exhibit decreased activity of neurons during investigation of the live mouse and increased activity during disengagement from this investigation (i.e., withdrawal). This pattern of neuronal activity was disrupted in mice with decreased expression of dHip astrocytic MOR. Compared to control mice, MOR KD also reduced social approach in mice treated with sub-threshold doses of the NMDA antagonist, MK-801. Our study indicates that decreased expression of astrocytic MOR in the dorsal hippocampus alters the activity of

pyramidal neurons during social approach and reduces social behavior in mice, possibly via affecting of secretion of glutamate by astrocytes. Our data suggests that astrocytic MOR might point to a new direction for treatment of abnormal social behaviors and affective states.

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Poster

PSTR263: Astrocytes in Animal Behaviors

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Topic: B.09. Glial Mechanisms

Support: Lafayette Parish Medical Society Endowed professorship
UL Lafayette undergraduate research minigrant
UL Lafayette GSO

Title: Fgfr1 inactivation in the central nervous system results in reduced lactation and altered tanycyte physiology

Authors: J. STAGRAY[1], **J. FISER**[2], C. HEALEY[2], A. FAUL[2], A. CHISTOSERDOV[3], J. MALAHMEH[2], *K. SMITH[2];
[1]Biol., Univ. of Louisiana at Lafayette, Lafayette, LA; [2]Dept. of Biol., Univ. of Louisiana at Lafayette, Lafayette, LA; [3]Univ. of Louisiana, Lafayette, Lafayette, LA

Abstract: Mouse models are a reliable model system for demonstrating behavioral and cellular phenotypes influencing maternal rearing of progeny. These behaviors and cellular phenotypes are governed through neuroendocrine functions. These endocrine functions relate to cognitive and emotional responses of the organism when they are subjected to environmental stressors. Poor maternal care can produce offspring that are more susceptible to various health conditions such as anxiety, depression, high blood pressure, and an increased rate of diabetes onset. FGFR1 is a receptor that is highly expressed in neural stem cells within the central nervous system (CNS) and in astroglia. FGFR1 is important for stem cell division, stem cell maintenance, neurogenesis, and tripartite synapses between glia and neuronal cell types. Within the 3rd ventricle (3V), tanycytes are distributed lining the ventral and medial walls. Tanycytes are a specialized astrocyte that possesses radial glial properties. Tanycytes can alter their phenotypes based on environmental stimuli to maintain homeostasis of the hypothalamus. We previously demonstrated that mice with an inactivation of *Fgfr1* in neural stem cells and their daughter cells results in an alteration of tanycyte morphology and proliferative capabilities. Our previous experiences with maintaining this mouse colony demonstrated an increase rate of pup mortality when mothers lacked *Fgfr1* compared to control littermate mothers without conditional

inactivation. In our current experiments, we examine the effects of *Fgfr1* inactivation on dams and their 1st litters. There was no observable behavioral difference when measuring nesting, exploring, feeding, or self-grooming. We found there was not a difference in the amount of time it took mothers to retrieve pups under stressful stimuli. We did find there was a distinction between groups with regards to the amount of progeny that possessed milk spots. This led us to evaluate lactation associated hormonal pathways involving prolactin and oxytocin. Prolactin has been found to interact with mammillary tissues to stimulate milk production and secretion. Meanwhile, oxytocin has been shown to induce maternal bonding associated behaviors towards progeny while also reducing memory associated phenotypes. Here we formally assess why mothers are not adequately feeding their progeny, and how this may molecularly be resulting in the increased pup mortality rates we have previously observed.

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Poster

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Topic: B.09. Glial Mechanisms

Support: Stanley Family Foundation

Title: Akap11 deficiency, a shared genetic risk for schizophrenia & bipolar disorder, dysregulates cyclic amp & lipid metabolism

Authors: *X. LIU, H. PRIBIAG, K. KIM, D. GRAYKOWSKI, B. SONG, J. LEVIN, M. H. SHENG;
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Abstract: A-Kinase Anchoring Protein 11 (Akap11) has been identified as a shared risk gene for Schizophrenia (SCZ) and bipolar disorder (BP), yet its specific role within the brain remains poorly understood. To gain mechanistic insights into Akap11 function, we conducted a comprehensive analysis involving single-cell RNA sequencing of mouse astrocytes, bulk RNA sequencing, and proteomics of cultured astrocytes, as well as metabolomics of mouse brain tissue and cultured astrocytes derived from Akap11 wild-type (WT), heterozygous (HET), and knockout (KO) mice. Our investigation unveiled several significant findings: (1) notable transcriptomic and proteomic alterations across various molecular pathways in Akap11 mutant mice and cultured astrocytes, (2) elevated protein levels of protein kinase A (PKA) subunits, (3) increased 3',5' cyclic AMP levels, (4) upregulation of lipid metabolic pathways, particularly fatty acid and cholesterol biosynthesis, and (5) accumulation of lipid species such as

lysophosphatidylcholine, phosphatidylcholine, and cholesterol esters. In addition, we provided cellular and biochemical evidence elucidating Akap11's involvement in regulating autophagy of PKA subunits, cAMP elevation, PKA activity, and lipid droplet accumulation. Furthermore, we demonstrated that Akap11 dysfunction in astrocytes impacts neuronal biology. These findings provide molecular insights into the complex cellular functions of Akap11 in the brain and its potential relevance to the pathophysiology of SCZ and BP.

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Poster

PSTR263: Astrocytes in Animal Behaviors

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Topic: B.09. Glial Mechanisms

Support: DA050180
TL1TR001437

Title: Importance of system xc-mediated astrocyte to neuron communication: implications spanning behavioral control to biological information processing

Authors: *G. J. SIMANDL[1,2], S. W. PECK[3], N. RADDATZ[3], S. BOSE[3], R. C. TWINING[3], B. MAUNZE[3], S. CHOI[3], D. A. BAKER[3];
[1]Marquette Univ., Milwaukee, WI; [2]Biomedical Sciences, Marquette University, Milwaukee, WI; [3]Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: Revealing the biological basis of behavioral control is key given the remarkable capacity for humans to display self-destructive behavior. Behavioral control is often depicted as the net integration of bottom-up and top-down processes. A compelling yet insufficiently explored idea is that higher-order cognition has an anatomical and a molecular basis. The premise for the latter is that evolutionarily new mechanisms were needed for the computational requirements of cognition but not needed for basic behavior regulation. This hypothesis can be tested by manipulating system x_c^- (Sxc), which is an evolutionarily new astrocytic glutamate release mechanism that regulates neuronal activity and contributes to a rodent model of maladaptive behavior - non-reinforced cocaine seeking. Here, we used a genetically modified rat model created to eliminate Sxc activity (MSxc rats) to determine which types of behavioral control are reliant on Sxc-mediated astrocyte to neuron signaling. To do this, MSxc and WT rat behavior were compared in paradigms that differentially require basic and complex behavioral control mechanisms. Adult male MSxc rats performed similarly to WT rats in simple tasks including visual/spatial discrimination, classical conditioning, operant responding, fear-

conditioning, hedonic-based feeding, cocaine self-administration, and anxiety-based impulse generation. In contrast, MSxc rats displayed significant differences from WT in gambling task, temporal/probabilistic discounting, and five-choice serial reaction task. Collectively, the behavioral abnormalities displayed by MSxc rats resemble those previously observed by others following impaired prefrontal cortical function. However, obtaining these results with a global manipulation of an astrocytic mechanism expressed throughout the brain is significant for several reasons. First, it builds upon evidence supporting the possibility that there is a molecular basis to higher-order brain function, which could lead to new approaches to understand and treat cognitive dysfunction. Second, it is relevant to the emerging understanding of astrocytes. Third, these findings may be pertinent to ideas being investigated by computational neuroscientists and others, including the suggestion that the unique intracellular signaling characteristics of astrocytes perform transformer-like information processing. Transformers "attend" to encoded information and assign weight to important items, while incorporating distinct temporal and spatial characteristics, similar to astrocytic maintenance of synaptic transmission.

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Poster

PSTR263: Astrocytes in Animal Behaviors

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Program #/Poster #: PSTR263.18/B51

Topic: B.09. Glial Mechanisms

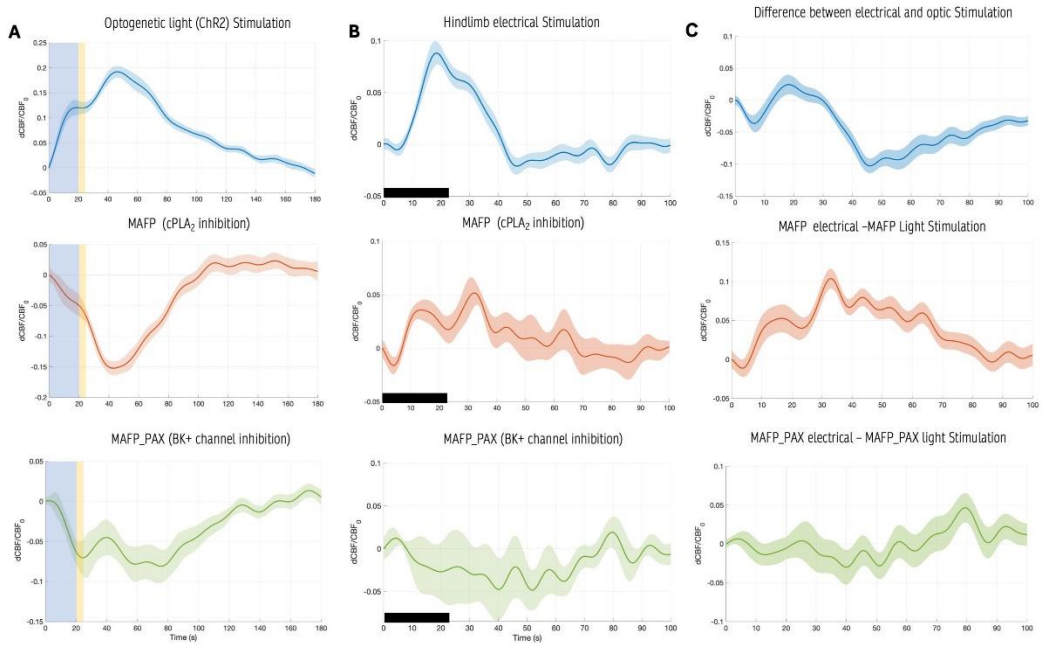
Support: Wallace Coulter Foundation

Title: Neurovascular coupling revisited by combining a pharmacological and optogenetic/hindlimb-stimulation approach

Authors: *N. PERROTTI, A. SUAREZ, J. J. RIERA;
Florida Intl. Univ., Miami, FL

Abstract: Previous studies define two contributors to neurovascular coupling: release of vasoactive substances from (1) neuronal terminals and (2) astrocytic Ca2+-signaling activating eicosanoid (cPLA2, Filosa et al. 2006) and K+-siphoning (BK channel, Metea et al. 2007) mechanisms. A recent study (Suarez et al. 2022, SfN), quantitaively evaluated contributor (2) using laser Doppler flowmetry (LDF) to measure regional cerebral blood flow (rCBF) changes evoked by channel rhodopsin activation selectively expressed in astrocytes after pharmacological manipulation. This study uses the same pharmacological agents to block contributor (2) while bringing some insights to contributor (1) by employing a hindlimb (HL) stimulation. We used WT mice (strain: C57BL/6J age: 20-35 weeks) undergoing electrical pulses (10 ms, 3 Hz, 2.2

mA, AM-System) for 25s to evoke a rCBF response like that observed by Suarez et al. rCBF was measured with LDF through an open craniotomy centered at the HL somatosensory cortex (S1) (N=5). We evaluated the effect of cPLA2 and BK channel inhibition individually and in union. Figure 1. Panel A: astrocytic CHR2-activation. Panel B: HL stimulation. (Top Panels) typical rCBF response after stimulus onset (Middle Panels) 15 min after (4uL) MAFP application evoked rCBFs show suppression of vasodilation under both paradigms (Bottom Panels) (4uL) Paxilline in conjunction with MAFP causes further sustained reduction in vasodilation. Panel C: Subtraction of WT response in electrical data from ChR2-Mlc1 response. (Top Panel) Time dynamic, intensity and scaling factor differences may explain the negative response shown when comparing stimulation paradigms under normal conditions. (Middle-Bottom Panels) Blocking the cPLA2 and BK channel removed most of rCBF suggesting it is mainly governed by astrocytic pathways. Future experiments entail blocking nitric oxide (NO) synthase with 7-nitroindazole to isolate neuronal-based NO contribution.



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Poster

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Support: NSF ID 000933778

Title: Astrocytic Contributions to Adaptive Stress Responses in the Medial Prefrontal Cortex

Authors: *N. LOOMBA[1], M. KWON[2], D. ZAIDI[2], Y. SIN[3], S. NASKAR[3], S. PATEL[3];
[1]Vanderbilt Univ., Nashville, TN; [2]Northwestern Univ. Feinberg Sch. of Med., Chicago, IL;
[3]Psychiatry and Behavioral Sci., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Astrocytic Contributions to Adaptive Stress Responses in the Medial Prefrontal Cortex Niharika Loomba[1], Michelle Wong[2], Danyal Zaidi[2], Yeonju Sin[2], Saptarnab Naskar[2], Sachin Patel[21]Vanderbilt Brain Institute, Vanderbilt University[2] Dept. of Psychiatry and Behavioral Sciences, Northwestern University Dysfunction in the medial prefrontal cortex (mPFC) is implicated in maladaptive fear responses to threats, a common symptom of various psychiatric disorders (Penninx et al., 2021). Most studies examining the role of the mPFC in responding to aversive stimuli have focused on neuronal mechanisms; however, non-neuronal cells compose up to half of the brain's cell population. Astrocytes, the most abundant non-neuronal cell type in the brain, interact with neurons in the tripartite synapse to regulate synaptic transmission. However, there is still a major knowledge gap in linking how these cells interact with neurons to drive maladaptive behaviors often displayed in psychiatric disorders. Using a combination of fiber photometry, optogenetics, and miniscope imaging in adult C57BL/6J mice, we investigated the role cortical astrocytes in responding to stressful stimuli to regulate defensive behaviors and coping mechanisms. We found astrocyte Ca2+ is correlated with a significant increase at the onset of struggle bouts in both immobilization stress and tail-suspension stress, suggesting a role in active coping mechanisms to stress. Furthermore, just prior to and during initiation of a struggle bout, neuron Ca2+ activity modestly rises and is immediately followed by a robust increase in astrocyte Ca2+. To further probe the relationship between neuron-astrocyte interactions, we optogenetically stimulated neurons while recording Ca2+ activity from astrocytes. Neuronal stimulation from frequencies ranging from 5-30Hz caused an increase in astrocyte Ca2+ activity. Together, our results indicate that astrocyte activity is correlated with active stress coping and neurons may interact with astrocytes to modulate behavioral responses to stressful stimuli. These findings will help elucidate the non-neuronal mechanisms underlying maladaptive behavioral responses present in psychiatric disorders.

Disclosures: N. Loomba: None. **M. Kwon:** None. **D. Zaidi:** None. **Y. Sin:** None. **S. Naskar:** None. **S. Patel:** None.

Poster

PSTR263: Astrocytes in Animal Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR263.20/B53

Topic: B.09. Glial Mechanisms

Support: Internal Support from Vassar College

Title: Astrocytic glutamine synthetase in spatial working memory in rats.

Authors: *C. A. MENGDEN, D. K. RAI-GERSAPPE, M. MARTINEZ, T. DOYLE, N. AJMAL, L. A. NEWMAN;
Psychological Sci., Vassar Col., Poughkeepsie, NY

Abstract: Astrocytes maintain neuronal homeostasis and regulate synaptic glutamate (Glu) and GABA, the major excitatory and inhibitory neurotransmitters in the central nervous system. Glu and GABA support learning and memory through synaptic plasticity. The astrocyte-specific enzyme, glutamine synthetase (GS), converts Glu and GABA to glutamine, which is necessary for maintaining neuronal Glu/GABA pools. We explored the role of GS in the hippocampus (HC) and prelimbic cortex (PrL), regions known to be involved in spatial working memory. We hypothesized that GS inhibition by methionine sulfoximine (MSO) will impair spatial working memory. Male and female Long-Evans rats were surgically implanted with HC or PrL bilateral guide cannulae. Following recovery, spatial working memory was assessed using spontaneous alternation: a twenty-minute, four-arm maze task measuring innate spatial navigation using extramaze cues. Fifteen minutes prior to each testing session, rats were intracranially microinjected with saline or MSO (0.4 mM, 2 mM, or 10 mM MSO) using a Latin square administration schedule. At least 48hr separated each session and novel extramaze cues were used each session. Percent alternation, the proportion of times when a rat entered all four arms within sets of five arm entries, was used to assess spatial working memory. Female rats' estrous cycle stage was assessed with vaginal smears. Preliminary results suggest that GS inhibition has a dose-dependent effect on percent alternation in the HC where there is a trend of spatial working memory improvement at low (0.4 mM) MSO concentration and deficits at high (10 mM) MSO concentration. The same effect is not seen in the PrL, where GS inhibition does not affect percent alternation. GS inhibition does not affect the number of arm entries, suggesting that motivation and movement are unaffected by MSO. Current results do not show any sex differences or effects of estrous cycle stage on percent alternation for either the HC or PrL group. This experiment supports a role for astrocytic GS in hippocampal working memory. The greater sensitivity of the HC to GS inhibition in spatial working memory may reflect a particular role for astrocytic support of spatial cognition in the HC when there is no delay involved in the task.

Disclosures: C.A. Mengden: None. **D.K. Rai-Gersappe:** None. **M. Martinez:** None. **T. Doyle:** None. **N. Ajmal:** None. **L.A. Newman:** None.

Poster

PSTR263: Astrocytes in Animal Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR263.21/B54

Topic: B.09. Glial Mechanisms

Support: Vassar College
Beckman Foundation Scholarship
Sigma Xi Grant
Carmichael lab

Title: Sex specific effects of chemogenetic activation of astrocytes when using microRNA targeting sequences on spatial working memory

Authors: *D. S. SERRANO[1], L. A. NEWMAN[2], Z. DING[1], T. DOYLE[1], M. MARTINEZ[1], J. BONANNO[3], J. LIN[4], J. D'ORAZIO[5], K. U. TANG[5], E. LI[6], S. FREILICH[7];
[1]Psychological Sci. and Neurosci. and Behavior Program, Vassar Col., Poughkeepsie, NY; [2]Dept. of Psychological Sci., Vassar Col., Poughkeepsie, NY; [3]Yale Univ., New Haven, CT; [4]Vassar Col., New York, NY; [5]Neurosci. and Behavior, Vassar Col., Poughkeepsie, NY; [6]NIH, Bethesda, MD; [7]Vassar Col., Poughkeepsie, NY

Abstract: Astrocytes influence metabolism, neurotransmitter activity, and ionic balance—fundamental components of synaptic plasticity necessary for memory. In our study, we sought to characterize the relationship between astrocytes and spatial working memory. We used a chemogenetic technique (Designer Receptors Exclusively Activated by Designer Drugs, or DREADDs) to activate astrocytes in the prelimbic cortex or hippocampus of Long Evans rats during a delayed spontaneous alternation (dSA) task. Bilateral injections of pAAV-GFAP-hM3D(Gq)-mCherry (DREADD-A), (PHP.eB)-GfaABC1D-DREADD hM3D-mCherry-4x6T-CW3SL (DREADD-miRNA-T) or a control virus (PHP.eB-GfaABC1D-smV5-4x6T7 or pAAV.GFAP.eGFP.WPRE.hGH) were given. Following two weeks to allow for expression, rats received intraperitoneal injections of either the corresponding hM3D(Gq) receptor agonist, compound 21 (C21), or the vehicle (saline) 30 minutes before dSA testing in a counterbalanced order with novel visual cues in each session. There was a significant interaction of drug administration, virus type, and sex on spatial working memory. Females with C21 activation who received the DREADD-A virus showed a significant impairment in SWM; this effect was not seen in the females who received the DREADD-miRNA-T virus or either of the control viruses. Males did not show improvements with C21 administration in the presence of controls or DREADD-A virus, but in those who received the DREADD-miRNA-T virus, we observed a trend of SWM improvement with C21 activation. Astrocytes expressing the DREADD-A virus showed greater GFAP branching than astrocytes that did not express the virus. This effect was greater in females suggesting the reactive states of the astrocytes might lead to some of the impairments in SWM in females. We are currently exploring whether the microRNA targeting cassette mitigates this effect. We also expect that the DREADD-miRNA-T will limit nonspecific expression of hM3D(Gq) as seen in previous studies in mice.

Disclosures: D.S. Serrano: None. **L.A. Newman:** None. **Z. Ding:** None. **T. Doyle:** None. **M. Martinez:** None. **J. Bonanno:** None. **J. Lin:** None. **J. D'Orazio:** None. **K.U. Tang:** None. **E. Li:** None. **S. Freilich:** None.

Poster

PSTR263: Astrocytes in Animal Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR263.22/B55

Topic: F.04. Neuroimmunology and Neurovirology

Support: NHLBI 1K01HL155240-01
NCATS 3UL1TR 002537-03W1
NCATS UL1TR002494

Title: Unraveling the Impact of Preeclampsia: Insights into Offspring Memory and Sex-Specific Glial Cell Dynamics

Authors: D. BRUMMOND[1], Y. LIU[1], O. BUNTON[1], E. JANKY[1], D. G. SCROGGINS[1], D. H. HECK[1], *S. M. SCROGGINS[2];
[1]Biomed. Sci., Univ. of Minnesota Med. Sch. Duluth Campus, Duluth, MN; [2]Biomedicanl Sci., Univ. of Minnesota Duluth Campus, Duluth, MN

Abstract: Preeclampsia (PreE), a hypertensive disorder during pregnancy, is a significant contributor to global fetal health risks. Among its long-term repercussions are increased chances of cognitive and behavioral issues in children born to affected mothers. Our research aims to delve into how PreE affects glial cell populations in offspring shortly after birth, and to assess its impact on offspring memory. To induce PreE in mice, we infused arginine vasopressin or saline to 10-week-old C57BL/6J virgin females via subcutaneous osmotic mini-pump throughout pregnancy. After natural parturition, we analyzed offspring at various timepoints post-birth for glia cell composition, brain-derived neurotropic factor levels, and concentrations of brain chemokines/cytokines. Brain tissue samples from the offspring were enzymatically digested to obtain single cell suspensions, which were then subjected to flow. Brain lysates were analyzed using a multiplex platform to explore changes in chemokines and cytokines associated with microglia/macrophages. Lastly, adult offspring were subject to the Plus-maze test. When feasible, we analyzed male and female offspring separately. Offspring from pregnancies affected by PreE exhibited reduced microglia and astrocyte populations one week after birth, regardless of sex. Interestingly, by the seventh week, we observed sex-specific alterations in microglia and astrocyte frequencies. In females, both microglia and astrocytes were significantly elevated in PreE-affected offspring. Conversely, in males, only astrocytes showed a significant increase at week seven. We found no significant differences in microglia/macrophage-associated brain chemokines or cytokines in males or females at week 12 post-birth. Moreover, only female offspring of PreE had elevated levels of brain-derived neurotropic factor at this timepoint. Lastly, offspring born from PreE-affected pregnancies displayed impaired spatial working memory in the spontaneous alternation Plus-maze task. Our results underscore the adverse effects of PreE on spatial working memory in offspring of affected pregnancies, as well as sex-specific fluctuations

in glial cell populations during early postnatal development. Ongoing investigations in our laboratory are aimed at broadening our comprehension of these developmental changes in glial cells and exploring potential alterations in function of microglia and astrocytes. Additionally, we are broadening our scope of behavioral and cognitive evaluations in offspring to deepen our understanding of how PreE impacts the health and development of offspring, both in the early stages and later in life.

Disclosures: D. Brummond: None. **Y. Liu:** None. **O. Bunton:** None. **E. Janky:** None. **D.G. Scroggins:** None. **D.H. Heck:** None. **S.M. Scroggins:** None.

Poster

PSTR264: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR264.01/B56

Topic: C.01. Brain Wellness and Aging

Support: Deutsche Forschungsgemeinschaft (Projektnummer 456368804)

Title: Quantification of mitochondrial redox status at the single-organelle level: interplay of mitochondrial morphology, age and gender

Authors: *S. POESCHEL, M. MUELLER;
Inst. Neuro- and Sensory Physiol., Univ. Med. Ctr., Göttingen, Germany

Abstract: Mitochondria, even on the level of an individual cell, show an enormous degree of morphological and functional heterogeneity. Furthermore, they dynamically adapt to the current metabolic status of their hosting cell. This also includes their redox conditions, as mitochondria are not only a prominent source of reactive oxygen species but also a target for oxidative stress. Much is still to be investigated about such subcellular redox-signaling in health and disease. Here we took advantage of a transgenic redox-indicator mouse model expressing roGFP (reduction-oxidation sensitive green fluorescent protein) in the mitochondrial matrix of excitatory projection neurons (*Wagener et al. 2016, Antioxid Red Signal 25: 41-58; Hanson et al. 2004, J Biol Chem 279: 13044-53*). Aiming at the visualization of individual neuronal mitochondria in acute brain tissue slices (400 µm thickness) and the quantification of their redox conditions by excitation-ratiometric two-photon microscopy, we chose to analyze axonal fiber tracts. After solving the issues of laser-radiation-mediated tissue bleaching and performing adequate redox sensor calibrations, we tested the mitochondrial redox-imaging approach in various brain regions (*corpus callosum, capsula interna, putamen*). Based on the promising results obtained, we finally focused on the putamen. To characterize the relationship between age, gender, morphology, and redox state of neuronal mitochondria, we compared mice on postnatal days p50, p150, p350, and p675. In general, the degree of roGFP oxidation was highest

in those mitochondria showing a more spherical morphology. A transition of mitochondrial morphology to a more spherical shape was provoked by exposure to 15 min of oxygen withdrawal. Furthermore, the fraction of spherical mitochondria increased with ageing. At age p350, this led to a significantly higher fraction of roundish mitochondria in female than in male mice. In addition, female mice at p150 showed a lower degree of roGFP oxidation than their male siblings. Both of these findings might be linked to the estrogen levels, which start to decrease in female mice with reproductive senescence around p300. In view of the pivotal role of mitochondria for cellular wellbeing and their involvement in various neurological conditions and disorders, this interplay of gender-, aging-, and mitochondrial-morphology related redox alterations may be crucial and deserve further detailed studies.

Disclosures: S. Poeschel: None. **M. Mueller:** None.

Poster

PSTR264: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR264.02/B57

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant (DP2GM146322)

Title: Identification of a highly dynamic pathway regulating mitochondrial RNA and cytosolic RNA crosstalk by super-resolution live microscopy

Authors: *A. WOODS, C. MOLAKAL, Y. C. WONG;
Northwestern Univ., Chicago, IL

Abstract: Mitochondria are highly dynamic organelles, which require proper regulation to maintain neuronal health. ALS is a devastating neurodegenerative disease characterized by the loss of upper and lower motor neurons. Of note, misregulation of both mitochondria and RNA have been proposed to contribute to the molecular mechanisms underlying ALS, suggesting that further understanding their crosstalk may shed light on ALS pathogenesis. Moreover, while mitochondria contain their own mtRNA, the mechanisms regulating mtRNA and its ability to be dynamically co-regulated with cytosolic RNA is still not well understood. Using live super-resolution microscopy over time, we identify a mitochondrial RNA binding protein which undergoes novel trafficking dynamics and phase separation dependent on mitochondrial function. Importantly, these dynamics were directly dependent on its ability to bind RNA. Interestingly, modulation of cytosolic RNA was further able to disrupt its proper trafficking dynamics. Finally, we find that trafficking dynamics of this mitochondrial RNA binding protein may be disrupted in models of ALS, potentially contributing to mitochondrial dysfunction in disease. In summary, our findings highlight a novel highly dynamic pathway for co-regulating

mitochondrial RNA and cytosolic RNA, which may have important implications for understanding mitochondrial homeostasis and ALS cellular mechanisms.

Disclosures: A. Woods: None. **C. Molakal:** None. **Y.C. Wong:** None.

Poster

PSTR264: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR264.03/B58

Topic: C.01. Brain Wellness and Aging

Support: DOD Grant PR211767

Title: In Vivo Mapping of Mitochondrial Function with 4D Oxy-wavelet MRI in Mitochondrial trans-2-enoyl-coenzyme A-reductase (MECR) Mice Modeling Mitochondrial Enoyl CoA Reductase Protein-Associated Neurodegeneration (MEPAN) Syndrome

Authors: D. CORTES[1], D. WEST[1], N. COULSON[1], K. SCHWAB[2], T. BECKER-SZURSZEWSKI[2], S. HARTWICK[1], D. MURDOCK[3], D. WALLACE[4], ***Y. WU**[1];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Univ. of Pittsburgh, pittsburgh, PA; [3]Children's Hosp. of Philadelphia, philadelphia, PA; [4]Children's Hosp. of Philadelphia, Swarthmore, PA

Abstract: To date, ~30 patients around the world have been identified with mitochondrial enoyl CoA reductase protein-associated neurodegeneration (MEPAN), a rare genetic disease characterized by childhood-onset of progressive dystonia, chorea, and/or ataxia, optic atrophy, and impaired speech due to dysarthria. MEPAN is caused by mutations in the mitochondrial trans-2-enoyl-coenzyme A-reductase (MECR) gene. *MECR* is part of the mitochondrial fatty acid synthesis (mtFAS) system. *Mecr* knockdown or deletion in cells resulted in severe loss of mitochondrial electron transport chain (ETC) components, reduced ETC activities, disturbed redox state and PPAR transcription system. There is no cure for MEPAN. Animal models are invaluable in modeling human diseases and therapeutic development. However, *there is a lack of sensitive and robust in vivo tools to map mitochondrial function in intact brains in a spatially specific manner, to guide mechanistic investigations and to evaluate therapeutic efficacy.* We successfully developed a novel functional MRI method, called 4D Oxy-wavelet MRI, which is capable of *in vivo* detection of mitochondrial ETC defects in intact live brains with high spatial resolutions. *A MEPAN* mouse model has been established carrying the patient variants of the 10-basepair deletion and 285-point mutation alleles. *In vivo* 4D Oxy-wavelet MRI found that live *MEPAN* mouse brains showed extensive *in vivo* mitochondrial abnormality in many brain regions, such as cerebellum, somatosensory cortex, isocortex, striatum, fimbria, thalamus, pallidum, hippocampus (dentate gyrus, CA2, CA3), and hypothalamus. Our preliminary testing showed that the *in vivo* 4D Oxy-wavelet MRI is capable

of spatial mapping of mitochondrial dysfunction in *MEPAN* mice. This can provide *in vivo* non-invasive surrogate biomarkers for robust and sensitive brain phenotyping in *MEPAN* mice, guiding mechanistic investigation and facilitating and accelerating preclinical therapeutic development.

Disclosures: D. Cortes: None. **D. West:** None. **N. Coulson:** None. **K. Schwab:** None. **T. Becker-Szurszewski:** None. **S. Hartwick:** None. **D. Murdock:** None. **D. Wallace:** None. **Y. Wu:** None.

Poster

PSTR264: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR264.04/B59

Topic: C.01. Brain Wellness and Aging

Support: National Research Foundation, Korea Grant 2020R1C1C1008033
Korean Diabetes Association Grant O.K., 2019F-4
Cooperative Research Program of Basic Medical Science and Clinical Science from Seoul National University College of Medicine and Seoul National University Hospital Grant 800-20240012

Title: Crosstalk between neuronal mitochondrial fission and IL-1b signaling in the progression of hypoglycemic neuronal damage with deficit in spatial memory

Authors: *J.-Y. JOO, S. LEE, M. SHIN, O. KWON;
Seoul Natl. University, Col. of Med., Seoul, Korea, Republic of

Abstract: Severe hypoglycemia (HPG) is a critical adverse effect of insulin therapy in diabetes, potentially leading to brain damage. Our study delves into the crosstalk between mitochondrial dynamics and neuroinflammation to understand hypoglycemic neuronal damage mechanisms and identify potential therapeutic interventions. Male C57BL/6 mice were fasted for 24 hours and HPG (below 20 mg/dL) was induced by intraperitoneal (i.p.) injection of insulin. HPG was induced for 5 hours and terminated by 25 % glucose solution i.p. injection. Mice were then sacrificed on day 1,4, and 7. Unbiased screening revealed the retrosplenial cortex (RSC) as vulnerable to HPG among other cortex regions, evidenced by elevated oxidative stress with 4-Hydroxynonenal immunohistochemistry on day 7. Progressive increases in oxidative stress and apoptosis, analyzed by terminal deoxynucleotidyl transferase dUTP nick end labelling staining, were observed in the RSC. While mitochondrial fragmentation, examined through transmission electron microscopy, immunoblotting, and immunohistochemistry, and inflammatory activation with IL-1β expression level were already significantly increased at day 1 whereas TNF-α and IL-6 expression levels were unchanged. Treatment with mitochondrial fission inhibitor (mdivi-1) or

IL-1 receptor antagonist (IL-1ra) effectively mitigated hypoglycemic neuronal damage. Notably, elevated mitochondrial fission was only significantly increased in neurons, not microglia and astrocytes, as analyzed by co-localization of phosphorylated dynamin-related protein, a marker of activated mitochondrial fission, with markers specific to neurons, microglia, and astrocytes. In vitro experiments with cell-type-specific regulation revealed that preventing mitochondrial fragmentation with mdivi-1 in SH-SY5Y, neuronal cells, and inhibiting IL-1 signaling with IL-1ra in either BV-2, microglial cells, or SH-SY5Y significantly prevented hypoglycemic damage. Morris water maze assessments confirmed the protective effects of these interventions against spatial memory impairment induced by HPG. Although the hippocampus is crucial for cognitive function, we found no significant increase in oxidative damage after HPG. No depressive or anxious phenotypes were observed in HPG-experienced mice compared to controls in the open field, tail suspension, and elevated plus maze tests. These results suggest that direct regulation of neuronal mitochondrial fission, combined with indirect modulation through the crosstalk between neuroinflammation with IL-1 signaling, could prevent hypoglycemic neuronal damage and spatial memory impairment.

Disclosures: J. Joo: None. **S. Lee:** None. **M. Shin:** None. **O. Kwon:** None.

Poster

PSTR264: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR264.05/B60

Topic: C.01. Brain Wellness and Aging

Support: MOST 110-2321-B-001-011 -
MOST 111-2321-B-001-010 -
NSTC 112-2321-B-001-008
MOST 110-2320-B-002 -025 -MY3

Title: The roles of the equilibrative nucleoside transporter-2 in regulating energy metabolism in the hippocampus and cortex of mice with LPS-induced neuroinflammation

Authors: T. CHU[1], C.-J. HO[2], K.-C. WU[3], Y. CHERN[4], *C.-J. LIN[1];
[1]Natl. Taiwan Univ. Sch. of Pharm., Taipei, Taiwan; [2]Sch. of Pharm., Natl. Taiwan Univ., Taipei, Taiwan; [3]Biomed. Translation Res. Center, Academia Sinica/School of Pharmacy, Natl. Taiwan Univ., Taipei, Taiwan; [4]Inst. Biomed Sci., Taipei, Taiwan

Abstract: Introduction Metabolic alteration in neuroinflammation is an important contributor to the pathogenesis of neurodegenerative diseases. The equilibrative nucleoside transporter 2 (Ent2) plays a critical role in maintaining the homeostasis of nucleosides, the building block of many intermediates involved in energy metabolism. Previously, Ent2 deletion has been demonstrated

to confer protection against lipopolysaccharide (LPS)-induced neuroinflammation and memory deficit. How Ent2 may regulate energy metabolism under neuroinflammation deserves to be further explored. **Objectives** This study was to investigate the impacts of Ent2 deletion on the metabolomic profile and mitochondria function in the cortex of mice with LPS-induced neuroinflammation. **Methods** $Ent2^{-/-}$ (C57BL/6-$slc29a2^{em1}$) mice were generated using the CRISPR-Cas9 technique. Both $Ent2^{-/-}$ mice and the wild-type (WT) littermate controls (10-13 weeks of age) were treated with 2.5 mg/kg (1.5×10^6 endotoxin units/mg) LPS via intraperitoneal administration for consecutive 7 days. Animals were sacrificed 24 hours after the last injection of LPS. Only male mice were used in this study to avoid gender effect. The metabolomic assay was conducted using UPLC-MS/MS analysis. Mitochondria function of brain slices was examined by the seahorse assay under glycemic and aglycemic conditions. **Results** In terms of oxygen consumption rate (OCR), LPS treatment significantly altered the mitochondria function by reducing basal respiration and ATP production under a glycemic condition and elevating basal respiration under an aglycemic condition in the cortex of the WT mice. Ent2 deletion reversed the change in basal respiration under both glycemic and aglycemic conditions in the cortex. On the other hand, LPS treatment tended to reduce the extracellular acidification rate (ECAR) in the cortex of the WT mice, whereas Ent2 deletion reversed the reduction in ECAR. The metabolomic analysis showed that LPS treatment increased the levels of inosine and the NAD+/NADH ratio in the cortex. Ent2 deletion normalized the changes in the levels of inosine and NAD+/NADH ratio. **Conclusion** These findings showed that LPS treatment can alter mitochondria function and energy metabolism in the cortex. Ent2 deletion may normalize the changes caused by LPS treatment.

Disclosures: T. Chu: None. **C. Ho:** None. **K. Wu:** None. **Y. Chern:** None. **C. Lin:** None.

Poster

PSTR264: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR264.06/B61

Topic: C.01. Brain Wellness and Aging

Support: College of Pharmacy, University of Rhode Island
College of Pharmacy Dean's Excellence Funding, University of Rhode Island
George & Anne Ryan Institute for Neuroscience, University of Rhode Island
First-Year Doctoral Fellowship, University of Rhode Island

Title: The impact of voluntary exercise on mitigating mtDNA mutations across different tissues

Authors: *H. M. TOBIAS-WALLINGFORD, S. BARTMAN, L. GASPAR, G. COPPOTELLI, J. M. ROSS;
George and Anne Ryan Inst. for Neurosci.; Col. of Pharm., Univ. of Rhode Island, Kingston, RI

Abstract: A wealth of studies supports the accumulation of mitochondrial DNA (mtDNA) mutations as one of the drivers of aging and age-related diseases. One of the best-studied models used to evaluate this relationship is the mtDNA mutator mouse, which expresses a proofreading deficient version of mtDNA polymerase-γ, (PolgA). This model accumulates mtDNA mutations and deletions, resulting in a premature aging phenotype, including reduced lifespan, weight loss, reduced fertility, canities (graying of fur), alopecia (hair loss), kyphosis (curvature of spine), osteoporosis, reduced subcutaneous fat, enlarged heart, anemia, sarcopenia (muscle wasting), and hearing loss. Previous work with this mouse model has investigated the impact of voluntary exercise on the aging phenotype and mtDNA mutation load. We showed that voluntary exercise improved the overall premature aging phenotype, decreased total mtDNA mutations, and normalized the proteomic landscape in this mouse model. It remains unclear, however, whether voluntary exercise directly decreased the mtDNA mutation load or rather stopped further accumulation, thereby preventing the progression of the aging phenotype. Using a Cre-LoxP recombination system to excise the mutated PolgA to stop *de novo* mtDNA mutations, we are studying the impact of voluntary exercise on pre-existing mtDNA mutations across different tissues. Ongoing studies utilizing methods, such as next generation sequencing, western blot, qPCR, and immunohistochemistry aim to further elucidate the role that exercise plays in counteracting the mitochondrial impairment associated with the aging process.

Disclosures: H.M. Tobias-Wallingford: None. **S. Bartman:** None. **L. Gaspar:** None. **G. Coppotelli:** None. **J.M. Ross:** None.

Poster

PSTR264: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR264.07/B62

Topic: C.01. Brain Wellness and Aging

Support: R01MH122706
R01MH116026

Title: Abnormal Brain Functions Introduced by Mitochondrial Disease: A Multimodal fMRI Study Across Sensory, Affective, and Cognitive Domains

Authors: *K. BO[1], C. KELLY[2], M. PICARD[3], T. D. WAGER[4];
[1]Dartmouth Col., Hanover, NH; [2]Columbia Univ. Irving Med. Ctr. (CUIMC), New York City,

NY; [3]Columbia Univ., New York, NY; [4]Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Cellular energy transformation by brain mitochondria is impaired by genetic mitochondrial DNA defects, causing mitochondrial diseases (MitoD) associated with neurological disorders. In the same way as brain lesions studies shed new light into the functional significance of anatomical brain regions, studying individuals with genetic lesions affecting mitochondria can shed light on their role in the pathophysiology of both normal and abnormal brain function. However, the impact of impaired mitochondrial energy transformation capacity on brain activity remains poorly understood. To address this gap, we scanned 29 participants with mitochondrial disease and 62 matched controls with functional magnetic resonance imaging (fMRI) during a series of tasks designed to probe multisensory visual and auditory perception, cold-induced pain, psychosocial stress (speech preparation), and working memory (N-back). Mass univariate analysis confirmed that all tasks evoked strong and significant activation of relevant brain areas, and the whole brain task activation map can be significantly decoded using support vector machine classifier (Paired classification accuracy >80%, and effect size cohen's d > 0.9 for all tasks), validating the quality of the data and analytic approach. Cross-validated Support Vector Machine-based classification revealed that brain activity during working memory task (60%, $p = 0.02$ in permutation test) and pain (58%, $p = 0.05$ in permutation test) significantly differentiated mitochondrial disease patients from controls, whereas multisensory and stress tasks were not different for healthy and disease. Finally, activation in an a priori working memory network was significantly correlated with a clinical measure of neurological symptoms (Columbia Neurological Score, $r = 0.25$, $p = 0.02$). These findings indicate that mitochondrial disease selectively impairs cognitive functions that demand high metabolic resources. This specificity suggests that functional brain processes related to working memory and pain are vulnerable to mitochondrial dysfunction.

Disclosures: K. Bo: None. **C. Kelly:** None. **M. Picard:** None. **T.D. Wager:** None.

Poster

PSTR264: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR264.08/B64

Topic: C.01. Brain Wellness and Aging

Support: NSF CAREER award number: 2045640
NIH Grant: R35GM150564

Title: Liproxstatin-1 attenuates neuronal lipid peroxidation by targeting GPX4.

Authors: *K. AGRAWAL[1], O. M. OGUNDELE[1], M. GARTIA[2];
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Abstract: Reactive oxygen species (ROS) are free radicals produced in normal cellular processes that drive mitochondrial energy respiration. In normal states, cells are equipped with radical scavengers that attenuate their negative impact on membrane integrity and cell health. In ferroptosis, iron (i.e., Fe^{2+}) reacts with hydrogen peroxide (H_2O_2) and produces hydroxyl radicals. ROS produced in this process propagates lipid peroxidation and causes cell death. This is implicated in the cause and progression of neurodegenerative disorders like Alzheimer's disease. Although the toxicity of ROS is established, some of the mechanisms that govern cell defense against ferroptosis-induced oxidative stress remain elusive. The current study aims to investigate a novel mechanism in neuronal cells that counteract ferroptosis-induced oxidative stress through the GSH-GPX4 pathway. In Neuro-2A cells pharmacological activation of lipid ROS was achieved by RSL-3 treatment in vitro. In ascending concentration gradients, there was a correlation between treatment concentration and cell survival assessed through a standardized MTT assay. However, treatment with Liprostatin-1, a molecule that enhances GSH (Glutathione)-GPX4 (Glutathione Peroxidase 4) levels, suppresses lipid ROS production and enhances cell survival of Neuro-2A cells. To verify that GPX4 is required for liprostatin-1-mediated cell survival, CRISPR-Cas9 knockout of GPX4 was performed in Neuro-2A cells, and further assessed in a cell survival assay with RSL-3 treatment. Detection of lipid peroxidation thresholds will be achieved by functional mitochondrial staining, glutathione, iron, cell ROX green, liperfluo, MDA assays. Annexin assay will also be performed to distinguish between ferroptotic and apoptotic cell death. In pharmacological experiments, the results showed that liprostatin-1 is protective against lipid ROS production that is induced by GPx4 inhibition through RSL-3. These results will be further verified through GPX4 knockout to ascertain that liprostatin -1 enhances radical scavenging downstream of GSH. Liprostatin-1 and other molecules that target GPX4 signaling are potential therapeutic targets for attenuating ferroptosis-linked neurodegenerative disorders, and oxidative stress in neurons.

Disclosures: K. Agrawal: None. **O.M. Ogundele:** None. **M. Gartia:** None.

Poster

PSTR264: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR264.09/B65

Topic: C.01. Brain Wellness and Aging

Support: FONDECYT Postdoctoral #3230131

Title: Abnormal mitochondrial quality control in human fibroblasts of APOE4

Authors: *S. A. NIÑO[1,2];
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Abstract: Ageing is the primary risk factor for most neurodegenerative diseases. APOE4 was the first genetic variation associated with decreased longevity. This association is likely linked to the neurotoxic effects of APOE4, namely its impact on mitochondrial plasticity, including interference of mitophagy, dysfunction of mitochondrial dynamics, and metabolic variations. These alterations affect mitochondrial quality control (MQC), which can lead to the accumulation of dysfunctional mitochondria and long-term impairment of cell function. However, the precise mechanism by which APOE4 affects mitochondrial dysfunction is unclear. Fibroblasts are connective tissue cells that show mitochondrial deficiencies similar to those of neuronal cells in patients with neurodegenerative processes; therefore, fibroblasts are proposed as a cellular model for studying mitochondrial biology in aging under a genetic context. This dissertation aims to determine the impact of APOE4 on the MQC of aged human fibroblasts. The project hypothesis focuses on APOE4 altering the rate of biogenesis/mitophagy, leading to mitochondrial dysfunction. Therefore, we performed primary cultures of aged human fibroblasts expressing the APOE3 allele, presenting sporadic AD, and with the APOE4. Human fibroblasts were obtained from Coriell Institute. Likewise, we focused on MCC to understand the impact of APOE4 on pathological aging. Alterations in the expression of TFAM and PGC1a transcripts, in addition to mtDNA damage, were observed by RT-qPCR. Mitochondrial membrane potential was analyzed by microscopy with TMRE, observing hyperpolarization by APOE4, while mitochondrial network formation was analyzed by producing a binary morphological skeleton with ImageJ software, where mitochondrial network formation was decreased for APOE4. Mitochondrial H2O2 levels were assessed through the HyPer7 sensor. Subsequently, the autophagic pathway was analyzed by transfecting fibroblasts with the mCherry-GFP-LC3 reporter, resulting in an apparent blockage of autophagic flux. To confirm autophagic blockade, western blots of essential mitophagy proteins were performed. We observed increased autophagic activity and early endosome formation in the APOE4. Our data further confirm the hypothesis of a prominent link between impaired mitophagy, impaired energy homeostasis, and endosomal system failures as a critical early event during APOE4-induced accelerated pathological aging. Combining central and peripheral measures of mitochondrial function can be more promising for future mechanistic disease stratification than applying a single biomarker.

Disclosures: S.A. Niño: None.

Poster

PSTR264: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR264.10/B66

Topic: C.01. Brain Wellness and Aging

Support: Fight for Sight UK
Glaucoma UK
Rosetrees Trust
Santen SenSyT PhD Studentship through UCL

Title: Atp synthase c-subunit leak channel is associated with aberrant cellular metabolism in glaucoma patients with the e50k optineurin mutation

Authors: *B. PETRITI[1,2,3], S. SUBRAMANIAN[1], P. LICZNERSKI[1], K.-Y. CHAU[3], E. A. JONAS[1];
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Abstract: The E50K Optineurin (OPTN) mutation is associated with normal tension glaucoma (NTG). Preliminary electron microscopy done by our group showed E50K mitochondria (Mt) to be fragmented, round, dilated and darker than controls. Such features are reported to be related to an immature type of metabolism, characterised by inner membrane proton leak, uncoupled phosphorylation, and increased glycolysis. We have shown an important source of leak channel activity to be composed of the c-subunit ring of ATP synthase. We hypothesised E50K Mt have a leak metabolism, in which assembled ATP synthase operates in reverse, consuming ATP to restore the inner membrane potential with the free c-subunit ring acting as the pore.We found that E50K cells have an accelerated cell growth rate and overall increased protein synthesis rate ($P<0.05$). Upon addition of oligomycin, E50K Mt depolarized whereas controls hyperpolarized, consistent with the E50K mutants manifesting reversal of ATP synthase into hydrolysis mode. E50K cells have increased OCR, glycolytic rate, NADH/NAD+ ratio, lactate levels and glycolytic ATP production (all $P<0.05$). E50K Mt exhibit an increase in ATP synthase c-subunit protein levels ($P<0.05$), while the beta subunit is relatively constant across cell lines. In conclusion, E50K mutant Mt depolarize upon oligomycin treatment suggesting the presence of an inner mitochondrial membrane proton leak with ATP synthase reversal consuming, rather than producing, ATP. To sustain the ATP demand, glycolysis is upregulated. The data support that the leak channel is via the c-subunit of ATP synthase. Presence of a leak leads to uncoupling of oxidation from phosphorylation and to an immature phenotype, as shown by increased cell and protein synthesis rate.

Disclosures: B. Petriti: None. **S. Subramanian:** None. **P. Licznerski:** None. **K. Chau:** None. **E.A. Jonas:** None.

Poster

PSTR264: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR264.11/B67

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant R01HD106197
NIH Grant P50HD105353
UW-Madison Vilas Faculty Award

Title: Metabolic differences in basal forebrain cholinergic neurons at birth in Down syndrome

Authors: *N. R. WEST[1], S. MACGREGOR[1], J. L. MARTINEZ[1], M. HOSSEINI[1], S. KNAACK[1], S. LIU[1], K. HANTHANAN ARACHCHILAGE[2], D. WANG[2], A. M. M. SOUSA[3], A. BHATTACHARYYA[4];
[2]Biostatistics and Med. Informatics, [3]Neurosci., [4]Cell and Regenerative Biol., [1]Univ. of Wisconsin-Madison, Madison, WI

Abstract: Cholinergic neurons of the basal forebrain (BFCNs) play an integral role in cognitive functions by providing the major cholinergic input into the cortex and regulate attention, learning, and memory. Degeneration of BFCNs correlates with memory loss in aging, and BFCN degeneration is exacerbated in Down syndrome (DS, T21), Alzheimer's disease (AD), and Dementia with Lewy Bodies, and Parkinson disease. However, little remains know about BFCN development. We performed single nucleus RNA-sequencing (snRNA-seq) of early human post-natal (age 0-2) unaffected basal forebrain tissue. snRNA-seq clustering analysis reveals cholinergic neurons, GABAergic interneurons, oligodendrocytes, astrocytes, and microglia are present in the basal forebrain. This is the first RNA-sequencing investigation of human basal forebrain tissue.

Little is known about early mechanisms contributing to the susceptibility of BFCNs in neurodegenerative diseases. We sought to determine if there are early characteristics of BFCNs that may promote their susceptibility later in life in the context of DS, where BFCN degeneration begins in the third decade of life and individuals have an early onset of AD. We performed single nucleus RNA-sequencing (snRNA-seq) of early human post-natal (age 0-2) DS basal forebrain tissue. snRNA-seq clustering analysis confirms that the same cell populations present in the unaffected control are present in DS, indicating no major changes in the cellular composition of the DS basal forebrain. Comparison of unaffected and DS snRNA-seq reveals genes and pathways related to cellular metabolism, protein homeostasis, and autophagy are altered in DS BFCNs. These results suggest that BFCN pathology begins during prenatal development in DS and may contribute to BFCN susceptibility later in life.

Because access to prenatal tissue is limited, we wanted to determine whether induced pluripotent stem cell (iPSC)-derived BFCNs are similar to in vivo BFCNs and if they can model BFCN development. Single cell RNA-sequencing (scRNA-seq) of T21 and isogenic control iPSC-derived BFCNs indicates that the iPSC differentiation results in a similar number of BFCNs from T21 and control cells. scRNA-seq also reveals that genes and pathways related to cellular metabolism, protein homeostasis, and autophagy are altered in T21 BFCNs. These results confirm that BFCN pathology begins early in DS development. The shared DEGs and pathways

between BF tissue and iPSC-derived BFCNs validates the use this iPSC model to study BFCN development in DS. Future work will utilize iPSC-derived BFCNs to probe the differences discovered in the RNA-seq analysis of DS tissue and T21 iPSC-derived BFCNs.

Disclosures: N.R. West: None. **S. MacGregor:** None. **J.L. Martinez:** None. **M. Hosseini:** None. **S. Knaack:** None. **S. Liu:** None. **K. Hanthanan Arachchilage:** None. **D. Wang:** None. **A.M.M. Sousa:** None. **A. Bhattacharyya:** None.

Poster

PSTR264: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR264.12/B68

Topic: C.01. Brain Wellness and Aging

Support: UNCF/Bristol-Myers Squibb E.E. Just Faculty Fund
Career Award at the Scientific Interface from the Burroughs Welcome Fund
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National Institutes of Health (NIH) Small Research Pilot Subaward (5R25HL106365-12) from the NIH PRIDE Program
Vanderbilt Diabetes and Research Training Center
Chan Zuckerberg Initiative (CZI) Science Diversity Leadership grant

Title: Three dimensional reconstructions of the amygdala and hypothalamus reveal changes in the MICOS complex and mitochondrial morphology in aging and Alzheimer’s disease

Authors: *B. Y. SHAO[1], A. G. MARSHALL[1], C. B. PALAVICINO-MAGGIO[2,3], D. TOMAR[4], A. KADAM[4], A. O. HINTON, Jr.[1];
[1]Vanderbilt Univ., Nashville, TN; [2]Basic Neurosci. Div., Harvard Univ., Cambridge, MA; [3]McLean Hosp., Belmont, MA; [4]Wake Forest Univ. Sch. of Med., Lewisville, NC

Abstract: The mitochondrial contact site and cristae organizing system (MICOS) complex have been implicated in regulating mitochondrial structure and pathology in models of aging (amygdala and hypothalamus) and Alzheimer's disease (AD), which is characterized by cognitive and motor impairments. However, the impact of MICOS and mitochondrial morphology on neuronal function in the amygdala and hypothalamus remains unclear.We examined postmortem brains from control and AD patients and confirmed abnormal protein accumulation. MICOS gene expression showed upregulation of MICOS (Mic27 and Mic10) in AD brains, suggesting a role in AD pathogenesis. Gene expression of MICOS and mitochondria-regulating genes (Opa1 and Drp1) was also performed in *D. melanogaster*, murine, and human models. In laboratory-evolved *D. melanogaster* brains, Opa1, Drp1, and MICOS (Mic19) was upregulated in the advanced aging cohort. In aged mice and humans, MICOS, Drp1 and Opa1 were downregulated

in the amygdala and hypothalamus, which is linked to lowered mitophagy.We also explored the impact of aging on mitochondrial morphology by performing three-dimensional (3D) reconstruction of mitochondria and axons from the amygdala and hypothalamus in 3-month-old versus 2-year-old mice (young versus old). We utilized serial block face-scanning electron microscopy and the Amira Software and found that mitochondria are increased in size and complexity in the amygdala and decreased in size and more spherical in the hypothalamus, suggesting better functionality in the hypothalamus. Mitochondrial transport in axons is important for maintaining neuronal function. Thus, we analyzed the 3D morphology of axonal and non-axonal mitochondria. The axonal mitochondria are larger with higher branching index in the hypothalamus of young mice and no changes in axonal mitochondria in the amygdala. This suggests higher functional capacity of mitochondria in the hypothalamus and age-related impairment of mitochondrial transport in the amygdala, common in neurodegenerative disease. We then explored the impact of mitochondria structural changes by analyzing calcium signaling in human embryonic kidney cells (HEK293), which express many neuronal genes. Knockout of MICOS genes *chchd6* (Mic25) and *mitofilin* (Mic 60) in HEK293 cells resulted in decreased calcium uptake rate and mitochondrial calcium capacity rate.

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Poster

PSTR264: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR264.13/B69

Topic: C.01. Brain Wellness and Aging

Support: the National Natural Science Foundation of China (32071040 to BL, 82071241 and 81871048 to LH)
Guangdong Basic and Applied Basic Research Foundation (2023B1515040019 to BL)
Guangdong Project (2017GC010590 to BL)

Title: Mitochondrial calcium uniporter (MCU) mediates aging-related abnormalities in metabolism-excitation coupling and cognitive impairment

Authors: *S. YANG[1], L. CHEN[2], L. HUANG[3], B. LI[4];
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Abstract: Aging is associated with cognitive decline; however, the precise neural mechanisms driving this connection remain ambiguous. We found that neuronal calcium transients in the cortical neurons of aged mice were significantly lower, indicating reduced neuronal excitability. To explore the influence of metabolism on this age-associated decline in neuronal excitability and cognitive impairment, we performed metabolomics analysis on the prefrontal cortex of aged mice. We found that purine catabolism was significantly enhanced in aged mice. The increase in purine catabolism led to a significant elevation of uric acid levels, effectively suppressing the firing frequency of action potentials. To investigate the effects of elevated uric acid on neuronal excitability and cognitive function *in vivo*, we increased the uric acid levels in the prefrontal cortex of adult mice with a diet rich in uric acid. We found that this significantly reduced the excitability of cortical neurons and resulted in apparent cognitive impairment. To further validate these findings, we employed febuxostat to inhibit uric acid production in aged mice. Intracerebroventricular injection of febuxostat effectively reduced uric acid concentrations, elevated neuronal excitability, and improved cognitive function in aged mice. These findings suggest that elevated uric acid levels are crucial in aging-related reductions in neuronal excitability and cognitive function. Furthermore, we examined the mechanism underlying the aging-induced abnormality in purine metabolism. Aging coincided with an increase in MCU expression in the mitochondria of cortical neurons. MCU upregulation resulted in heightened mitochondrial Ca^{2+} signaling, aberrant mitochondrial morphology, disrupted metabolic networks, increased purine catabolism, elevated uric acid production, reduced neuronal excitability, and impaired cognitive function in mice. In contrast, the reduction of MCU expression reversed these processes. These results suggest that MCU upregulation is a critical factor in aging-related impairments.

Disclosures: S. Yang: None. **L. Chen:** None. **L. Huang:** None. **B. Li:** None.

Poster

PSTR264: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR264.14/B70

Topic: C.01. Brain Wellness and Aging

Title: Analyzing stress induced ATF4 heterodimerization via proximity-labeling proteomics

Authors: *M. HATZIMINADAKIS, R. JAGANNATHAN;
The Taub Inst. for Res. on Alzheimer's Dis. and the Aging Brain, Columbia Univ., New York, NY

Abstract: The transcription factor (TF) ATF4 is the central downstream effector of the Integrated Stress Response (ISR) and a master regulator of cellular homeostasis in response to cellular stressors such as proteotoxic and oxidative stress. The ISR is a convergent signaling

pathway, funneling diverse upstream signals to a single shared effector, ATF4. ATF4 is expressed in multiple neurodegenerative diseases including Alzheimer's disease (AD) and Parkinson's disease (PD), where it can promote both pro-survival and pro-death transcriptional programs. How ATF4 transcriptional flexibility is regulated in response to different types of cellular stress, or in different neurodegenerative diseases, is not known. ATF4 is an obligate dimeric TF, meaning it must form a complex with other TFs in order to be transcriptionally active. We hypothesized that the transcriptional effect of ATF4 in different contexts may be regulated in part by its interactome, or the other TFs and regulatory molecules it complexes with. To test this hypothesis, we leveraged a technique called proximity-labeling (PL) proteomics, which is a rapid, sensitive technique for the identification of protein interactomes in which a protein of interest is tagged with an enzyme that chemically labels nearby proteins with biotin, followed by affinity purification and mass spectrometry to identify the labelled proteins. We developed an ATF4-PL construct utilizing the engineered ascorbate peroxidase, APEX2, and expressed this construct in HEK 293T cells exposed to different cell stress conditions, such as ER stress, proteasome inhibition, and mitochondrial stress. Through this approach, we have identified and validated several stress-responsive ATF4 heterodimerization partners, including the AP-1 TF, c-Jun, and others. Ongoing experiments are focused on elucidating how these different heterodimerization partners modulate ATF4 transcriptional activity in different stress conditions. In the future, we plan to apply similar methodologies to interrogate the ATF4 interactome in different neurodegenerative disease models. We expect that ATF4 combinatorial regulation may help explain clinical differences between and within neurodegenerative diseases and highlight novel targets for therapeutic intervention.

Disclosures: M. Hatziminadakis: None. **R. Jagannathan:** None.

Poster

PSTR264: Metabolism, Oxidative Stress, and Cellular Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR264.15/Web Only

Topic: C.01. Brain Wellness and Aging

Title: Mitochondrial metabolic efficiency is a regulator of tissue and neuronal aging in C. elegans

Authors: *A. ALI[1], S. BALAKRISHNAN[1], H. BAE[1], A. ABULIMITI[1], M. TSUJISHITA[1], C. WHITTLE[2], M. BARKOULAS[3], A. BROWN[4], P. J. SMITH[5], K. N. ALAVIAN[1];
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Abstract: Mitochondrial dysfunction is a key feature of the aging process, and multimorbidity associated with old age. While many studies have explored the role of the mitochondrial activity, biomass and dynamics in the ageing process, a direct connection between mitochondrial metabolic (coupling) efficiency and longevity or healthspan has not been explored. Our recent work indicates that a decline in mitochondrial metabolic efficiency, measured as the ratio of cellular ATP produced over oxygen uptake, with age, is central to impairments in key functions such as energy homeostasis and anaplerosis. We have observed that modulation of inner membrane ion leak currents is a key determinant of metabolic efficiency across life course in multiple organ systems. Our studies in C. elegans suggest that regulation of this mechanism is critical for maintaining physiological resilience and physical activity in old animals. This work also suggests that this mechanism of metabolic efficiency is required for the benefits of metabolic of exercise in a novel model of physical activity. We have also developed high throughput assays for identifying nutraceuticals and pharmacologial compounds that can specifically target this mechanism to enhance healthy ageing and extend lifespan.

Disclosures: A. Ali: None. **S. Balakrishnan:** None. **H. Bae:** None. **A. Abulimiti:** None. **M. Tsujishita:** None. **C. Whittle:** None. **M. Barkoulas:** None. **A. Brown:** None. **P.J. Smith:** None. **K.N. Alavian:** None.

Poster

PSTR265: Memory Circuit Alterations in Alzheimer's Disease

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR265.01/B71

Topic: H.08. Learning and Memory

Support: Alzheimer Society of Canada Proof of Concept Grant
CFI Leaders Opportunity Fund

Title: Abnormal hippocampal sharp wave ripple dynamics in TgCRND8 mice

Authors: *Y. SUN[1], S. CHEKHOV[2], S. MARGARIAN[2], P. E. FRASER[3,4,5], K. TAKEHARA-NISHIUCHI[2,1,5];
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Abstract: Alzheimer's disease (AD) is a progressive neurological disease associated with the decline in episodic memory. Throughout the hippocampus, transient bursts of oscillatory activity known as sharp wave ripples (SWR, 100-250 Hz) occur during NREM sleep and awake rest. Critical for memory consolidation, many studies using mouse models of AD reveal abnormalities in SWRs and other associated neural activity. Previously, we reported disruptions of dorsal CA1

spike dynamics associated with SWRs in the TgCRND8 model with AD-related amyloidosis and spatial memory deficits. To supplement these findings, we examined how hippocampal SWR dynamics and the cross-region interactions with cortical spindle and delta oscillations are affected during NREM sleep. Using wire electrodes, we recorded local field potential activity from the dorsal CA1 hippocampus and anterior cingulate cortex (ACC) of 3-5 month-old wildtype (WT) and TgCRND8 mice. Recordings were performed during rest periods before and after animals were subjected to an object location memory task. We extracted oscillatory patterns from the recorded signals and found no change in the incidence rate and amplitude of cortical spindle and delta events in the TgCRND8 mice compared to WT mice. Differences in incidence rate of SWRs however were dependent on the applied detection thresholds. TgCRND8 mice had a higher proportion of large amplitude SWRs in comparison to WT mice and the overall average SWR amplitude was increased. Furthermore, both SWR and delta incidence rate increased after experience comparably for the TgCRND8 and WT mice. We then examined the temporal coordination of these network patterns and found no difference in the event and amplitude cross-correlations of SWR-spindle, SWR-delta or delta-spindle between TgCRND8 and WT mice, or before and after experience. However, fine-tuned nesting of SWRs in spindle troughs was disrupted in TgCRND8 mice. SWRs from TgCRND8 mice co-occurred around spindle peaks rather than spindle troughs as observed in WT mice. Overall, aberrant oscillatory patterns in the TgCRND8 amyloidosis model were specific to hippocampal SWRs, suggesting a possible impairment in local inhibition that connects our previously found disruptions of SWR-spike phase modulation with abnormal SWR dynamics.

Disclosures: Y. Sun: None. **S. Chekhov:** None. **S. Margarian:** None. **P.E. Fraser:** None. **K. Takehara-Nishiuchi:** None.

Poster

PSTR265: Memory Circuit Alterations in Alzheimer’s Disease

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR265.02/B72

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NRF-2022R1A2C2009265
NRF-2022M3E5E8018421
NRF-2021R1A6A3A01086526
R01 MH099073
R21 AG067008-01

Title: Altered CA1 and CA3 place cell spiking patterns in APP/PS1 Alzheimer’s disease mice

Authors: *S. PARK[1], M.-J. PARK[2], E. KIM[3], H. RHIM[2], J. J. KIM[3], Y. HUH[4], J. CHO[5]; [1]Ewha Women's Univ., Seoul, Korea, Republic of; [2]Korea Inst. of Sci. and Technol., SEOUL,

Korea, Republic of; [3]Psychology, Univ. of Washington, Seattle, WA; [4]Catholic Kwandong Univ., Incheon, Korea, Republic of; [5]Brain Cognitive Sci., Ewha Womans Univ., Seoul, Korea, Republic of

Abstract: The hippocampus is susceptible to neurodegenerative disorders such as Alzheimer's disease (AD). The APPswe/PSEN1dE9 (APP/PS1) transgenic mouse model is widely used to study the pathology of AD and previous research established AD-associated impairments in hippocampal-dependent learning and memory. However, the neurophysiological mechanisms underlying cognitive dysfunctions in AD is less well known, so we investigated place cell activities of CA1 and CA3 hippocampal subregions in APP/PS1 mice. Behaviorally, APP/PS1 mice demonstrated impaired spatial recognition memory compared to wild-type(WT) mice in the object location test. Physiologically, the spatial representation ability of APP/PS1 CA1 and CA3 place cells deteriorated compared to the WT. The activity of the APP/PS1 CA1 place cells were more altered than the APP/PS1 CA3 place cells compared to the WT place cells. Burst firing patterns were also altered in both the APP/PS1 CA1 and CA3 place cells. Additionally, theta, low-gamma, and high-gamma rhythms were significantly attenuated in the CA1 and CA3 of APP/PS1 mice compared to the WT. Our results suggest that altered APP/PS1 CA1 and CA3 place cell activities, burst firing, and rhythms collectively contribute to impaired hippocampal-dependent spatial learning and memory in AD.

Disclosures: S. Park: None. **M. Park:** None. **E. Kim:** None. **H. Rhim:** None. **J.J. Kim:** None. **Y. Huh:** None. **J. Cho:** None.

Poster

PSTR265: Memory Circuit Alterations in Alzheimer's Disease

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR265.03/B73

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Natural Science Foundation of China (82371198)
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National Natural Science Foundation of China (81971004)
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Title: BACE1 in fast-spiking PV interneurons non-uniformly regulates the plasticity of hippocampal CA1 pyramidal neurons.

Authors: *Y. ZHENG, Z. CHEN, L. LI;
Capital Med. Univ., Beijing, China

Abstract: BACE1 is a promising drug target for treating Alzheimer's disease (AD). However, clinical trials of BACE1 inhibitors failed to show efficacy and even led to cognitive worsening.

This suggests that BACE1 may play a role in regulating cognition-relevant neural circuits. Recently, we reported that parvalbumin-positive inhibitory interneurons (PV INs) in hippocampal CA1 express BACE1 at a high level and the CA1 fast-spiking PV INs with BACE1 deletion exhibited an enhanced response of postsynaptic N-methyl-D-aspartate (NMDA) receptors to local stimulation on CA1 oriens, with average intrinsic electrical properties and fidelity in synaptic integration with our mouse strain with conditional knockout of BACE1 in PV neurons (PV-Cre;$BACE1^{fl/fl}$). Intriguingly, the BACE1 deletion reorganized the CA1 recurrent inhibitory motif assembled by the heterogeneous pyramidal neurons (PNs) and the adjacent fast-spiking PV INs from the superficial to the deep layer. Moreover, the conditional BACE1 deletion impaired the AMPARs-mediated excitatory transmission of deep CA1 PNs, while the superficial CA1 PNs exhibited a moderate increase in the AMPARs-mediated excitatory postsynaptic currents. Further rescue experiments confirmed that these phenotypes require the enzymatic activity of BACE1. In the present study, we further sought to investigate whether the fast-spiking PV INs regulate the long-term potentiation (LTP) of these two subtypes of CA1 PNs in a non-uniform way, and how BACE1 is involved in this microcircuit working model. Our results showed that BACE1 deletion in the PV INs decreased the high-frequency stimulation (HFS)-induced plasticity of deep CA1 PNs while increasing that of the superficial ones. We also used channelrhodopsin expressing AAV to manipulate the PV INs, specifically in acute hippocampal slices and found that BACE1 deletion made the PV INs lose a stable response to the HFS-LTP during the induction and expression of CA3-CA1 synaptic plasticity. Using an inhibitory optogenetic AAV-eNpHR, we explored the mechanism underlying how PV INs with or without BACE1 expression tune the CA3-CA1 plasticity. We observed that inhibiting PV INs temporally contributed to the HFS-LTP of CA3-CA1 projection. Our findings suggest that BACE1 helps maintain the homeostasis of the CA1 microcircuit motif in synaptic plasticity related to hippocampus-dependent learning and memory. We propose a neuron-specific working model of BACE1 in regulating CA1 microcircuits and enabling the hippocampal CA1 as a computational unit with spatial and temporal precision.

Disclosures: Y. Zheng: None. **Z. Chen:** None. **L. Li:** None.

Poster

PSTR265: Memory Circuit Alterations in Alzheimer’s Disease

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR265.04/B74

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSFC Grant 82371198

Title: Diversity of CA1 pyramidal plasticity modulated by fast-spiking PV interneurons in an AD mouse model

Authors: *Z. CHEN, L. LI, Y. ZHENG;
Capital Med. Univ., Beijing, China

Abstract: Alzheimer's disease (AD) is a refractory disease with complex pathogenesis. The synaptic degeneration and decline in plasticity in the hippocampal circuit play a central role in AD. Previous studies have elucidated the role of hippocampal long-projecting circuits in the AD brain. Nevertheless, how the hippocampal microcircuits are involved in the AD process is unclear. The known pieces of evidence revealed that the fast-spiking parvalbumin (PV) positive basket interneurons (PVBCs) connect the adjacent deep CA1 pyramidal neurons (deep CA1PNs) and superficial CA1 pyramidal neurons (superficial CA1PNs) to form a recurrent inhibitory microcircuit relevant to learning and memory. In this study, we examined how PVBCs regulate CA1 plasticity in an AD mouse model, taking into account neuronal heterogeneity in the CA1 microcircuit.We found that the single-cell long-term potentiation (LTP) of superficial CA1PNs was selectively impaired, while deep CA1PNs remained normal in 5xFAD mice at six months, compared to their wild-type littermates. The CA1 PVBCs functioning as filters at higher frequencies were damaged in the 5xFAD mouse brain. In addition, the amplitude of NMDAR-EPSCs in PVBCs was enhanced in the mouse model brain. Intriguingly, the administration of memantine in acute hippocampal slices rescued the diminished LTP in superficial CA1PNs of 5xFAD mice compared to wild-type mice. Therefore, we propose that the CA1 fast-spiking PVBCs are significantly more susceptible to AD-like microenvironments. The dysfunction of filter property and postsynaptic NMDAR response might cause the decline in synaptic transmission and LTP of CA1PNs in AD-like progress. Modulating electrophysiological properties and NMDAR functions in PVBCs may improve cognitive behaviors by retuning the working mode of the PVBC-CA1PNs microcircuit in the AD brain. The study may pave a promising path to a combination of drug therapies and non-invasive administration for AD.

Disclosures: Z. Chen: None. **L. Li:** None. **Y. Zheng:** None.

Poster

PSTR265: Memory Circuit Alterations in Alzheimer's Disease

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR265.05/B75

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: EPSRC/Wellcome grant EP/W024020/1
EPSRC grant EP/Y020316/1

Title: Amyloid pathology reduces dynamic range and disrupts neural coding in 5xFAD mice

Authors: *M. GO[1], K. E. CLARKE[2], Y. LI[1], S. V. PRADO[1], B. TEIXEIRA[1], S. R. SCHULTZ[1];

[1]Bioengineering, Imperial Col. London, London, United Kingdom; [2]Biomed. Engin., Univ. of Melbourne, Melbourne, Australia

Abstract: Alzheimer's Disease (AD) is characterized by impairment in memory and cognition, and by aberrant neuronal activity notably in the vicinity of amyloid plaques. To understand how amyloidosis in AD changes circuit properties affecting memory encoding and recall, we examined the relationship between neuronal activity, distance to amyloid plaques, and spatial memory readout from hippocampal CA1 place cells. We performed two-photon calcium imaging in head-fixed mice navigating an air-lifted circular track. Cells were labelled with jGCaMP7s while amyloid plaques were labelled with Methoxy-x04. We studied 5xFAD (AD model) mice and their wildtype littermates divided into two age groups: young (2-3 months) and old (6.5-10 months), with 6 mice in every group. We found that during non-running periods, neuronal activity in CA1 was higher in 5xFAD mice compared to wildtype (WT) littermates in both young (WT: mean 4.10 a.u./min SEM 0.06, n=5309 cells; 5xFAD: 4.50±0.06 a.u./min, n=4836; $p<0.0005$ by Kruskal-Wallis test) and old (WT: mean 3.19±0.07 a.u./min, n=2548 cells; 5xFAD: 3.68±0.05 a.u./min, n=9666; $p<0.0005$) groups. Moreover, neurons close to amyloid plaques (<20µm in old, <40µm in young) had elevated activity. Old 5xFAD mice ran faster than old WT mice but showed significantly impaired dynamic range of neuronal activity in response to speed (WT: 9.25±0.19 a.u./min; 5xFAD: 7.43±0.06 a.u./min, $p<0.0005$). Compared to old WT cells, old 5xFAD cells had less spatial information and lower dynamic range of activity in response to location. In young mice, both spatial information and dynamic range in response to location were higher in 5xFAD cells compared to WT cells. However, young 5xFAD place cells had less spatial information and lower dynamic range (in response to location) near plaques, suggesting that disrupted neural coding starts there.
We then analyzed how these changes in circuit properties affect memory encoding and recall. Compared to WT mice, old 5xFAD mice required more laps around a familiar track for cells to exhibit place tuning, indicating impaired memory recall. Impaired memory encoding in a novel environment was similarly observed in 5xFAD mice in both age groups. Old 5xFAD place fields for a familiar track were less stable within a trial compared to old WT place fields. The same was observed in a novel environment where intra-trial stability for old 5xFAD place fields was even lower. Our study provide insight into the progression of spatial information coding deficits in 5xFAD mice and how these affect learning of new spatial memories versus recalling of old.

Disclosures: M. Go: None. **K.E. Clarke:** None. **Y. Li:** None. **S.V. Prado:** None. **B. Teixeira:** None. **S.R. Schultz:** None.

Poster

PSTR265: Memory Circuit Alterations in Alzheimer's Disease

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR265.06/B76

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG057665-01A1
CIHR PJT156179 1018245 410009513
CIHR PJT173409 1022274 410013821
CIHR PJT191806 1028209 410020048
CRC-2018-00042

Title: Investigating acute effects of deep brain stimulation of the hippocampus in late-stage Alzheimer's disease pathology

Authors: *A. TREVISIOL[1], T. BECKETT[2], J. MCLAURIN[3], B. STEFANOVIC[4];
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Abstract: Despite recent diagnostic advances, Alzheimer's disease (AD) is still predominantly detected in patients after significant brain atrophy has already occurred, posing a great challenge for disease-modifying treatments, and effective management of clinical symptoms. Deep Brain Stimulation (DBS) has shown promising results in various neurological disorders with reports of increased cognitive performance, but its effects on the local neuronal network are poorly understood and only a handful of studies have explored its potential as a treatment for late-stage AD. Our study aimed at understanding how DBS affects the local neuronal network, by using linear multichannel electrodes, lowered into the hippocampus (HIP) of lightly anesthetized TgF344-AD rats, in the late stage of the disease process (16 months of age). Using sub-millisecond switching, we alternated a series of DBS parameters (high vs. low stimulation frequency with different amplitudes) with extracellular neuronal response recording. Local field potentials (LFP) analysis pre- vs. post-DBS demonstrated that the AD-induced attenuation in power (e.g. -26.6% ±13.1% for the delta band in the dorsal HIP in TgF344-AD rats), and coupling of the hippocampal oscillations could be transiently potentiated by the higher DBS frequency (e.g. +40.0% ±12.4% increase in delta power for TgF344AD vs +29.5% ±11.2% for controls at 10 Hz and 0.2 mC/cm^2/pulse), whereas low-frequency (1 Hz) stimulation had little effect. The relative increase in power in the TgF344-AD rats affected the lower frequency bands (delta, theta and alpha, 1-12 Hz) while the DBS-induced increase in the modulation index occurred in all combinations of low-band phase and gamma amplitudes. The analysis of the spiking activity in the 0.75-3 kHz band revealed that the TgF344-AD rats' neurons increased their firing both during and after DBS, but the increase in synchronization to the stimulation frequency observed following DBS offset was less pronounced in TgF344AD rats' vs. nTg rats' neurons. These findings indicate that higher DBS frequency may be an effective way to ameliorate hippocampal neuronal network dysfunction in late-stage AD pathology. Further investigation is warranted to explore the potential long-term benefits of chronic DBS treatments in the clinic.

Disclosures: A. Trevisiol: None. **T. Beckett:** None. **J. McLaurin:** None. **B. Stefanovic:** None.

Poster

PSTR265: Memory Circuit Alterations in Alzheimer's Disease

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR265.07/B77

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CCAD2024-002-1

Title: Early retrosplenial cortex hyperexcitability in the 5xFAD mouse model of Alzheimer's Disease pathology

Authors: *L. CHEN[1], D. MACCHIA[2], P. DERDEYN[1], C. KAPPEL[2], K. BEIER[3];
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Abstract: To develop treatments to prevent or halt cognitive decline in Alzheimer's Disease (AD), it is essential to intervene early in the disease. Defining when and where early disease-related changes happen in the brain thus is a central goal of our research. Neuronal hyperexcitability has been implicated both in AD patients and in mouse models of AD pathology and is a key driver of AD pathogenesis. Therefore, brain region-specific hyperexcitability serves as a promising target for early treatment. The entorhinal cortex (ENT)-hippocampus circuit has been a major focus in AD research since its dysfunction contributes to key AD symptoms such as impaired spatial memory. However, it is not clear whether AD-related cellular and circuit abnormalities begin there in the ENT or arise from upstream inputs that then influence ENT function. To identify input populations that may cause ENT dysfunction, we used a rabies virus retrograde tracing approach to map changes in ENT inputs in the 5xFAD mouse model of AD pathology. We observed increased inputs from retrosplenial cortex (RSC) to ENT at a prodromal time point (2 months of age) in 5xFAD mice compared to controls, prior to the onset of plaque formation and memory impairment, indicating increased RSC activity. Consistently, we found *in vivo* hyperactivity *using* fiber photometry and *in vitro* neuronal hyperexcitability using whole-cell patch clamp recordings in RSC layer 5 (RSC^{L5}) cells of 5xFAD mice at the prodromal time point, while spontaneous synaptic activity was largely normal at this time. Furthermore, performing *in vivo* calcium imaging with a head-mounted miniature microscope on a linear track revealed increased frequency and amplitude of calcium events and decreased spatial information in RSC^{L5} cells of 2-month-old 5xFAD mice compared to controls, confirming our observation of cellular hyperexcitability, and revealing impaired spatial coding at this prodromal time point. In conclusion, our results show early cellular hyperexcitability and disrupted spatial coding in RSC^{L5} cells in 5xFAD mice at a prodromal time point, which may drive deficits in cellular functions and information coding locally and downstream later during AD progression.

Disclosures: L. Chen: None. **D. Macchia:** None. **P. Derdeyn:** None. **C. Kappel:** None. **K. Beier:** None.

Poster

PSTR265: Memory Circuit Alterations in Alzheimer's Disease

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR265.08/B78

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Canadian Institutes of Health Research - CIHR
Canada Research Chairs - CRC

Title: Alterations in Neuronal Stochasticity and Circuit Functionality in the Fischer 344 Transgenic Rat Model of Alzheimer's Disease

Authors: *K. CHEN[1], E. PINEAU[3], M. KOLETAR[1], A. TREVISIOL[1], J. S. HE[4], M. HILL[2], J. MCLAURIN[5], B. STEFANOVIC[6];
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Abstract: As the global population ages, the prevalence of dementia and its impact on families, society, and healthcare systems is rising. Alzheimer's Disease (AD) is the most common cause of dementia. Yet, some elders who exhibit the pathological hallmarks of AD— amyloid deposition and hyperphosphorylated tau—do not show symptoms of dementia. This intriguing phenomenon of brain resilience suggests that factors beyond traditional pathological markers may play a role in the etiology of cognitive impairment in AD. One such factor could be the stochasticity in neuronal reactivity. Recent studies indicate that healthy brain function undergoes continuous remodeling of neuronal functional properties even in environmentally and behaviorally stable conditions. This basal plasticity likely leads to ongoing, activity-independent synaptic changes that contribute to the stochastic responsiveness of neurons to stimulation. Evidence also shows that reduction in volatility of neuronal responses to repeated stimulation is associated with impaired information processing. We hypothesize that a lower trial-dependent variation in the neurons activated by a repetitive stimulus leads to greater impairment upon AD progression. Here we employed ultra high-density Neuropixels probes to estimate the neuronal stochasticity in the somatosensory cortex and the hippocampus. Our findings indicate that impaired TgF344AD rats displayed altered across-trial neuronal response rate, reduced number of responsive neurons, and diminished high-frequency oscillatory activity in the hippocampus compared to their non-transgenic littermates. This work may identify a novel target for presymptomatic AD interventions, and early stratification of AD patients by their susceptibility to dementia.

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Poster

PSTR265: Memory Circuit Alterations in Alzheimer's Disease

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR265.09/B79

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA Grant K01AG066847
NIA Grant P01AG052350
NSF OIA-2121164

Title: Characterizing Neurodegeneration of Subiculum Neuron Cell Types in the 5xFAD Mouse Model of Alzheimer's Disease

Authors: M. PACHICANO, N. KHANJANI, M. BECERRA, Z. SMITH, B. BRENINGSTALL, ***M. S. BIENKOWSKI**;
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by aggregation of amyloid and tau pathology across distinct brain regions. The unique distribution patterns of AD pathology and its spread suggest that some brain regions and their constituent cell types are more susceptible/resilient to AD neurodegeneration and cell death. Neurodegeneration of the subiculum (SUB) is a critical biomarker of AD clinical symptoms, but it is unknown if SUB cell types are differentially affected by AD. The 5xFAD mouse model of AD has been shown to present a clear progressive timeline of AD pathology with elevated levels of amyloid precursor protein at 2 months of age followed by amyloid plaque accumulation in SUB and cortex, and significant neurodegenerative cell loss in the SUB by 8 months of age. Our previous work creating the mouse Hippocampus Gene Expression Atlas (HGEA; Bienkowski et al, 2018) identified the laminar organization of four unique SUB cell types each with unique gene expression and connectivity. Additionally, the laminar organization of SUB cell types is also present in human, suggesting conserved cell type homology across evolution (Bienkowski et al, 2021). Guided by the HGEA, we hypothesized that SUB neurons in 5xFAD mice differentially undergo cell type specific changes to gene expression, connectivity, and morphology across progressive disease timepoints before cell death. Using RNAscope single molecule fluorescent *in situ* hybridization (smFISH), we identified that SUB_3 cell types in the ventral SUB of male and female 5xFAD mice are highly susceptible to AD cell death by late stage of the disease and earlier timepoints are characterized by changes to gene expression. Using anterograde viral tracers, we have found that SUB axons display axonopathy and anterogradely-labeled axons in the fornix white matter tract are significantly diminished compared to littermate control at 8 months of age. Finally, retrograde labeling of distinct SUB cell type projection neuron populations using G-deleted rabies viral tracing revealed neurodegenerative characteristics of soma and dendritic morphology that become progressively more common and severe across age

timepoints. Together, these data provide advanced characterization of AD neurodegeneration within the SUB and its constituent neuronal cell type populations. Future studies will investigate the underlying cellular and molecular processes mediating SUB cell type susceptibility to AD neurodegeneration.

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Poster

PSTR265: Memory Circuit Alterations in Alzheimer's Disease

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR265.10/B80

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Ministry of Science, Innovation and Universities, PID2022-141733NB-I00/AEI/10.13039/501100011033/ FEDER, UE
FPU20/02632, Ministry of Science, Innovation and Universities

Title: Impact of early social recognition disruption on episodic memory in 3xTg Alzheimer's disease mice.

Authors: *A. TERUEL SANCHIS[1], M. VILA-MARTÍN[2], C. SAVARELLI BALSAMO[3], L. JIMÉNEZ[1], M. SANCHO ALONSO[3], J. MARTÍNEZ -RICÓS[3], V. TERUEL-MARTI[3], E. LANUZA[2];
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Abstract: Alzheimer's Disease (AD) is commonly associated with pronounced memory challenges, particularly in spatial tasks. Yet, the integrated memory that involves both spatial and social elements is not well-understood. This research explores initial social recognition difficulties in the triple transgenic mouse model of AD (3xTgAD), examining how these issues align with AD's pathological evolution. Female 3xTgAD mice, aged between 2-4 months and 6-8 months, were subjected to a social recognition assay. By the age of 6-8 months, these mice showed notable difficulties in identifying unfamiliar mice. Immunofluorescence methods targeting beta-amyloid and tau proteins, as well as comprehensive brain analysis, were conducted at 3, 6, 9, and 12 months. The findings revealed premature intracellular beta-amyloid build-up within the hippocampus and significant extracellular accumulations by 9-12 months, linking these biological markers with the observed social recognition issues. Additionally, calcium imaging was used to assess dCA1 place cell responses to urinary cues, crucial for rodent social

and territorial behavior. Results indicated strong cell activity in younger mice, while those aged 6-8 months showed a substantial reduction in dCA1 place cell engagement, suggesting difficulties in integrating social cues into hippocampal memory circuits. These results underscore the emergence of social recognition deficits before significant beta-amyloid deposition in 3xTgAD mice. The evident decline in dCA1 place cell function related to social recognition challenges points to potential early indicators of AD progression, enriching our understanding of the disease's effects on comprehensive memory systems.Funding: Ministry of Science, Innovation and Universities, PID2022-141733NB-I00/AEI/10.13039/501100011033/ FEDER, UE. A Teruel-Sanchis is a predoctoral fellow of the FPU20/02632 program of the same Ministry

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Poster

PSTR265: Memory Circuit Alterations in Alzheimer’s Disease

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR265.11/B81

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG067049 to K.D. from the National Institute on Aging (NIA)

Title: Increasing PSD-95 palmitoylation rescues memory deficits in Alzheimer’s disease model mice

Authors: *Y. DU[1], K. PRINKEY[2], A. PHAM[2,3], M. DINATA[2], K. B. DORE[2];
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Abstract: Background: Synaptic loss is the earliest change in Alzheimer’s disease (AD) and serves as its primary biomarker. The scaffolding protein PSD-95, crucial for synaptic integrity, is significantly depleted in AD. PSD-95 association with post-synaptic membranes is regulated by palmitoylation, a reversible process modulated by specific enzymes including ABHD17, which depalmitoylates PSD-95. *In vitro* studies have demonstrated that inhibition of ABHD17 can counteract Aβ-induced synaptic depression and dendritic spine changes.
Methods and Results: In this study, we used PF11, an intrabody that specifically binds to palmitoylated PSD-95, and observed a significant reduction (40-50%) in palmitoylated PSD-95 in the hippocampus of 6 to 9-month-old female APP/PS1 mice, a model of Alzheimer's disease, with no change in total PSD-95 levels. This reduction was not evident in male mice. Moreover, treatment with Palmostatin B, which inhibits PSD-95 depalmitoylation, restored palmitoylated PSD-95 levels in a dose-dependent manner in AD mice, indicating this drug can cross the blood-

brain barrier and act on synapses in the brain. Importantly, Palmostatin B injections in 9-month-old female AD mice rescued memory deficits observed in the Morris Water Maze test but did not improve the performance of male mice. Moreover, electrophysiological recordings of miniature excitatory synaptic currents (mEPSCs) revealed the synaptic transmission deficits in AD female mice can be restored by Palmostatin B injections. This rescue effect on synapses was also confirmed by assessing dendritic spine density and morphology using fluorescent dye DiI labeling.
Conclusion: Our data show that proteins implicated in Alzheimer's disease pathogenesis significantly reduce PSD-95 palmitoylation, which might indicate a previously unknown step in the pathophysiology of Alzheimer's disease. Furthermore, the palmitoylation status of PSD-95 plays a critical role in the pathophysiology of Alzheimer's disease. Enhancing PSD-95 palmitoylation in the hippocampus can reverse memory deficits and synaptic dysfunction in older, symptomatic AD model mice, identifying ABHD17 as a promising target for AD therapeutics.

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Poster

PSTR265: Memory Circuit Alterations in Alzheimer's Disease

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR265.12/B82

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant AG081931

Title: Mechanisms of context encoding in APP/PS1 mice are rescued by increasing cerebral blood flow by eliminating capillary stalls

Authors: *L. BERKOWITZ[1,2], R. TODOROVA[3], R. E. HARVEY[3], D. CABUS[2], J. LETENDRE[2], J.-L. SHIMIZU[2], X. DONG[2], N. TEHRANI[2], J. JIA[2], S. ROTH[2], A. FERNANDEZ-RUIZ[4], N. NISHIMURA[2], C. B. SCHAFFER[2];
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Abstract: Spatial disorientation is one of the earliest symptoms of Alzheimer's disease (AD). The hippocampal formation creates a cognitive map as place cells form firing fields linked to specific locations within an environment, supporting spatial orientation. In rodent models of AD-like pathology, place cells have been shown to exhibit broadened tuning and altered responses to environmental changes. Additionally, events known as sharp wave ripples (SWRs), that coordinate the firing of hippocampal neurons, are disrupted in mouse models of AD. Previously,

our lab found that treatment with antibodies against Ly6G increases cerebral blood flow by reducing the number of capillaries with stalled blood flow. This led to improved performance on spatial memory tasks. Here, we investigate the effect of anti-Ly6G treatment on neural mechanisms associated with cognitive map stability across contexts. We investigated the association between anti-Ly6G treatment and cognitive map stability in 7-9-month-old APP/PS1 mice and wild-type (WT) controls by recording neural activity in hippocampus area CA1 using 64-channel silicon probes. Neuronal activity was recorded while mice explored open field arenas in one of two environmental contexts, A or B, and during pre- and post-task sleep. We detected place cells, identified as neurons with significant spatial information in their firing rate maps; neural assemblies, groups of neurons with correlated firing during context exploration as revealed by independent component analysis; and SWRs using local field potential recordings from pyramidal and stratum radiatum layers of CA1. Place cells from WT mice showed higher within-context (AA or BB) than between-context (AB) ratemap correlations. In contrast, place cells from APP/PS1 were unstable and remapped within and between environments, suggesting that the instability of the hippocampal network may underlie context encoding deficits. APP/PS1 mice also had a reduced rate and duration of awake SWRs compared to controls, and neural assemblies showed reduced reactivation in SWRs during sleep. Following treatment with anti-Ly6G antibodies, context discrimination by place cells, duration of awake-SWRs, and assembly reactivation during SWRs in post-task sleep increased in APP/PS1 mice. These results highlight the association between SWR dynamics and modified place cell stability, a potential mechanism contributing to the instability of the cognitive map in AD. Importantly, stabilizing the cognitive map following anti-Ly6G treatment suggests treatments to increase CBF may mitigate spatial disorientation and memory impairments in AD.

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Poster

PSTR265: Memory Circuit Alterations in Alzheimer's Disease

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR265.13/B83

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Hunsberger Startup Package

Title: Sex differences in memory retention and social behaviors after social isolation in Alzheimer's disease mice

Authors: *L. TOENNIES[1], K. KAPLAN[2], N. FERRARA[3], H. C. HUNSBERGER[4];
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Abstract: Alzheimer's disease (AD), the leading cause of dementia, disproportionately impacts women compared to men. Early detection and intervention strategies are needed to reduce future disease risk. Loneliness and isolation represent significant risks in dementia transition. Maintaining strong social connections is associated with reduced mortality rates and a lower risk of AD. An increasing prevalence of loneliness and AD after the COVID-19 pandemic proposes an urgent need to better understand the connection between isolation and AD progression within the context of sex differences. Following isolation or group housing of AD and control mice at 2-3 months of age, we measured social interaction and fear memory.Interestingly, we observed increased memory retention in isolated AD female mice compared to group-housed female and male mice. Our results provide the first evidence of potential memory enhancement after acute isolation. Previous studies were only performed in male rodent models and have not investigated an earlier time point before AD pathology. Using immunohistochemistry targeting c-Fos, PV interneurons, and inflammatory markers, we aim to understand the brain circuitry involved, how aging and long-term isolation impact these results, and whether this memory enhancement remains intact in other memory tasks.

Disclosures: L. Toennies: None. **K. Kaplan:** None. **N. Ferrara:** None. **H.C. Hunsberger:** None.

Poster

PSTR265: Memory Circuit Alterations in Alzheimer’s Disease

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR265.14/B84

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01ES032163

Title: Effects of systemic estradiol depletion on cognitive function in the 3xTg-AD mouse model

Authors: *S. AKTUNA[1,2], H. GRITTON[3], M. M. MAHONEY[3];
[1]Univ. of Illinois at Urbana-Champaign, Urbana, IL; [2]Neuroscience, University of Illinois at Urbana-Champaign, Urbana, IL; [3]Comparative Biosci., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Alzheimer’s Disease (AD) is a debilitating neurodegenerative disease characterized by declines in cognition and memory and is estimated to affect more than 44 million people worldwide. AD pathophysiology includes the accumulation of amyloid beta (Aβ) plaques and

neurofibrillary tangles that are thought to contribute to neuronal loss. Vulnerability to early onset is also associated with mutations of genes in the amyloid and tau pathways including APP, PSEN1, PSEN2 and MAPT. Furthermore, the increased prevalence of AD in women is linked to the depletion of circulating sex steroid hormones including estradiol that is significantly reduced following menopause. Here we tested the hypothesis that estradiol depletion through ovariectomy would accelerate the severity and progression of memory impairments in 3xTg-AD mice compared to intact females and males. AD model mice (3xTg-AD) homozygous for three mutant alleles: APPSwe, PsenI, and tauP301L:(B6;129-Tg(APPSwe,tauP301L)1Lfa Psen1tm1Mpm/Mmjax) underwent testing across several memory tasks at 3 months of age. We assessed memory retention performance in the Barnes Maze, which has 3 days of training consisting of 4 trials per day and a probe trial conducted 48 hours later, Novel Object Recognition Task, Y-maze Spontaneous Alternation Task, and Fear Conditioning (Context and Tone). We found that at 3-month old ovariectomized females showed decreased spontaneous alternation behavior in the Y Maze, indicating a deficit in spatial working memory and in cued fear-condition memory. Interestingly, we also found that 3xTg-AD males showed evidence of deficits in acquisition on the Barnes Maze as early as 3 months of age. Future work will test animals at 5 months and 11 months of age to examine if estradiol depletion has a compounding affect that accelerates the decline in memory compared to non-ovariectomized animals.

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Poster

PSTR266: Alzheimer’s Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.01/B85

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Cell-type-specific modulation of mitochondrial function by acetylcholine signaling: a cancer cell-based model to study cholinergic malfunction-based neurodegeneration in Alzheimer's disease with a focus on mitochondrial localization of muscarinic acetylcholine receptors.

Authors: *M. DIVI[1], M. G. SABBIR[2];
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder. Several hypotheses have been proposed to explain the molecular pathogenesis of AD. Notably, the cholinergic hypothesis suggests that cholinergic signaling, crucial for cognition, is diminished in AD brains, underlying cognitive malfunction. Cholinergic neurons use Acetylcholine (ACh) as a neurotransmitter and have two primary receptor classes: the muscarinic ACh receptors

(mAChRs), which have five subtypes (CHRM1-5), and nicotinic ACh receptors. Among the mAChRs, CHRM1 is predominantly expressed in the cortex and hippocampus, and its significant reduction in a cohort of AD patients' brains highlights its role in AD. In a recent study using Chrm1 knockout mice, we demonstrated that Chrm1 is localized in neuronal mitochondria and that the loss of Chrm1 differentially affected mitochondrial ultrastructure, respiratory supercomplex assembly, post-translational modifications of oxidative phosphorylation-associated proteins, and respiratory function in a brain tissue-specific manner (cortex versus hippocampus). Building on these findings, we hypothesize that all mAChRs are localized in the mitochondria and can modulate mitochondrial function through ACh signaling. This hypothesis was tested by studying the localization of transiently expressed fluorescence-tagged mAChRs in cultured primary mouse dorsal root ganglion neurons. Furthermore, ACh-mAChR-mediated modulation of mitochondrial function was analyzed using different prostate cancer (PC) cells. The prostate gland is innervated by the autonomic nervous system, and four PC cell lines (DU145, PC3, 22RV1, and LNCap) displaying varying expressions of mAChRs were characterized for respective mAChR expression using reverse transcription polymerase chain reaction. ACh-induced mitochondrial metabolism in mAChR-positive or negative PC cells was assessed by measuring mitochondrial oxygen consumption (respiration) and extracellular acidification rate (glycolysis). Our findings revealed that all mAChRs are associated with fluorescence protein-labeled mitochondria in neuronal cells, and we found a correlation between mAChR expression and ACh-mediated modulation of mitochondrial respiration in PC cells, suggesting that mAChRs regulate mitochondrial function, thereby influencing cell growth and survival. The implication of these findings is that it provides a model system to further study the intricate molecular signaling pathway controlling ACh-mAChRs mediated regulation of mitochondrial signaling that is highly relevant in understanding dysfunction of cholinergic synapse in AD brains.

Disclosures: M. Divi: None. **M.G. Sabbir:** None.

Poster

PSTR266: Alzheimer’s Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.02/B86

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NIA 1RF1AG065628

Title: Dysregulated RyR-Ca^{2+}signaling drives mitochondrial dysfunction in AD neurons

Authors: *W. GALLEGOS[1], S. H. MUSTALY[2], I. -. SEKLER[3], R. A. MARR[4], G. E. STUTZMANN[5];
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Med. and Sci., Chicago, IL; [3]Ben Gurion Univ., Beer-Sheva, Israel; [4]Neurosci., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL; [5]Neurosci., Rosalind Franklin Univ. /Chicago Med. Sch., North Chicago, IL

Abstract: The transfer of Ca^{2+} between the endoplasmic reticulum (ER) and mitochondria acts as an integratory mechanism between neuronal activity and bioenergetic output. The efflux of ER-Ca^{2+} in response to neuronal signaling is regulated by the ER-localized ryanodine receptor (RyR) and inositol triphosphate receptor (IP_3R), which can directly supply Ca^{2+} to the cytosol and mitochondria. In Alzheimer's disease (AD), a "leaky" phenotype of the RyR is observed and results in unregulated and excessive ER-Ca^{2+} release with detrimental effects on synaptic function, protein handling, and memory encoding. Due to the proximity and reliance of the mitochondria on tightly regulated ER-Ca^{2+} signaling, we hypothesize that dysregulated RyR-Ca^{2+} signaling directly alters mitochondrial functions and contributes to maladaptive Ca^{2+} handling, increased oxidative stress, dysfunctional mitophagy, and increased susceptibility to apoptosis. To test this, we measured mitochondrial functions in human-induced neurons (HiNs) derived from AD and nonAD patients. Changes in mitochondrial functions were monitored under live cell fluorescent microscopy in tandem with genetically encoded Ca^{2+} indicators and organelle-targeted fluorophores. We utilized these biosensors to compare mitochondrial Ca^{2+} uptake, membrane potential, superoxide production, and mitophagy between AD and nonAD HiNs. In AD HiNs, mitochondrial Ca^{2+} levels, membrane potential, and superoxide production were significantly higher than in nonAD HiNs, along with defective mitophagy. Furthermore, evoked RyR-Ca^{2+} release generated by caffeine perfusions resulted in significantly elevated mitochondrial Ca^{2+} uptake and mitochondrial membrane depolarization relative to nonAD HiNs. The attenuation of RyR-Ca^{2+} signaling with Ryanodex reduced observed mitochondrial defects, decreased superoxide production, and restored mitophagy in AD HiNs. Additionally, increased susceptibility to apoptosis was observed in AD HiNs with increased caspase 3 activity and an increased pool of readily releasable cytochrome c.Our data demonstrate that dysregulated RyR-Ca^{2+} signaling can act as an upstream mechanism of mitochondrial dysfunction. The complete or partial rescuing of mitochondrial functions through pharmacological interventions emphasizes the importance of dysfunctional RyR-Ca^{2+} signaling as a driving factor for mitochondrial dysfunction and provides a potential therapeutic target for combating cellular dysfunction in early AD.

Disclosures: W. Gallegos: None. **S.H. Mustaly:** None. **I.-. Sekler:** None. **R.A. Marr:** None. **G.E. Stutzmann:** None.

Poster

PSTR266: Alzheimer's Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.03/B87

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: the Cecil H. and Ida Green Distinguished Chair fund

Title: Selective role of altered heme homeostasis in Alzheimer’s disease pathogenesis

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Abstract: Alzheimer’s disease (AD) is the most common type of dementia. About 6 million Americans are currently living with AD, and the number of people beyond age 65 living with the disease doubles every 5 years. AD can be divided into familial and sporadic cases. AD with familial autosomal dominant inheritance is rare, accounting for <1% of cases, whereas sporadic late-onset AD is the most common form. Despite the intense studies of the molecular, biochemical and cellular mechanisms of the disease for decades, the true aetiology and pathogenesis of AD remain unknown. Here, to gain insights into the molecular events underpinning the true cause of AD, we examined and compared the molecular features of neuronal cells derived from patients with familial AD vs sporadic AD, using human iPSC-derived neural stem cells. We characterized the parameters in heme homeostasis, oxidative metabolism, and oxidative stress in neuronal cells. Our data indicate that neuronal cells from sporadic AD, not familial AD, exhibit dramatic changes in heme homeostasis and oxidative energy metabolism. This suggests that the initiating molecular events underlying sporadic and familial AD may be distinct, with altered heme homeostasis and oxidative metabolism being the dominant factor in sporadic AD pathogenesis. Current experiments are underway to further illuminate the broad molecular alterations in neuronal cells derived from sporadic AD.

Disclosures: L. Zhang: None.

Poster

PSTR266: Alzheimer’s Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.04/B88

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1T32HD071866
NIH Grant 1K99AG078402-01
UAB Nathan Shock Center Pilot Grant 2021

Title: Sex-specific multisystem alterations in metabolome in aged TgF344-AD rats

Authors: *S. GIRISH KUMAR[1], A. BANERJEE[3], M. BABAR[2], P. CHENNUPATI[2], J. P. CARCAMO DAL ZOTTO[2], A. CATON[2], S. DING[4], A. HERNANDEZ[5];

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Abstract: Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by cognitive decline and neuronal dysfunction, as well as increased risk of metabolic impairment and disruption of gut microbiota composition. Moreover, we recently demonstrated systemic metabolic dysfunction may be driven by these alterations in gut microbiome composition. However, how specific metabolite production and utilization is altered with AD, and whether this is tissue specific, remains unknown. Therefore, the primary objective of this study is to assess metabolic perturbation within both the central nervous system and intestine through untargeted metabolomic investigation using an aged transgenic rat model of AD (TgF344-AD). These rats exhibit AD-like neuropathology, metabolic impairment, and cognitive decline that progressively worsen with age, making it a suitable model for our studies. Small and large intestinal content, along with hippocampal and frontal cortical samples, were taken from 19-month-old TgF344-AD and WT rats. Samples were analyzed using liquid chromatography/mass spectrometry (LC/MS). Data normalization and multivariate partial least squares discriminate (PLS-DA) analyses, including Variable importance in projection (VIP) and Principal Component (PCA) analyses, were performed using MetaboAnalyst 6.0. PCA analysis revealed a statistically significant separation of metabolite profiles between TgF344-AD and WT groups in intestinal and brain tissues, demonstrating regional specificity of metabolic-related alterations in AD. Several individual metabolites contributed to the differential metabolite profiles between TgF344-AD and WT groups, including several neurotransmitters and essential amino acids that were up or down regulated uniquely across tissues. Notably, glutamate was upregulated within the hippocampus, but downregulated within frontal cortex. These data align with regional differences in excitatory signaling that occur with aging and AD. Moreover, there were significant differences in metabolome across sex as well as interactions between sex and genotype, such that metabolite profiles differed across genotype differently in male and female subjects. These data demonstrate AD-related impairments in the metabolome of aged subjects include a broad range of metabolites and are sex- and region-specific. Moreover, this work further highlights targeting the gut for therapeutic interventions aimed at alleviating AD-related dysfunction.

Disclosures: S. Girish Kumar: None.

Poster

PSTR266: Alzheimer's Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.05/B89

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Sex differences in brain glucose metabolism in Alzheimer's Disease

Authors: *M. RAMEZAN[1], A. C. SHIN[2];
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Abstract: Alzheimer's disease (AD) is a prevalent health concern, with women constituting about two-thirds of its cases. Beyond classic hallmarks, including amyloid-beta (Aβ) plaques and neurofibrillary tangles, brain glucose hypometabolism is underscored as an early biomarker. Women exhibit distinct alterations in glucose metabolism in various brain regions compared to men. Also, research suggests a decrease in the activity of key glycolytic enzymes before mitochondrial dysfunction occurs in the hippocampus of female mice. Despite its potential involvement in AD progression and evidence of it being a primary region of metabolic changes in AD, the role of the hypothalamus in AD is often overlooked. Aging women but not men have shown glucose hypometabolism in their hypothalamus. We aimed to investigate and compare glucose metabolism, specifically glycolysis, between male and female hypothalamic neuronal cell lines in AD-like conditions. To do this, we cultured mHypoA-BMAL1-WT male and female adult mixed hypothalamic cell lines. First, cells were incubated with varying concentrations of mouse Aβ peptide to mimic AD conditions. Annexin V-FITC/PI cytotoxicity assay was run to determine the least Aβ concentration which can produce significant cytotoxicity in cells. We then used the Seahorse Glycolysis Stress Test to determine how each sex responds to challenging energetic conditions by increasing their glycolysis rate, as measured by Extra Cellular Acidification rate (ECAR) (n=4 in each group). This test also measures Oxygen Consumption Rate (OCR) to assess aerobic respiration, simultaneously. We found that Aβ with a dose of 5μM significantly lowered cell viability by inducing apoptosis. There was a trend toward glycolysis as a preferred pathway to meet the energy demand in female vs. male and Aβ vs. control cells. Glycolytic capacity had a declining trend in Aβ-treated cells vs. healthy cells and in males vs. females. Interestingly, the ability of cells to increase glycolytic activity when faced with an energetic crisis, known as glycolytic reserve, was significantly reduced by Aβ treatment in females (ECAR = 0.13 ± 0.01 in Aβ vs. 0.48 ± 0.05 mpH/min/cell in control), but not in males. Moreover, the energy map indicated that Aβ-treated female hypothalamic neurons have the lowest energetic profile. Our data provide evidence that Aβ treatment as well as the female sex reduce the ability of hypothalamic neurons to use glycolysis in times of energetic adversity and may increase preference toward glycolysis when in non-stress conditions. This can partly explain the sex differences in AD progression in the hypothalamus.

Disclosures: M. Ramezan: None. **A.C. Shin:** None.

Poster

PSTR266: Alzheimer’s Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.06/B90

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ADRD Supplement R01NS095872-05S1

Title: Chemosensitivity Patterns of APP/PS1 Mice at Different Life Stages

Authors: *B. PATEL[1], A. JONES[1], B. KREITLOW[2], G. F. BUCHANAN[3];
[1]Univ. of Iowa, Iowa City, IA; [2]Neurosci., Univ. of Iowa, Iowa City, IA; [3]Neurol., Univ. of Iowa, Iowa City, IA

Abstract: Alzheimer's disease (AD) is a neurological disorder characterized by accumulation of brain pathology and worsening dementia. More than 6 million Americans are living with AD, and the numbers are only rising. There is currently no cure for AD, and the only help available to patients is few medications to relieve symptoms. As AD progresses, it is associated with a number of comorbidities, including respiratory dysfunction. Several animal models exist that recapitulate AD pathology, such as amyloidopathy, but whether these have respiratory dysfunction is unknown. In this experiment, we hypothesized that the Amyloid Precursor Protein/ Presenilin 1 (APP/PS1) mouse will display respiratory dysfunction that will be more prominent as mice age, and will be associated with pathological changes in the brainstem. In this ongoing study, young (2-4 mos) and older (> 6 mos) APP/PS1 mice were instrumented for EEG/EMG recording to assess sleep-wake state, allowed to recover, and presented with 7% CO2 or room air stimuli during wake or sleep while measuring EEG and breathing. Brains were collected after trials for immunohistochemistry to examine the accumulation of amyloid plaques within the brainstem So far, older mice demonstrate impaired arousal and ventilatory responses to CO2. Furthermore, a few mice have been found deceased in full hindlimb extension indicating seizure-related death. Continuing work will include more trials in mice of both age groups, trials in wildtype control animals, and assessment for brainstem pathology.

Disclosures: B. Patel: 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.; R01NS095872-05S1. **A. Jones:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; R01NS129722, F31NS113479. **B. Kreitlow:** 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.; R01NS095872-05S1, R01NS129722-01S1. **G.F. Buchanan:** 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.; R01NS095872-05S1, R01NS129722-01S1, R01NS129722.

Poster

PSTR266: Alzheimer’s Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.07/B91

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NRF-2022K1A3A1A20015190
NRF-2022R1A2C1011996
NRF-2022K2A9A1A01098131
22RB1130
RS-2023-00302751
2020-0-01343

Title: Calorie restriction attenuates Alzheimer's disease pathology in 5xFAD mice

Authors: *S. AHN**[1,2], S. YU****[1,2], C. JEONG[1,2], H. CHOI[1,2], S. SUN[1,2], E. SEO[1,2], H. HU[1,2], H. SHIN[3], S. PARK[3], H. SEO[1,2];
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Abstract: Calorie restriction (CR) is a natural dietary therapy that has been shown to improve health and extend lifespan. CR affects synaptic proteins in hippocampal CA3 and spatial learning ability. CR ameliorates age-related behavioral deficits in the AD mouse model. However, the cellular mechanisms of CR in AD pathological environments are not clearly understood yet. In this study, we used the 5x FAD mice which is the Alzheimer's disease mouse model to find the protective effect and molecular mechanism of CR in AD. Mice at the age of 14 months were fed either ad libitum (AL) or CR diet (CR, 80% at 1st week,70% at 2nd week, and 60% CR at 3rd and 4th week) for 4 weeks. After 4 weeks feed, we performed multiple behavioral tests, including Morris water maze test, elevated plus maze test, and tail suspension test to measure the cognitive function of 5xFAD mice. CR group showed the increased mitochondrial enzyme activities and oxidative phosphorylation levels. We also found that glucose restriction (5.5mM) increase cell viability of the HT-22 hippocampal cells under the hypoxia conditions. It also increased the pro-inflammatory gene expressions in BV2 microglia cells under the hypoxia conditions. These results suggest that CR attenuate AD pathology by affecting mitochondria activities and neuro-inflammatory responses in the brain

Disclosures: S. Ahn**:** None. **S. Yu****:** None. **C. Jeong:** None. **H. Choi:** None. **S. Sun:** None. **E. Seo:** None. **H. Hu:** None. **H. Shin:** None. **S. Park:** None. **H. Seo:** None.

Poster

PSTR266: Alzheimer's Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.08/B92

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG082362
T32GM008541
K01AG062683

Title: Disruption of astrocyte lysosome function and lipid metabolism by Alzheimer's disease risk variant APOE E4

Authors: *J.-G. S. ROSA[1], L. QIAN[1], A. PELLETIER[1], S. PENG[1], R. HUANG[1], T. TCW[1], A. SCRIVO[2], A. CUERVO[2], J. TCW[1];
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Abstract: ApoE is a lipid carrier that in the brain is primarily secreted by astrocytes for lipid export to neurons. Polymorphisms in the *APOE* gene have strong associations to late-onset Alzheimer's disease (AD) risk, with the *APOE E4* (*APOE4*) variant increasing AD risk up to 15-fold in homozygotes relative to carriers of the neutral allele *APOE E3* (*APOE3*). While *APOE4* has been shown to induce intracellular lipid accumulation and elevated cytokine secretion in astrocytes, the underlying mechanisms remain poorly understood. To better understand the impact of *APOE4* on astrocyte function, we performed RNA-seq of isogenic *APOE* 4/4 and *APOE* 3/3 astrocytes derived from human induced pluripotent stem cells (hiPSC) from AD *APOE* 4/4 patients (N=3), finding that the lysosome was among the top most significantly down-regulated pathways in *APOE* 4/4 astrocytes. This result was validated using snRNA-seq data from the Seattle Alzheimer's Disease Brain Cell Atlas, which showed that astrocytes from the dorsolateral prefrontal cortex and middle temporal gyrus of postmortem AD *APOE4*-carriers (N=84 donors) exhibit significant down-regulation of lysosome and autophagy pathways. As the lysosome is a key site for regulation of cellular growth and catabolism, we hypothesized lysosomal dysfunction may underlie toxic lipid accumulation found in *APOE* 4/4 astrocytes. Here, we utilized isogenic *APOE* 3/3 and 4/4 astrocytes derived from a male and female hiPSC line in experiments utilizing fluorescent reporters, flow cytometry, immunocytochemistry, and Western blot. We found *APOE* 4/4 astrocytes exhibited reduction of lysosomal acidification, proteolytic activity, lipid catabolism, and expression of lysosome membrane proteins. Macroautophagy, a process by which intracellular macromolecules are trafficked to the lysosome for degradation during stress, was significantly reduced in *APOE* 4/4 astrocytes. Mechanistically, we found *APOE* 4/4 astrocytes have increased signaling by mTORC1, a master growth regulator implicated in aging and AD. Finally, inhibition of mTORC1 rescued lipid, inflammation, and lysosome phenotypes in *APOE* 4/4 astrocytes. These results provide an underlying mechanism of lipid accumulation in *APOE* 4/4 astrocytes and further implicates mTORC1 signaling in the pathophysiology of AD.

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Poster

PSTR266: Alzheimer's Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.09/B93

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG067330
NIH T32DK007665

Title: Adiponectin pathway activation reverses tau pathology and electrophysiological defects

Authors: *E. R. MCGREGOR[1], O. RIPPENTROP[2], D. LASKY[3], R. M. ANDERSON[1], M. V. JONES[4];
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Abstract: The specific mechanisms of cognitive decline and dementia associated with Alzheimer's disease (AD) remain unclear; however, changes in brain mitochondrial metabolism are coincident with functional decline associated with AD. Direct links between pathology and cellular energetics have not been established, nor have therapeutic strategies explored this connection. Here, we interrogate the therapeutic potential of mitochondrial targeting via the adiponectin receptor activator AdipoRon (AR). Adiponectin is an adipose-tissue-derived hormone previously linked to metabolic syndrome and aging, and adiponectin receptors are expressed in all cell types of the brain. Here, we show that AR clears neurofibrillary tangles (NFTs), a hallmark of AD, and rescues diverse tauopathy-associated defects in primary neurons. Specifically, AR reduced levels of phospho-tau and lowered NFT burden by a mechanism requiring AMPK, an energy-sensing kinase linked to adiponectin signaling. The transcriptional response to AR extended beyond the expected reprogramming of mitochondrial metabolism, influencing postsynaptic receptors, cellular maintenance, and homeostatic pathways. At the organelle level, activation of the autolysosomal pathway involved increased protein levels of LC3 and p62 and was dependent on AMPK. Regarding metabolism, the negative consequences of NFTs on mitochondrial activity and ATP production were corrected. Furthermore, AR restored decreases in dendritic complexity caused by tauopathy, and this effect was dependent on AMPK and the stress-responsive kinase JNK. Whole-cell patch-clamp experiments identified NFT-associated defects in electrophysiological passive parameters (e.g., resting potential, resistance) and active parameters (e.g., action potential firing), both of which were corrected by AR. The translatability of the NFT clearance and electrophysiological phenotypes observed in

cell culture findings to an in vivo system was conducted. A four-month AR feeding paradigm (50mg/kg diet daily fed) administered to hTau P301S mice was sufficient to prevent Tau-associated deficits in CA1 pyramidal cell spike firing and spike parameters (i.e., spike amplitude and width). Together, these data reveal a neuronal intracellular network linking mitochondrial function to cellular maintenance processes and electrical aspects of neuronal function that can be targeted via adiponectin receptor activation.

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Poster

PSTR266: Alzheimer's Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.10/B94

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 1RF1AG079503-01

Title: Intravital imaging of neuronal energy metabolism in APP/PS1 at single cell resolution

Authors: *A. N. MARTINEZ MEJIA[1], J. HAN[1], J. DERDOY[2], M. GOYAL[3,4], A. Q. BAUER[3,1], S. HU[1], J.-M. LEE[4,3,1,2];
[1]Biomed. Engin., [2]Neurosci., [3]Radiology, [4]Neurol., Washington Univ. in St. Louis, St. Louis, MO

Abstract: Human FDG PET studies demonstrate that altered glucose metabolism is an early event in the pathogenesis of Alzheimer's disease (AD), occurring soon after the deposition of amyloid plaques. It is unclear, based on these observational studies, whether this altered metabolism is reversible, or which cell types are contributing to the changes. In this study, we use two-photon fluorescence lifetime imaging (TP-FLIM) to measure changes in nicotinamide adenine dinucleotide (NAD(P)H) and flavin adenine (FAD) in the brains of living APP/PS1-Thy1-RGECO female mice (varying ages) under isoflurane anesthesia. NAD(P)H and FAD, metabolites critical to glycolysis and mitochondrial metabolism, have intrinsic fluorescent characteristics that permit measurement of bound vs. free forms using FLIM. Given that protein-bound NAD(P)H is thought to be relatively stable over time, the ratio of free vs bound ($NAD(P)H_{free/bound}$) NAD(P)H can partly reflect the ratio of glycolytic vs oxidative metabolism. Furthermore, a higher NAD(P)H-to-FAD (N/F) ratio is typically associated with more glycolysis. Together these ratios provide a quantitative measure of a metabolic shift towards or away from glycolysis. We analyzed N/F and $NADH_{free/bound}$ in excitatory neurons relative to their distance from the amyloid plaque. We found that regardless of age, neurons near the plaque have lower N/F and $NADH_{free/bound}$ ratios, whereas those further away have a higher N/F and

NADH $_{free/bound}$; suggesting that neurons closer to the plaque have less glycolysis and altered metabolism than those further away from it. Further characterization and validation are needed, but these data provide evidence that we can measure metabolites in living mice at single cell resolution. Future studies will examine other cell types and determine whether changes in metabolism are reversible with the reduction of plaque size/load using anti-Abeta antibodies.

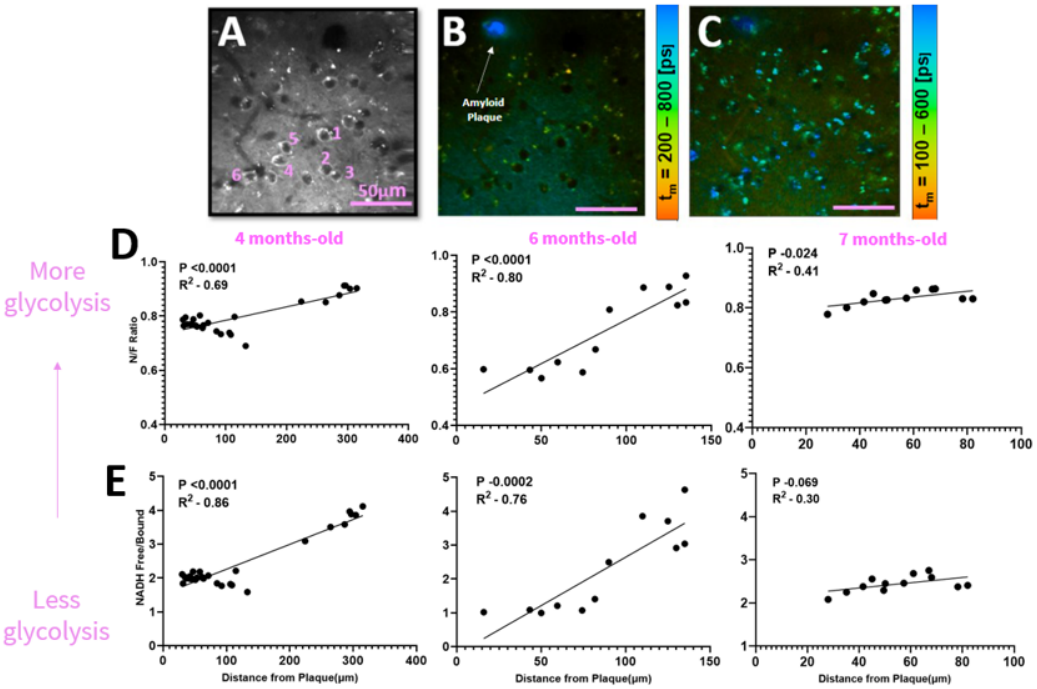


Figure 1. Representative field of view of **A)**Thy1-RGECO labeled neuronal Intensity image **B)**NAD(P)H and Methoxy-x04 TP-FLIM image and **C)** FAD TP-FLIM image **D)** Representative linear regression analysis showing N/F ratios of Thy1-labeled neurons relative to their distance from plaque of a 4-months old, 6-months old, and 7-months old mouse, respectively. **E)** Representative linear regression analysis showing NADH $_{Free/Bound}$ ratios of Thy1-labeled neurons relative to their distance from plaque of a 4-months old, 6-months old, and 7-months old mouse, respectively.

Disclosures: A.N. Martinez Mejia: None. **J. Han:** None. **J. Derdoy:** None. **M. Goyal:** None. **A.Q. Bauer:** None. **S. Hu:** None. **J. Lee:** None.

Poster

PSTR266: Alzheimer's Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.11/B95

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA Grant T32AG061897
NIA Grant R37AG053589

Title: Single-nuclei sequencing in ovariectomized rat hippocampus identifies estrogen-sensitive brain cell sub-populations and pathways

Authors: *J. W. MCLEAN, T. WANG, Y. SHANG, R. D. BRINTON;
Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: Estrogen acts as a master regulator of systems biology in the female brain, coordinating metabolic, immune, and neuronal homeostasis. Loss of estrogen during the menopausal transition is associated with declining brain glucose metabolism and increasing neuroinflammation, which may contribute to neuronal vulnerability and lead to the doubled lifetime risk of Alzheimer's disease (AD) in women. However, the neuronal and glial cell type-specific response to estrogen deficiency in hippocampus, a brain region critically affected in AD, is incompletely understood.

To identify hippocampal cells and pathways affected by estrogen loss, 5-month-old female Sprague Dawley rats were randomly assigned to sham ovariectomized (SHAM), ovariectomized plus vehicle (OVX), or OVX with 17β-estradiol (OVX+E2). Animals were weighed before and weekly after surgery. Animals were sacrificed at 5 weeks post-surgery.

Nuclei were isolated from dorsal hippocampus and two biological replicates per treatment group were pooled and a 10X Genomics Chromium Single Cell 3' kit was used to generate a single-nuclei cDNA library for each condition. Libraries were sequenced on an Illumina NovaSeq6000, and demultiplexed FASTQ files were analyzed using CellRanger 7.1.0 and Loupe Browser 8.0. After filtering out nuclei with >5% mitochondrial reads, established cell type-specific marker genes allowed annotation of distinct clusters of major brain cell types including excitatory neurons, inhibitory neurons, astrocytes, oligodendrocytes, oligodendrocyte precursor cells (OPCs), microglia, and endothelial cells.

Single-nuclei transcriptomics identified a reduction in cell percentage of a neuron cluster expressing *Slc17a6* (vGlut2) in OVX hippocampus, which was partially rescued in OVX+E2, relative to control. Additional subpopulations affected include other inhibitory and excitatory neuron clusters, as well as oligodendrocytes. Bulk analysis indicated KEGG pathways altered in OVX condition included antigen processing, cholesterol metabolism, insulin resistance, and glutamatergic synapse pathways.

These findings support previous reports of estrogen loss affecting neuroinflammation, bioenergetics, and synaptic function and are being elucidated in ongoing studies to identify cell-type specific pathway contributions. Outcomes will advance an understanding of brain cell-specific responses to estrogen loss and help identify specific drivers of brain vulnerability in the estrogen-deficient female brain.

Disclosures: J.W. McLean: None. **T. Wang:** None. **Y. Shang:** None. **R.D. Brinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuTherapeutics, LLC.

Poster

PSTR266: Alzheimer's Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.12/B96

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIA grant P01-AG026572
Women's Alzheimer's Movement to RDB

Title: Large-scale proteomics analysis of Alzheimer's Disease plasma blood reveals sex and APOE specific signatures

Authors: *S. MERLINI, R. D. BRINTON, F. VITALI;
Univ. of Arizona, tucson, AZ

Abstract: Age, sex, and APOE4 genotype are key non-modifiable risk factors for Alzheimer's disease (AD), with APOE4 significantly increasing risk, especially in women. The APOE-sex (APOE-SX) interaction underscores the need for personalized AD treatments. We analyzed plasma blood proteomic data from the UK Biobank to identify sex and APOE-specific protein and pathway signatures. Plasma samples were analyzed for participants diagnosed with AD and controls. After propensity-score matching on age and education level, 199 AD cases (133 females, F; 66 males, M) and 199 controls (104 F, 95 M) from APOE3/3, 3/4 and 4/4 individuals were retained. Protein levels were normalized using z-scores. For each APOE-SX condition, differentially expressed proteins (DEPs, p-value < 0.05) between AD and controls were identified using linear regression with empirical Bayes estimators to calibrate the per-protein variance using information from all the proteins. Gene Set Enrichment Analysis (GSEA) was subsequently conducted using Gene Ontology Biological Processes (GO-BP) accounting for DEP fold change. Redundant GO-BP terms (adjusted p-values<0.05) were removed by computing GO-BP semantic similarity (cut-off of 0.6). Comparison analyses were then conducted to identify common and unique DEPs and enriched GO-BPs across APOE-SX conditions. Across all APOE-SX conditions, F4/4 exhibited the greatest number of DEPs (n=741), while M-44 had the fewest (n=102), compared to matched controls. Proteomic analysis revealed DEPs specific and common across all conditions. For example, CCL8, involved in immunoregulatory processes, was overexpressed only in females. GSEA revealed the highest numbers of AD-enriched pathways in APOE3/3 carriers (n=37-40 GO-BPs), whereas M3/4 and M4/4 exhibited the fewest (n=13 and 17). Both unique and common GO-BPs were found related to APOE-SX signatures, such as downregulated pathways related to protein kinase activity were specific to F4/4, while intracellular transport and receptor signaling common to F3/4, F4/4, and M4/4. Notably, no common DEPs or GO-BP terms were found across all APOE-SX conditions. These findings suggest APOE-SX specific proteomic signatures and altered biological processes in AD, supporting the development of personalized therapeutics considering APOE-SX interaction.

Disclosures: S. Merlini: None. **R.D. Brinton:** None. **F. Vitali:** None.

Poster

PSTR266: Alzheimer's Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.13/B97

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIH Grant P01AG026572

Title: Therapeutic potential of midlife combination therapy in hAPP hAPOE risk model of Alzheimer's Disease

Authors: *G. TORRANDELL[1], H. VAN ROSSUM[2], A. PARKER[1], R. D. BRINTON[3]; [1]Univ. of Arizona, Tucson, AZ; [2]Neurosci., Univ. of Arizona, Tucson, AZ; [3]Ctr. for Innov in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: The preclinical phase of Alzheimer's Disease can begin decades prior to the onset of clinical symptoms. During this stage, pathophysiological changes that drive hallmark beta amyloid and tau accumulation occur. These changes include brain and peripheral inflammation along with dysregulated glucose and lipid metabolism. Targeting these systems of biology through FDA-approved therapeutics could be an effective strategy to address early AD risk mechanisms thereby reducing the burden of AD. Our previous analyses indicated that lipid-lowering, glucose metabolism regulators, and anti-inflammatory therapeutics resulted in statistically lower AD risk. To investigate the impact of combination therapeutics on AD pathology, we utilized a late onset AD risk mouse model expressing humanized (h) hAPP hAPOE3/3 and hAPP hAPOE4/4 genes. Male and female mice, beginning at 15 months of age, were exposed to midlife interventions involving lipid-lowering, glucose-regulating, or anti-inflammatory treatments, either as monotherapy or in combination, for 90 days. Treatments were administered daily through the mice's chow. A control group was fed a calorie-matched diet without drug treatment. Mice underwent cognitive assessments using novel object recognition (NOR) before and after treatment and weekly nesting tests, as well as monthly body composition measurements using EchoMRI. At day 90, plasma metabolic markers were evaluated using a clinical blood analyzer. Additionally, immunophenotyping in peripheral blood and meningeal cells was conducted using flow cytometry and brain microglial activation was assessed by immunohistochemistry (IHC). Amyloid beta levels were measured in plasma using MSD and in brain via IHC. Results from this study demonstrated that while each therapeutic alone effectively targeted its respective biological system, combination therapy produced a synergistic effect, leading to greater amyloid beta clearance, reduced neuro- and peripheral inflammation, and enhanced metabolic homeostasis. Additionally, combination therapy rescued cognitive decline after 3-month treatment. The response to treatment was influenced by sex and APOE genotype,

with combination therapy proving most effective in those at higher risk for AD: females carrying the APOE4 allele. Observational studies support the use of FDA-approved risk factor therapeutics to reduce the risk of AD, with a greater benefit when used in combination. This study highlights the therapeutic potential of combination therapy targeting key drivers of AD pathology and supports a precision medicine approach tailored to individual factors such as sex and APOE genotype.

Disclosures: G. Torrandell: None. **H. Van Rossum:** None. **A. Parker:** None. **R.D. Brinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); RDB is President of NeuTherapeutics, LLC..

Poster

PSTR266: Alzheimer's Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.14/B98

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIA grant P01AG026572
Center for Innovation in Brain Science

Title: Accelerated midlife endocrine aging in hAPOE4/4 females

Authors: *T. WANG, Z. MAO, Y. SHANG, N. DELATORRE, J.-P. WIEGAND, R. D. BRINTON;
Univ. of Arizona, Tucson, AZ

Abstract: Age, female sex and the *APOE4* allele are among the top risk factors for Alzheimer's disease (AD), with a stronger *APOE* link to AD in women. ApoE4 significantly increases the odds ratio of AD in women compared to men and accelerates rates of cognitive decline more in women. This *APOE*-sex interaction also pertains to *APOE* mouse models where apoE4 induces more severe neurodegeneration and cognitive deficits in female mice. However, the mechanisms by which these two risk factors converge to disrupt brain function remain elusive.Herein, we used a translational rodent model we developed as per STRAW criteria to investigate the effect of *APOE* genotype on biological transformations associated with female midlife endocrine aging. 6-, 9- and 15-month *hAPOE3/3* and *hAPOE4/4* female mice were stratified into 3 different endocrine aging groups based on vaginal cytology profiles: regular cyclers (consistent 4-5 day cycles), irregular cyclers (of 6-9 day cycles), and acyclic (no cycling >9 days). The endocrine status, plasma hormone and biometric profiles, as well as brain bioenergetic function, transcriptomics, myelination and neuroinflammation status were characterized.Our findings indicated that systems biology of endocrine aging initially identified in the perimenopausal rat model were replicated in the *hAPOE3/3* mouse model. In contrast, *hAPOE4/4* females exhibited

accelerated endocrine aging with an increased magnitude of systemic and brain metabolic dismantling while compensatory adaptive responses were compromised. Further, the accelerated and amplified bioenergetic crisis in *hAPOE4/4* females was accompanied by increased immune activation and demyelination.Outcomes of these analyses provide a plausible mechanistic pathway underlying the greater risk of AD in *APOE4* females, thus providing a rational mechanistic precision medicine approach to intervene during midlife to prevent or delay the onset of the prodromal / preclinical stage of AD.

Disclosures: T. Wang: None. **Z. Mao:** None. **Y. Shang:** None. **N. Delatorre:** None. **J. Wiegand:** None. **R.D. Brinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); President of NeuTherapeutics, LLC.

Poster

PSTR266: Alzheimer's Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.15/B99

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIH Grant 1T32AG061897
NIH Grant R01AG057931
Funding The University of Arizona Center for Innovation in Brain Science

Title: Investigating neuroinflammatory profiles across midlife: Translational potential for novel Alzheimer's Disease risk model

Authors: *N. DELATORRE[1,2,3], H. VAN ROSSUM[1,4,3], R. D. BRINTON[3];
[2]Med. Pharmacol., [3]Ctr. for Innov in Brain Sci., [4]Neurosci., [1]Univ. of Arizona, Tucson, AZ

Abstract: Late-onset Alzheimer's disease (LOAD) has a decades-long prodromal phase. Age, sex, and *APOE*4 genotype are the leading non-modifiable risk factors. Current Alzheimer's disease (AD) animal models typically incorporate mutations that accelerate AD pathology, focusing on end-stage disease rather than risk or the prodromal phase. We propose the use of a novel risk factor model to better elucidate biological shifts during midlife that could indicate the prodromal stage of AD.
Previous lab findings in a humanized-*APOE* (h*APOE)* mouse model demonstrated sex-driven inflammatory profiles and metabolic shifts in female h*APOE* mice compared to aged-matched males. Additional lab findings indicated that the human mid-life females' inflammatory transcriptomic profile closely resembles the LOAD brain, while the mid-life males diverged. Based on the observed sex-driven inflammatory profiles, we hypothesize that the addition of a non-modifiable risk factor to our mouse model, such as humanized amyloid precursor protein

(hAPP), will induce stronger genotypic inflammatory profile differences through midlife. Transgenic h*APOE* ε3/3 and ε4/4 KI mice (Jackson Lab) were bred with homozygous B6(SJL)-App tm1.1Aduci/J animals, also acquired from Jackson Lab, resulting in h*APP*+h*APOE* (ε3/3 & ε4/4) mice. Mice of both sexes were aged to 12, 15, and 18 months and sacrificed (human equivalent ~ 43,50 and 56-year-old respectively). Flow cytometry on the left brain hemisphere was used to assess microglial functionality and lymphocyte phenotyping (N=265). Microglial activity (CD68) demonstrated a significant interaction between genotype:age ($p=0.00026$) and sex:genotype ($p= 0.027$). Female h*APP*+h*APOE*-ε3/3 had increased microglial activation at all ages while males showed increased activation only at 18 months. h*APOE*-ε4/4 mice demonstrated low activation across age and sex. There was also a significant sex:genotype:age interaction in microglia ability to phagocytose (pHrodo) ($p=0.039$) and antigen presentation (MHCII) ($p<0.0001$). Microglia's ability to phagocytose decreased with age, with h*APOE*-ε4/4s having the lowest across all groups. At 15m there was a subtle increase in MHCII in females while increased expression was observed in h*APOE*-ε3/3 mice at 18 months. These data indicate a repressed immune response during midlife aging in h*APP*+h*APOE*-ε4/4s mice. Additionally, h*APOE*3/3s had an increased activation profile. These findings highlight a need for a more thorough understanding and to consider midlife-related changes in inflammatory systems and their contribution to LOAD risk. Future studies must include examining translatability to the aging human brain.

Disclosures: N. Delatorre: None. **H. Van Rossum:** None. **R.D. Brinton:** None.

Poster

PSTR266: Alzheimer's Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.16/B100

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIH/NIA P01-AG026572 to RDB

Title: Cns-active drug intervention ameliorates cognitive function in 5xfad mouse model of alzheimer's disease

Authors: *H. CORTES-FLORES[1], C. FRENCH[1], R. D. BRINTON[2];
[2]Ctr. for Innov in Brain Sci., [1]Univ. of Arizona, Tucson, AZ

Abstract: Alzheimer's Disease (AD) is characterized by a long preclinical stage where pathological changes start to emerge. Among those changes, neuropsychiatric-related biomarkers can emerge. Preclinical AD dysregulations within the central nervous system (CNS) that are shared with neuropsychiatric diseases include disruption of neurotransmitter systems, neuronal loss, synaptic dysfunction, and amyloid beta accumulation. FDA-approved drugs for

neuropsychiatric diseases that target these systems, directly or indirectly, could delay disease progression and improve clinical outcomes of AD. Previous analysis in our lab showed that specific CNS-active drug treatments were associated with a significant decrease in AD risk. 5xFAD mice were utilized to assess the impact of CNS-active drugs, alone and in combination, on AD development. Six-week-old male mice received daily treatment with either a norepinephrine reuptake inhibitor, a GABA receptor positive allosteric modulator, or both. Animals were treated via oral gavage for two months. Novel object recognition (NOR) test and body composition analysis were conducted before and after drug intervention. Additionally, nesting assessments and body weight measurements were performed weekly. Following treatment completion, animals were sacrificed, and brains were collected for immunohistochemistry profiling in the hippocampal region. Plasma was used for biochemical analysis. Results from NOR tests indicated that CNS-active drug interventions preserved cognitive function and reduced anxious behavior compared to untreated controls, especially with the combination therapy intervention. Treated mice exhibited changes in fat and lean mass percentages relative to controls, while no significant differences emerged in glucose, triglycerides, or total cholesterol levels in plasma. Immunohistochemistry analyses were utilized to identify differences in the profile of AD markers associated with the administered therapeutics. Findings of this study support the use of CNS-active medications to preserve cognitive function in the context of AD, particularly when using a drug combination approach that targets multiple systems. Targeting early CNS dysregulations associated with AD via FDA-approved therapeutics could provide an efficient strategy to moderate disease progression. Furthermore, the benefits of using CNS-active medications in early versus late stages of AD reinforces the need for precision medicine strategies for AD. This work was supported by NIH / National Institute on Aging funding P01AG026572 to RDB, and the Center for Innovation in Brain Science to RDB.

Disclosures: H. Cortes-Flores: None. **C. French:** None. **R.D. Brinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuTherapeutics, LLC..

Poster

PSTR266: Alzheimer’s Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.17/B101

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: P01AG026572
Center for Innovation in Brain Science

Title: Introduction of APP induces bioenergetic deficits within an aged humanized APOE risk model of Alzheimer's disease: An FDG-PET analysis

Authors: *A. BHATTRAI[1], A. RAIKES[2], J. W. MCLEAN[2], R. D. BRINTON[2];
[2]Ctr. for Innovation in Brain Sci., [1]Univ. of Arizona, Tucson, AZ

Abstract: Late-onset Alzheimer's disease (LOAD) affects around 95% of the clinical dementia population aged 65 and older and is influenced by genetics, age, sex. LOAD is associated with cognitive, morphological, and bioenergetic changes. GWAS studies have identified Apolipoprotein ε4 (APOE4) as the strongest genetic risk factor, in addition to other prominent risk genes such as amyloid precursor protein (APP). APOE4 confers upto 15-fold higher risk in females compared to male APOE3/3s. Preclinical research often uses familial AD risk factor mouse models, which impact <5% of the clinical population, limiting clinical translatability. Herein, we investigated the impact of a single strong AD risk factor gene (APP) combined with LOAD-specific risk factors on brain and peripheral bioenergetics. Aged (23-25 months) humanized *APP*/*APOE* (APP carrier) and h*APOE* (APP non-carrier) mice with *E3/3, E3/4 and E4/4* genotype underwent metabolic and body composition screening including fasting blood glucose (FBG) and ketone (FKB) measurement, EchoMRI, and ^{18}F-FDG-PET. Cerebral FDG-PET standardized uptake values (SUV_R) were normalized to pons. For analysis, mice we classified as APOE4 carriers and non-carriers, based on the presence of E4 allele. All data were analyzed with APP carrier x APOE carrier x sex analyses of variance followed by post-hoc Bonferroni correction. APP carriers had a significantly lower SUV_R compared to non-carriers ($p = 3.375e-08$). This was primarily driven by the difference between the APP and non-APP APOE-E4 carriers ($p = 1.2e-05$). Females also had lower brain glucose uptake than males, though not significantly ($p = 0.075$). APP carriers additionally had lower FBG ($p= 2.2e-16$) than non-carriers with a trend towards sex differences ($p = 0.07$). Finally, APP carriers had higher FKB levels than non-carriers ($p = 2.05e-5$), with female APP carriers having higher FKB levels than all non-carriers ($p = 0.003$). In a mouse cohort at a comparative human age of ~70 years, lower brain SUV_R in APP carriers, specifically in the E4s, coupled with lower FBG indicates ongoing bioenergetic deficits and the inability to meet energetic demands via glycolysis. This bioenergetic profile is also observed in clinical populations. Greater FKB in APP carriers suggests a shift toward utilizing fatty acids to meet bioenergetic demands. These results highlight the metabolic impact of interaction between h*APP* and the APOE E4 allele in these aged mice, providing important evidence for the interplay between AD genetic risk factors and bioenergetic dysfunction.

Disclosures: A. Bhattrai: None. **A. Raikes:** None. **J.W. McLean:** None. **R.D. Brinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuTherapeutics LLC.

Poster

PSTR266: Alzheimer's Disease: Energy Homeostasis

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR266.18/B102

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIH/NIA P01AG026572 to RDB

Title: Brain morphometry is altered by chronological and endocrinological aging: Evidence from a humanized APOE risk model of Alzheimer's disease

Authors: *A. C. RAIKES, A. BHATTRAI, T. WANG, R. D. BRINTON;
Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: Menopause is a multi-systemic perturbation across endocrine, metabolic, and immune-inflammatory systems. Despite known effects on these systems, the impact on the brain and Alzheimer's disease (AD) risk remains incompletely understood, limiting preventative therapeutic development. Here, we investigated the impact of the menopausal transition on brain morphometry in a late-onset AD mouse model.
6-, 9- and 15-month humanized APOEε3/ε3 (JAX #29018), ε4/ε4 (JAX #27894), and ε3/ε4 (bred in-house) female mice were stratified into 3 endocrine aging groups based on vaginal cytology profiles: regular (consistent 4-5 day cycles) or irregular cyclers (6-9 day cycles), and acyclic (no cycling >9 days). Animals were euthanized on estrous day and transcardially perfused. Ex-vivo MRI included high resolution volumetric scans. Regional and total brain volumes were determined using an existing mouse-specific brain atlas. Total brain volume differences were modeled with a linear model for chronological/endocrinological aging groups and hAPOE genotype. Regional volumes were modeled with a hierarchical Bayesian mixed effects model for chronological/endocrinological aging groups and hAPOE genotype as well as total brain volume. Total brain volume increased with advancing endocrinological age ($F = 4.159$, $p = 0.005$). Additionally, hAPOE4 carriers exhibited greater overall brain volume compared to hAPOE3/3 ($F = 4.093$, $p = 0.048$), while no differences were observed between hAPOE3/4 and hAPOE4/4. These total brain volume differences were specifically observed between the 6M mice and the 15M-Irreg ($p = 0.008$) and 15M-Acyc ($p = 0.038$) mice, driven by a 4% increase in total brain volume in hAPOE4 mice. Across all chronological/endocrinological aging groups, after accounting for total brain volume and hAPOE genotype, increased volume was observed in the 6M-Reg mice in cortical regions whereas increased volume was observed in the 15M animals in brainstem, midbrain, subcortical, and white matter regions, as well as the ventricles. The magnitude of these unique, group specific differences above and beyond simply having larger brains was generally small ($< 1mm^3$ on average) but carried moderate to strong evidence for the observed effects ($|P+| > 0.81$).
These findings indicate a shift toward larger cortical and smaller subcortical regions and are consistent with human studies demonstrating reduced regional brain volume, cortical thickness and surface area in early menopause. Though these differences were small in magnitude, they provide evidence of an endocrinologically-driven shift in brain morphometry which may precipitate the onset of Alzheimer's disease.

Disclosures: A.C. Raikes: None. **A. Bhattrai:** None. **T. Wang:** None. **R.D. Brinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuTherapeutics.

Poster

PSTR267: Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

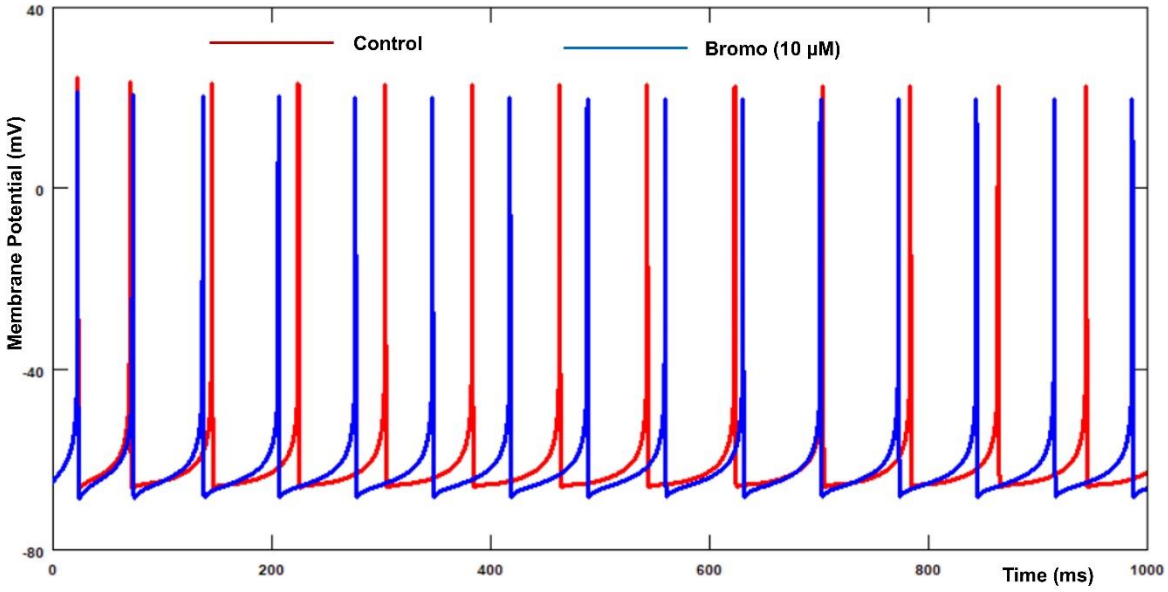
Program #/Poster #: PSTR267.01/B103

Topic: C.03. Parkinson's Disease

Title: Computational investigation unveils the interplay between dopamine receptors and calcium channels within subthalamic neuronal cells in the context of Parkinson's Disease

Authors: *C. MAHAPATRA[1,2], A. KAUR[3];
[1]Indian Inst. of Technol. Bombay, Nayagarh, India; [2]University of California San Francisco, San Francisco, CA; [3]Mol. and Cell. Biol., Univ. of California, San Francisco, union city, CA

Abstract: Recent experimental findings indicate a possible interplay between dopamine D2 receptors (D2R) and N-type calcium (Ca2+) channels, influencing the firing patterns of STN cells situated in the basal ganglia. This computational investigation seeks to clarify the fundamental mechanisms of this interplay and its impact on the dynamics of resting membrane potential (RMP) and firing patterns in Parkinson's Disease (PD). The research utilizes a three-pronged quantitative modeling strategy. Initially, it incorporates biophysical characteristics of different ion channels within STN cells gleaned from prior studies. Next, it formulates equations to depict the GPCR pathway's influence on cAMP levels, affecting the conductance of N-type Ca2+ channels. Lastly, it employs simulations of diverse pharmacological compounds to uncover fresh biological understandings.Employing the dopamine receptor agonist Bromocriptine (Bromo) at a concentration of 10 µM, the investigation explores the modified kinetics of N-type calcium (Ca2+) channels and consequent alterations in firing patterns. Findings indicate that Bromo influences the activation curves of N-type Ca2+ channels, shifting their half-activation potential towards more positive values. Dopamine agonists lead to a decrease in action potential frequency, attributed to diminished window current resulting from D2R activation and reduced conductance of N-type Ca2+ channels. Figure 1 depicts the impact of dopamine agonists on simulated firing patterns, with a current injection of 100 pA lasting for 1 ms. It vividly illustrates the decreased firing frequency (depicted by the blue solid line) observed under the influence of dopamine agonists. Activation of D2R receptors reduces the window current necessary to maintain the RMP, counteracted by a decrease in Ca2+ channel conductance.The research proposes potential therapeutic approaches utilizing Ca2+ channel agonists for treating PD, potentially serving as alternatives to dopamine in specific pathological contexts while also boosting spatial memory performance.



Disclosures: C. Mahapatra: None. **A. Kaur:** None.

Poster

PSTR267: Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR267.02/B104

Topic: C.03. Parkinson's Disease

Support: JSPS KAKENHI Grant Number JP22K06814

Title: Colocalization of Melanocortin 1 Receptor and Attractin in the Substantia Nigra

Authors: *A. EHARA, N. TOKUDA;
Dokkyo Med. Univ., Tochigi, Japan

Abstract: Melanocortin 1 receptor (MC1R) is known to control coat color with Attractin in the skin. Recently, it was reported that MC1R is present in the substantia nigra (SN) and that its deficiency causes Parkinson's disease-like dopaminergic neurodegeneration. However, the detailed localization of MC1R in a brain and whether MC1R colocalized with Attractin remain unknown. In this study, we histologically analyzed the localization and characteristics of MC1R cells in rat SN. In situ hybridization revealed that MC1R mRNA was weakly expressed in the SN pars compacta (SNc) cells and strongly expressed in the SN pars reticulata (SNr) cells. Double In situ hybridization chain reaction combined with immunofluorescent staining was used to characterize MC1R-expressing cells. These cells co-expressed Attractin mRNA and were

tyrosine hydroxylase (TH)-positive dopaminergic neurons in the SNc and parvalbumin (PV)-positive inhibitory neurons in the SNr. Immunostaining confirmed that MC1R-immunopositive cells showed the same localization pattern as mRNA-expressing cells. Fluorescence multiple immunostainings showed that MC1R-positive cells in the SNc were TH-positive, and those in the SNr were PV-positive. Furthermore, the majority of MC1R-positive cells in both regions were Attractin-positive. Subcellular localization showed that MC1R was present in the cytoplasm and the plasma membrane. In the cytoplasm, MC1R was rod-shaped and Attractin was dot-shaped, both in close proximity to each other. The results showed that MC1R co-localized with Attractin in dopamine neurons in the SNc and inhibitory cells in the SNr, suggesting that MC1R and Attractin act cooperatively as a regulatory system of nigrostriatal dopamine neurons in the SN.

Disclosures: A. Ehara: None. **N. Tokuda:** None.

Poster

PSTR267: Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR267.03/B105

Topic: C.03. Parkinson's Disease

Support: Aligning Science Across Parkinson's ASAP-020505
Instituto de Salud Carlos III Grant FI21/000919 and CP19/00200 and FIS PI23/00672
Fundacion Tatiana Perez de Guzman el Bueno
Ministerio de Ciencia e Innovación y Universidades Grant PID2019-111045RB-I00

Title: Girk2 / Aldh1a1 expression defines vulnerable nigrostriatal dopaminergic neurons in the primate brain: relevance for Parkinson's disease

Authors: N. LÓPEZ GONZÁLEZ DEL REY[1], N. HERNANDEZ-PINEDO[1], M. CARRILLO DÍAZ[1], M. DEL CERRO LEGAZ[1], N. ESTEBAN GARCÍA[1], M. H. MONJE[2], I. TRIGO-DAMAS[1], M. MARIN[3], J. L. LANCIEGO[4], C. CAVADA[5], J. A. OBESO[1], ***J. BLESA**[1]; [1]Fundacion de Investigacion HM Hospitales, Madrid, Spain; [2]Feinberg Sch. of Med., Univ. Autónoma De Madrid, Chicago, IL; [3]Fundación de Investigación HM Hospitales, Madrid, Spain; [4]CNS Gene Therapy Program, CIMA - Univ. of Navarra, Pamplona, Spain; [5]Anatomía, Histología y Neurociencia, Univ. Autónoma de Madrid, Madrid, Spain

Abstract: The differential vulnerability of dopaminergic neurons of the substantia nigra pars compacta is an unresolved subject in Parkinson´s disease. Recent studies in mice reveals the existence heterogenous subpopulations of nigral dopaminergic neurons. In monkeys, the

molecular phenotypes of dopaminergic neurons are poorly characterized. Here, we carried a detailed histological study disclosing the anatomical distribution of different molecular phenotypes within identified midbrain neurons in monkeys (i.e., Calbindin, Girk2, Alhd1a1) and their selective vulnerability in a progressive MPTP monkey model. Our data show that in the ventral tier of the substantia nigra pars compacta, neurons rich in Aldh1a1 and Girk2 are intermingled, whereas calbindin is the marker best identifying the most resistant neurons located in the dorsal tier and ventral tegmental area. Loss of Aldh1a1+ neurons in the ventral tier was progressive and related to the degree of parkinsonism of MPTP-treated monkeys. Also, Aldh1a1+ neurons projects massively to the striatum, specifically to the putamen, while Aldh1a1- neurons give rise to nigropallidal projections. Moreover, Aldh1a1+ staining was strikingly evident throughout dendrites processes with very long vertical trajectories extending through the substantia nigra pars reticulata and colocalized with dense striatal afferent fibers that extend dorsally within the same region. In conclusion, vulnerable nigrostriatal-projecting neurons in monkeys can be properly identified by using Aldh1a1 and Girk2 as distinctive markers. This could help to understand which factors are most relevant to explain selective neuronal vulnerability in PD and perhaps contribute to specific therapeutic developments.

Disclosures: N. López González del Rey: None. **N. Hernandez-Pinedo:** None. **M. Carrillo Díaz:** None. **M. Del Cerro Legaz:** None. **N. Esteban García:** None. **M.H. Monje:** None. **I. Trigo-Damas:** None. **M. Marin:** None. **J.L. Lanciego:** None. **C. Cavada:** None. **J.A. Obeso:** None. **J. Blesa:** None.

Poster

PSTR267: Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR267.04/B106

Topic: C.03. Parkinson's Disease

Support: Aligning Science Across Parkinson's [ASAP-020505]

Title: Absence of locus coeruleus noradrenergic neuronal loss in a progressive monkey model of Parkinson's Disease

Authors: *M. CARRILLO DÍAZ[1], N. HERNANDEZ-PINEDO[2], I. PÉREZ-SANTOS[3], M. CIORRAGA[2], N. MERCADO-GARCÍA[2], J. A. OBESO[2], C. CAVADA[3], J. BLESA[2]; [1]Network Ctr. for Biomed. Res. on Neurodegenerative Dis. (CIBERNED), Inst. Carlos III, Madrid, Spain; [2]Fundación de Investigación HM Hospitales, Madrid, Spain; [3]Anatomía, Histología y Neurociencia, Univ. Autónoma De Madrid, Madrid, Spain

Abstract: Parkinson's disease is one of the most common movement disorders whichpathology is mainly correlated with the loss of dopaminergic neurons in the substantia nigra pars compacta.

However, beyond this structure, there are other regions susceptible to Parkinson's-associated degeneration such as the locus coeruleus. The locus coeruleus, located in the pons, is the major group of noradrenaline-producing neurons in the brain and exhibits pathological changes in the brain of Parkinson´s disease patients, particularly those who evolved into dementia. In this study, we used dopamine-beta-hydroxylase (DBH) immunocytochemistry to assess noradrenergic neuronal loss in the locus coeruleus in a progressive monkey model of Parkinson's disease. We analyzed the brains of control monkeys and monkeys who, following treatment with MPTP remained asymptomatic, monkeys who recovered after showing mild parkinsonian signs, and monkeys with stable parkinsonism. Unbiased stereological estimations of neuron numbers were achieved using the optical fractionator. All analyses were performed within the same subjects in which dopaminergic degeneration in the substantia nigra had been studied earlier; we employed equal processing and analysis parameters, thus allowing for reliable data comparisons. This allowed us to compare the noradrenergic pathology in the locus coeruleus with the dopaminergic pathology in the substantia nigra in the same subjects. Our data reveal that, despite substantial loss of dopaminergic neurons, there is no apparent neuronal loss in the locus coeruleus in any of the MPTP-treated groups. These findings show that under the same experimental situation, cell loss in dopaminergic and noradrenergic brainstem groups do not follow analogous patterns. The observation that there is no noradrenergic denervation in a model that best resembles Parkinson's disease has potential pathophysiological implications.

Disclosures: M. Carrillo Díaz: None. **N. Hernandez-Pinedo:** None. **I. Pérez-Santos:** None. **M. Ciorraga:** None. **N. Mercado-García:** None. **J.A. Obeso:** None. **C. Cavada:** None. **J. Blesa:** None.

Poster

PSTR267: Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR267.05/B107

Topic: C.03. Parkinson's Disease

Support: Aligning Science Across Parkinson´s [ASAP-020505]

Title: Immunocytochemical distribution of the Cannabinoid Receptor 1 (CBR1) in the non-human primate basal ganglia: relevance for Parkinson's disease

Authors: *N. HERNANDEZ-PINEDO[1], M. CARRILLO DÍAZ[2], M. CIORRAGA[1], N. MERCADO-GARCÍA[1], C. CAVADA[3], J. A. OBESO[1], J. BLESA[1];
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Abstract: The endocannabinoid system and its main signaling hub, the type-1 cannabinoid receptor (CBR1), which is the principal cannabinoid receptor expressed in the brain, are reported to be implicated in diverse neurodegenerative disorders, such as Parkinson's disease (PD). However, with most studies focusing on rodents, the distribution of this receptor in the non-human primate basal ganglia is not well documented; this underlines the importance of species-specific translational studies. Here, we investigated the location of CBR1 in the basal ganglia of macaque monkeys. A map of the expression and distribution of the CBR1 receptor was generated using a specific antibody. We observed intense immunoreactivity primarily in axons and boutons. CBR1 exhibited considerable heterogeneity in density and laminar distribution in cortical regions. The hippocampus also contained a high density of CBR1 axons consistent with previous reports. In the basal ganglia, the striatum contained very faint immunoreactivity. The *globus pallidus (pars interna* and *externa)* and the *substantia nigra pars reticulata*, exhibited the most intense CBR1 immunoreactivity of the brain, while the thalamus and the subthalamic nucleus appeared to be completely devoid of CBR1. This suggests a possible modulatory role of CBR1 basal ganglia output. Additionally, dense CBR1-immunoreactive axonal bundles were detected in the ventral *substantia nigra pars compacta*, overlapping with the dendrites of ventrally projecting dopaminergic neurons. CBR1s have been suggested to be mainly located in gabaergic striatonigral and glutamatergic subthalamonigral terminals. This suggests that CBR1 may also be involved in modulating dopaminergic neurons in the *substantia nigra* ventral tier. These data enhance the anatomical distribution and potential understanding of CBR1 in the primate basal ganglia.

Disclosures: N. Hernandez-Pinedo: None. **M. Carrillo Díaz:** None. **M. Ciorraga:** None. **N. Mercado-García:** None. **C. Cavada:** None. **J.A. Obeso:** None. **J. Blesa:** None.

Poster

PSTR267: Parkinson’s Disease: Dopamine and Non-Dopamine Pathways

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR267.06/B108

Topic: C.03. Parkinson’s Disease

Support: São Paulo Research Foundation (FAPESP) Grant 2017/00003-0
National Council for Scientific and Technological Development (CNPq) Grant 312009/2022-4

Title: Nitric oxide donor increases medium spiny neurons responsiveness to cortical drive in L-DOPA-induced dyskinesias

Authors: *F. E. PADOVAN-NETO[1], D. RIBEIRO[2], R. GUIMARÃES[2];
[1]Univ. of São Paulo, Ribeirao Preto, Brazil; [2]Univ. of São Paulo, Ribeirão Preto, Brazil

Abstract: L-DOPA is the classical gold standard treatment for Parkinson's disease (PD). However, its chronic administration can lead to the development of L-DOPA-induced dyskinesias (LIDs). Dysregulation of the nitric oxide-cyclic guanosine monophosphate (NO-cGMP) pathway in striatal networks has been linked to deficits in corticostriatal transmission in LIDs. This study explored the impact of the NO donor sodium nitroprusside (SNP) on behavioral and electrophysiological measures in sham-operated and 6-hydroxydopamine (OHDA)-lesioned rats. Our findings revealed that systemic SNP administration enhanced stepping test performance in dyskinetic rats without influencing AIMs incidence. Furthermore, SNP substantially increased the spike probability of MSNs in response to excitatory corticostriatal transmission driven by electrical stimulation of the primary motor cortex, with a more pronounced effect in dyskinetic animals. These findings highlight the critical role of the NO signaling pathway in facilitating the responsiveness of striatal MSNs in both the intact and dyskinetic striatum. The study suggests that SNP has the potential to enhance L-DOPA's effects in the stepping test without exacerbating AIMs, thereby offering new possibilities for optimizing PD therapy. In conclusion, this study highlights the involvement of the NO signaling pathway in the pathophysiology of LIDs.

Disclosures: F.E. Padovan-Neto: None.

Poster

PSTR267: Parkinson’s Disease: Dopamine and Non-Dopamine Pathways

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR267.07/B109

Topic: C.03. Parkinson’s Disease

Support: 1R01NS122226-01

Title: The influence of interventional genetic knockdown of the serotonin transporter on L-DOPA-induced behaviors and transcriptional changes in hemiparkinsonian rats

Authors: *G. MCMANUS[1], A. CENTNER[1], J. HUANG[1], C. R. BISHOP[1], F. P. MANFREDSSON[2];
[1]Psychology, Binghamton Univ., Binghamton, NY; [2]Translational Neurosci., Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Dopamine replacement therapy with L-3,4-dihydroxyphenylalanine (L-DOPA) has remained the gold-standard treatment for the hypokinetic motor symptoms of Parkinson’s disease (PD) for several decades. Unfortunately, chronic use leads to the development of severe treatment-induced side effects known as L-DOPA-induced dyskinesia (LID). The mechanisms underlying LID are complex, but accumulating research has highlighted the involvement of aberrant neuroplasticity within raphe-striatal serotonin (5-HT) circuits. This compensatory neuroplasticity may be beneficial in earlier stages of PD but becomes maladaptive and

precipitates LID with continued dopamine (DA) loss in later stages. The 5-HT transporter (SERT) has emerged as an intriguing target for anti-dyskinetic adjuncts as it has been shown to traffic DA in the DA-denervated striatum and is upregulated in the brains of dyskinetic patients and animal models of LID. The current study employs an interventional genetic knockdown of SERT (SERT-KD) to further characterize its role in LID expression and LID-associated transcription factors. Adult male and female 6-hydroxydopamine-lesioned Sprague-Dawley rats were administered sub-chronic L-DOPA daily for 2 weeks to establish LID. Following the last day of L-DOPA treatment, animals underwent a second surgery where they received either adeno-associated virus 9 (AAV9) short-hairpin RNA driven SERT-KD (SERT-shRNA) or control scrambled shRNA AAV9-GFP (SCR-shRNA). LID reinstatement and motor performance were assayed over an additional 2 weeks of sub-chronic L-DOPA treatment. Raphe and striatal tissue were collected for reverse-transcription polymerase chain reaction (RT-PCR) analysis of known Parkinsonian and dyskinetic gene expression. We hypothesized that this genetic intervention would reduce LID expression by targeting a primary presynaptic mechanism in LID development wherein the pulsatile release of DA from 5-HT cells is attenuated. Behavioral data showed significant reductions in LID across the second treatment period in SERT-KD animals compared to SCR-shRNA controls that were correlated with commensurate changes in pro-dyskinetic mRNA expression including *cFOS* and prodynorphin (*PPD*). This suggests a reorganization of pro-dyskinetic circuitry following SERT-KD knockdown that further supports SERT as an important mechanism in the development and expression of LID. This study provides a promising avenue for developing novel therapies to optimize PD treatment.

Disclosures: G. McManus: None. **A. Centner:** None. **J. Huang:** None. **C.R. Bishop:** None. **F.P. Manfredsson:** None.

Poster

PSTR267: Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR267.08/B110

Topic: C.03. Parkinson's Disease

Support: NSERC RGPIN - 2020-06757

Title: Exploring the effects of dopaminergic profiles on muscle representation

Authors: *F. ADAMS[1], S. FOGLIA[2], K. RAMDEO[3], C. DRAPEAU[1], C. V. TURCO[1], A. J. NELSON[4];
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Abstract: Exploring the effects of dopaminergic profiles on muscle representation

AUTHORS: F.C. ADAMS[1], S.D. FOGLIA[2], K.R. RAMDEO[1], C.C DRAPEAU[1], C.V TURCO[3], A.J. NELSON[1,2]

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The organization of the primary motor cortex (M1) can be non-invasively assessed using transcranial magnetic stimulation (TMS) combined with frameless neuro-navigation. This technique involves single pulses of TMS delivered pseudo-randomly over a pre-defined grid, centered over the motor hotspot of the target muscle. By simultaneously recording motor evoked potentials (MEP) from the target muscles, this information can be used to create a "motor map". Motor maps allow quantification of characteristics such as area, and center of gravity (CoG) of the target muscle. Previous research has shown that dopamine modulates functional organization of motor cortical structures in the brain (Macedo-Lima et al., 2021). This study aimed to assess how dopaminergic bioavailability contributes to the representation of muscles in the primary motor cortex (M1). Motor corticospinal maps were obtained 32 right-handed males (mean age 24.38 ± 3.2 years) by delivering 80 suprathreshold TMS pulses over a 6 x 6 cm grid centered over the first dorsal interosseous (FDI) motor hotspot of the left M1. Saliva samples were obtained for analysis of the following dopaminergic genes: COMT, DRD2, DRD1, ANKK1, DAT1. For each gene, participants received a score of 0 if they possessed the allele associated with reduced dopamine levels in the brain or a score of 1, if they possessed the allele linked to increased dopamine levels. Scores from the 5 genes were summed to calculate each participant's overall genotype score. We used the overall score and individual genotypes to determine if one's genetic dopaminergic predisposition contributed to M1 muscle representation by separating participants that had a high dopaminergic bioavailability and low dopaminergic bioavailability. Preliminary results suggest dopaminergic genetic predisposition modulates CoG_x and area of the motor corticospinal map.

Disclosures: F. Adams: None. **S. Foglia:** None. **K. Ramdeo:** None. **C. Drapeau:** None. **C.V. Turco:** None. **A.J. Nelson:** None.

Poster

PSTR267: Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR267.09/B111

Topic: C.03. Parkinson's Disease

Title: Effects of multimodal serotonin and dopamine compounds on Levodopa-induced dyskinesia in hemiparkinsonian rats

Authors: *E. SARINICK[1], E. VALLE[2], S. DEMUS[2], C. BUDROW[2], M. DAWIDOWSKI[3], M. WROBEL[3], M. COYLE[2], C. R. BISHOP[2];
[1]Binghamton Univ., Vestal, NY; [2]Psychology, Binghamton Univ., Vestal, NY; [3]Dept. of Drug Technol. and Pharmaceut. Biotech., Med. Univ. of Warsaw, Warsaw, Poland

Abstract: Parkinson's Disease (PD) is a progressive neurodegenerative movement disorder arising from loss of nigrostriatal dopamine (DA) neurons, resulting in rigidity, tremors, and bradykinesia. While levodopa (L-DOPA), is commonly prescribed as a gold-standard therapy to combat motor deficits, long term use can precipitate adverse effects including abnormal involuntary movements (AIMS) known as L-DOPA induced dyskinesia (LID). Previous studies suggest that compounds with affinities at the serotonin (5-HT) 1A receptor (5-HT1AR), serotonin transporter (SERT), and DA D2 receptor (D2R) may reduce LID while maintaining the motor efficacy of L-DOPA. Therefore, in the current preclinical study, novel experimental compounds (4f, 4n, and 6d) with varying affinities for 5-HT1AR, SERT and D2R were assessed for their efficacy in reducing LID. To do this, male and female adult Sprague-Dawley rats (N = 9) received unilateral 6-hydroxydopamine into the medial forebrain bundle to render them hemiparkinsonian. Following recovery, lesioned rats, as determined by forepaw adjusting steps (FAS) tests, were rendered dyskinetic through daily L-DOPA treatment (6 mg/kg, s.c) for 2 weeks. For Experiments 1, 2, and 3, compounds 4f, 4n, and 6b (0, 5, 10, 20 mg/kg, i.p.) were counterbalanced and acutely injected in a within subjects design, where AIMs and FAS were implemented to assess alterations in LID and motor efficacy, respectively. Preliminary results revealed that 4f was without effect against LID, but maintained L-DOPA efficacy. We hypothesize that 4n and 6b, which ostensibly have balanced actions at our targets, will dose-dependently reduce LID. Such findings would support new compounds with optimized profiles that will improve long term PD treatment.

Disclosures: E. Sarinick: None. **E. Valle:** None. **S. Demus:** None. **C. Budrow:** None. **M. Dawidowski:** None. **M. Wrobel:** None. **M. Coyle:** None. **C.R. Bishop:** None.

Poster

PSTR267: Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR267.10/B112

Topic: C.03. Parkinson's Disease

Support: P50 DA044121-01A1
R01MH110556
1R01NS119690-01
ASAP-020600

Title: Mapping the projectome of DA subtypes.

Authors: *O. A. MORENO-RAMOS[1], D. D. A. RAJ[2], A. VIGOTSKY[1], E. P. PHELAN[4], D. A. DOMBECK[3], J.-F. POULIN[6], A. V. APKARIAN[5], R. AWATRAMANI[1];
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Abstract: Midbrain dopamine (mDA) neurons display a plethora of functions, including movement, reward learning, and motivation. The diverse functions of mDA neurons can be explained partially by their intrinsic heterogeneity, which has been shown recently using single-cell RNA sequencing. We sought to examine whether molecular heterogeneity correlates with anatomical projection differences. Using markers derived from single-cell data, our lab has shown a topographic projection mapping of molecularly distinct midbrain DA neurons from the Substantia Nigra pars compacta (SNc) and the Ventral Tegmental Area (VTA), using different intersectional genetic labeling strategies. We have revealed distinct genetically defined DAergic projection patterns to various forebrain regions. We seek to substantially extend and refine previous studies and provide a systematic quantitative anatomic characterization of genetically defined mDA subtype projections in the striatum. To do so, we 1. Utilized intersectional and subtractional (complementary) reporter viruses injected in SNc or VTA to determine the exclusivity of projections 2. Used new driver mouse lines based on newer single-cell taxonomic schemes, allowing greater access to specific subpopulations of DA neurons, and 3. Developed an axonal density quantification method to parcellate the striatum according to the mDA subtype projections. Utilizing our genetic driver mouse lines, we show across each genotype and analyzed a biased axonal distribution in all sections, demonstrating the striatum can be subdivided based on the distinct projections of the DA subtypes. This DA subtype projectome map will serve as a guiding resource to understand better circuitry, functional roles of mDA neurons, and manipulation of specific mDA subtypes.

Disclosures: O.A. Moreno-Ramos: None. **A. Vigotsky:** None. **D.A. Dombeck:** None. **J. Poulin:** None. **A.V. Apkarian:** None. **R. Awatramani:** None.

Poster

PSTR267: Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR267.11/B113

Topic: C.03. Parkinson's Disease

Support: PPMI data funded by Michael J. Fox Foundation and funding partners

Title: The relationship between dual task gait parameters and dopaminergic function in Parkinson's disease: insights from the Parkinson's progression marker initiative

Authors: *J. S. BARAJAS, E. OFORI;
Col. of Hlth. Solutions, Arizona State Univ., Phoenix, AZ

Abstract: The addition of a secondary task can worsen walking performance in people with Parkinson's disease; some of these changes include reducing gait velocity, arm swing asymmetry, reducing stride length, and increasing gait variability, which can increase risk of falls. Executive deficits in Parkinson's disease are associated with dopaminergic dysfunction in both the striatum and cortex. It is not clear what aspects of dual tasking are impacted as a function of dopaminergic availability. Thus, the purpose of this analysis is to determine whether dopamine active transporter in Parkinson's disease relates to specific dual task gait parameters. Data were obtained from the Parkinson's Progression Marker Initiative (PPMI) database (www.ppmi-info.org). Thirty people newly diagnosed with Parkinson's disease had a dopamine active transporter (DaT) imaging scan to assess dopamine transporter density using single photon emission computed tomography (SPECT). The SPECT imaging procedure was performed at the individual sites using DaTscan as the dopamine transporter; the measurements for this analysis were the striatal binding ratio (SBR) of the caudate, putamen and anterior putamen referenced to the occipital lobe. For the gait protocol, participants wore Axivity Ax6 Sensors and Opal Sensors while (1) walking at their preferred walking speed for one minute and (2) walking at their preferred walking speed for one minute while serial subtracting by 3's. Gait smoothness and rigidity were determined by measures of walking speed, cadence, asymmetry, and jerk. Changes in gait (dual task – single task) were used in the statistical analysis. Multi-level modeling was used to explore the relationship between dual task gait parameters and the SBR of the regions of interest. Changes in cadence were associated with the SBR of the putamen (p=.004) and the anterior putamen (p=.033), such that increases in the putamen SBR and decreases in the anterior putamen SBR were related to increases in change in cadence. Changes in right-sided jerk were associated with the SBR of the caudate (p=.007) and the anterior putamen (p=.038), such that increases in the caudate SBR and decreases in the anterior putamen SBR were related to increases in right-sided jerk. Dopamine active transporter in Parkinson's disease relates to changes in cadence and right-sided jerk while dual task walking. Further, dopamine dysfunction in the anterior putamen may be important for regulating the smoothness of dual task gait. Insight into dopaminergic function and dual task gait can inform clinicians on how to identify patients with increased risk for mild cognitive impairment and/or Parkinson's disease dementia.

Disclosures: J.S. Barajas: None. **E. Ofori:** None.

Poster

PSTR267: Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR267.12/B114

Topic: C.03. Parkinson's Disease

Support: Parkinson's Foundation Postdoctoral Fellowship for Basic Scientists Award PF-PRF-839073
NIH Grant R01NS9580

Title: Reduced striatal M4-cholinergic signaling following dopamine loss differentially contributes to parkinsonian and levodopa-induced diskinetic behaviors

Authors: *B. E. NIELSEN[1], C. FORD[2];
[1]Pharmacol., Univ. of Colorado - Anschutz Med. Campus, Aurora, CO; [2]Pharmacol., Univ. of Colorado, Aurora, CO

Abstract: A dynamic equilibrium between dopamine (DA) and acetylcholine (ACh) is essential for striatal circuitry and motor function, as imbalances are associated with Parkinson's disease (PD) and levodopa-induced dyskinesia (LID). DA and ACh interact at multiple levels, including the direct modulation of striatal output cells, the medium spiny neurons (MSNs), through G-protein-coupled receptors. Conventional theories posit that ACh levels are pathologically elevated following DA loss, predicting an enhancement in muscarinic M4 ACh receptor signaling in direct pathway MSNs that would contribute to an overall inhibition of movement in PD. However, whether and how those changes in ACh levels translate to specific alterations in striatal M4-cholinergic transmission and motor behavior, remains unknown. To address this, we combined two-photon imaging and brain slice electrophysiology to specifically disentangle ACh release and downstream signaling through M4-receptors in parkinsonian mice. We surprisingly found that synaptic release of ACh was unaltered in response to DA lesion, but the strength of transmission at muscarinic M4-receptor synapses on direct pathway MSNs was decreased, as a result of reduced postsynaptic M4-receptor function. We developed two strategies that effectively rescued the impaired cholinergic signaling: overexpression of M4-receptors and ablation of Regulator of G-protein Signaling 4 (RGS4) to prolong M4-receptor downstream signaling cascade selectively in direct pathway MSNs. Remarkably, restoring M4-cholinergic transmission led to a partial alleviation of both, parkinsonian motor balance and coordination deficits as well as levodopa-induced dyskinetic behavior, revealing an unexpected prokinetic effect in the absence of DA while the canonical antikinetic role of M4-receptors was observed when DA was present. Our findings indicate that the decrease in M4-function constitutes a cell-subtype and synapse-specific adaptation in response to DA loss with broad implications at striatal circuit level, playing a previously unnoticed role in the pathophysiology of PD and the progression to LID, and representing a promising therapeutic target for these movement disorders.

Disclosures: B.E. Nielsen: None. **C. Ford:** None.

Poster

PSTR268: Parkinson's Disease: Human Studies: Genetics and Diagnostic

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: /

Topic: C.03. Parkinson's Disease

Support: MJFF-020020

Title: Are all Parkinson's disease freezing of gait episodes the same? Characterizing the neural signature of freezing via an immersive virtual reality paradigm

Authors: *M. MILLER KOOP;
Cleveland Clin., Cleveland, OH

Abstract: Are all Parkinson's disease freezing of gait episodes the same? Characterizing the neural signature of freezing via an immersive virtual reality paradigm

Disclosures: M. Miller Koop: None.

Poster

PSTR268: Parkinson's Disease: Human Studies: Genetics and Diagnostic

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR268.01/B115

Topic: C.03. Parkinson's Disease

Support: Canadian Institute of Health Research (CIHR) (PJT-173540)

Title: Use of machine learning for identification of Parkinson's Disease and mild cognitive impairment through neuroimaging and biofluid biomarkers: a study from the PPMI cohort

Authors: *A.-G. DENNIS[1,2], R. CHEN[1,2], P. GERRETSEN[1,3], A. STRAFELLA[1,2,3];
[1]Univ. of Toronto, Toronto, ON, Canada; [2]Krembil Brain Institute, Toronto, ON, Canada; [3]Centre for Addiction and Mental Health, Toronto, ON, Canada

Abstract: Parkinson's Disease (PD) is a neurodegenerative disorder resulting in both motor symptoms, such as bradykinesia, rigidity, tremor, and gait difficulties, and a variety of nonmotor symptoms, like cognitive impairment and behavioural complications. During PD progression, cognitive ability declines, resulting in mild cognitive impairment (MCI). PD and MCI have been explored with neuroimaging-based biomarkers; however, relying on these biomarkers alone can sometimes be ineffective because of large individual differences in brain activity. Thus, combining biofluid biomarkers, allowing also for proteomic differences, can help in a better biological definition of the disease. Since current diagnostic tests for PD and MCI focus on individual biomarkers, misdiagnoses can be frequent. This research aims to combine neuroimaging and biofluid data as biomarkers for PD and MCI progression, with the goal of developing a more efficient method of predicting disease states and symptoms. Using the support

vector machine (SVM) and random forest (RF) machine learning techniques, models were created based on neuroimaging and biofluid biomarkers for a subset of PD and healthy subjects (HC) from the Parkinson's Progression Markers Initiative (PPMI) dataset. Striatal binding ratios (SBRs) of the caudate and anterior putamen extracted from DaT-SPECT imaging were used as neuroimaging biomarkers. Proteomic concentrations of beta-amyloid-42, alpha-synuclein, total-tau, phosphorylated-tau-181, and neurofilament light derived from cerebrospinal fluid (CSF) represented the biofluid biomarkers. When differentiating subjects with PD from HC, both SVM and RF techniques perform with high accuracy when only using neuroimaging. These techniques did not perform as well when proteomic biomarkers from CSF were used alone, or when SBRs were applied to detect MCI. We expected that due to the link between alpha-synuclein/neurofilament light and presence of PD, these models would perform better in comparison to other models when detecting PD. However, we found that models combining SBRs with beta-amyloid-42 concentrations tended to have higher performance. When detecting MCI, models combining SBRs with either phosphorylated-tau-181 or total-tau performed better than others. Based on these results, diagnostic performance may be improved through combining neuroimaging with biofluid-based biomarkers to distinguish subjects with PD from HC and subjects with MCI from subjects with normal cognitive abilities. This study's next steps involve investigating differences in performance between SVM and RF to determine which technique is best suited to the analysis.

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Poster

PSTR268: Parkinson's Disease: Human Studies: Genetics and Diagnostic

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR268.02/Web Only

Topic: C.03. Parkinson's Disease

Support: Chaya Charitable Fund
Michael J. Fox Foundation
Biogen, Inc

Title: P2rx7, an adaptive immune response gene, is associated with parkinson's disease risk and age at onset

Authors: *S. SHANI[1], M. GANA WEISZ[2], T. GUREVICH[3], N. GILADI[4], R. ALCALAY[2], A. ORR-URTREGER[5], O. GOLDSTEIN[2];

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Abstract: Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the accumulation of α-synuclein in Lewy bodies and the degeneration of neurons in the substantia nigra. PD patients carrying mutations in genes associated with the disease, such as *LRRK2* or *GBA1*, often exhibit distinct clinical and pathological features. Mounting evidence supports a role for the adaptive immune response in PD. We aimed to evaluate the genetic contribution of the adaptive immune response in three PD groups: *GBA1*-PD, *LRRK2*-PD, and non-carriers-PD (NC-PD). **Methods:** A list of adaptive immune response genes was extracted from the Molecular Signature Database (MSigDB). Subsequently, differentially expressed genes (DEGs) associated with PD were identified using four GEO datasets, and analyzed separately. DEGs overlapping with the adaptive immune response genes were further evaluated through whole-genome sequencing of 201 unrelated Ashkenazi Jewish (AJ) PD patients. Potential pathogenic variants were identified, and *P2RX7* was selected for further assessment. Eight *P2RX7* variants were genotyped in a cohort of 1200 AJ PD patients (unrelated and consecutively recruited), followed by rare variants burden analysis (allele frequencies (AF) <= 0.01). Risk analysis and Age at onset (AAO) analyses were performed for the common variants (AF > 0.01). AFs were compared to AJ non-neuro cases in the gnomAD database. Subsequently, variants associated with PD were further examined in an independent AJ cohort from AMP-PD database. **Results:** Four adaptive immune DEGs, *CD8B2, P2RX7*, *IL27RA*, and *ZC3H12A*, were identified. *P2RX7,* which harbored eight variants, underwent further assessment. Burden analysis, which included five rare variants, showed no significant difference between PD and controls. Among the three common variants, one variant showed an association with PD risk in NC-PD (allelic OR=1.147, p=0.015) and in the entire cohort (allelic OR=1.145, p=0.005). Another variant was associated with PD risk in *LRRK2*-PD (allelic OR=2.095, p=0.037), while a third was associated with early AAO in *LRRK2*-PD (p=0.014). In the secondary cohort of AMP-PD, ORs of the two PD risk variants were consistent with our results, though statistical significance was not achieved, probably due to the small control sample size. **Conclusions:** Variants within *P2RX7* may be linked to both PD risk and AAO. Considering *P2RX7* has been suggested as a potential therapeutic target for neurodegenerative diseases, including PD, our data provide additional support for this proposition and suggest a potential interaction between *P2RX7* and *LRRK2*.

Disclosures: S. Shani: None. **M. Gana weisz:** None. **T. Gurevich:** None. **N. Giladi:** None. **R. Alcalay:** None. **A. Orr-Urtreger:** None. **O. Goldstein:** None.

Poster

PSTR268: Parkinson’s Disease: Human Studies: Genetics and Diagnostic

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR268.03/B116

Topic: C.03. Parkinson's Disease

Support: CDMRP- W81XWH-22-1-0609

Title: Microbiome dysbiosis in Parkinson's disease is associated with elevated production of neurotoxic Trimethyl Amine metabolites.

Authors: *D. MONDHE[1], N. JAYABALAN[1], A. G. KANTHASAMY[2], R. GORDON[1]; [1]Queensland Univ. of Technol., Brisbane, Australia; [2]Biomed Sci, Iowa Ctr. for Advanced Neurotoxicology, Univ. of Georgia, Athens, GA

Abstract: Multiple cohort studies indicate that gut microbiota composition is significantly altered in people with Parkinson's disease (PD). However, the functional and pathological significance of gut dysbiosis in PD remains poorly understood. Gastrointestinal dysfunction is a common complaint in PD patients, often preceding clinical diagnosis and typically accompanied by increased gut mucosal permeability. In this study, we evaluated the changes in circulating gut microbiota metabolites in PD patient biofluids and used high-resolution functional metagenomics to determine changes in microbial composition and pathways in PD patients (n = 17) compared to an age-matched healthy cohort (n = 60). We found changes in multiple microbial metabolites in our PD cohort, including Trimethylamine (TMA), a brain-permeable metabolite of bacterial-origin with a positive association with the rate of PD progression and cognitive decline. We also found changes in polyamines and amino acid metabolism in PD patient plasma and urine samples. TMA and its metabolites were also altered in both PD patient blood and urine in a cohort of healthy (n = 31) and PD (n = 31) individuals. We also uncovered elevated levels of neurotoxic Formaldehyde (FA), in our PD patient cohort. Our high-resolution functional metagenomics studies demonstrate for the first time that TMA-generating bacteria, as well as bacterial enzymatic pathways responsible for its generation to be increased in PD patients. Our mechanistic studies also revealed that circulating TMAO can accelerate synuclein aggregation and immune activation in microglia and PBMCs. We also uncovered a trend towards loss of beneficial bacteria which produce anti-inflammatory metabolites, such as butyrate, in PD patients. Taken together, our results provide novel mechanistic insights into how gut dysbiosis and altered microbial metabolism can drive disease progression in PD.

Disclosures: D. Mondhe: None. **N. Jayabalan:** None. **A.G. Kanthasamy:** None. **R. Gordon:** None.

Poster

PSTR268: Parkinson's Disease: Human Studies: Genetics and Diagnostic

Location: MCP Hall A

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Program #/Poster #: PSTR268.04/B117

Topic: C.03. Parkinson's Disease

Support: NIH BRAIN Initiative U01 NS117839

Title: Effects of phase-targeted stimulation on bradykinesia in Parkinson's Disease

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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder. Motor symptoms include bradykinesia and rigidity, the severity of which correlates with the power of local field potential oscillations in the beta frequency band in the subthalamic nucleus and phase-amplitude coupling (PAC) between the phase of beta-band oscillations and the amplitude of gamma-band oscillations in the motor cortex. PAC in PD has been shown to decrease in a dose dependent manner in response to both therapeutic dopaminergic medication and deep brain stimulation (DBS). Recently, it has also been shown that PAC can be modulated by stimulation pulses delivered to the motor cortex that target a specific phase of the beta-band oscillations. This study aims to determine whether this same cortical phase-targeted stimulation (PTS) can alleviate PD motor symptoms, particularly with a quantitative measurement of bradykinesia. Finger tapping and grasping tasks were captured using a LEAP infrared motion sensor during DBS implantation procedures in human participants with PD. Prior to DBS implantation, a 63-channel high density electrocorticography (ECoG) array was placed over the hand region of the motor cortex which was able to simultaneously record neural signals and deliver stimulation pulses, confirmed during intraoperative CT and 3D reconstruction of the ECoG array relative to the cerebral cortex. Each task was run three times for three different conditions - no stimulation (baseline), peak targeting stimulation, and trough targeting stimulation. When compared to baseline, both peak and trough stimulation appear, on average, to increase finger tap amplitude and opening and closing velocity and to decrease the variability of the amplitude and frequency of repetitive hand movements. Our current results suggest that phase targeted cortical stimulation is a viable, less invasive option for treating PD motor symptoms, allowing less invasive stimulation than DBS without interrupting normal cortical processing and motor control.

Disclosures: K. Wyse-Sookoo: None. **Y. Salimpour:** None. **J. Kaur:** None. **W.S. Anderson:** None. **K.A. Mills:** None.

Poster

PSTR268: Parkinson's Disease: Human Studies: Genetics and Diagnostic

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR268.05/B118

Topic: C.03. Parkinson’s Disease

Title: Functional connectivity of pre-motor area and supplementary motor area increases during movement with sensory cues in people with Parkinson's disease

Authors: A. K. ZADEH[1], N. SADEGHBEIGI[2], H. SAFAKHEIL[3], S. K. SETAREHDAN[4], ***L. ALIBIGLOU**[5];
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Abstract: Individuals with Parkinson's disease (PD) often show enhanced performance in motor tasks when provided with external sensory cues. Previous research studies have extensively explored how different sensory cues influence motor abilities in people with PD. However, the underlying neural mechanisms driving these improvements and how they affect the functional connectivity within the motor network remain poorly understood.
This study aimed to investigate differences in the activation pattern and functional connectivity of the primary motor cortex (M1), supplementary motor area (SMA), and pre-motor area (PMA) between people with PD and neurologically healthy individuals (control group) during a wrist motor task. Ten people with PD and ten age- and sex-matched control subjects participated in this study. Each participant performed a repetitive wrist flexion and extension task under auditory-cued and visually-cued conditions. The changes in oxygenated hemoglobin and deoxyhemoglobin concentrations in motor areas were measured using functional near-infrared spectroscopy (fNIRS). Electromyograms from wrist muscles and kinematics of the wrist movements were also recorded. The fNIRS results demonstrated significantly higher neural activity in the PMA of PD patients compared to controls ($p=0.006$), with elevated activities also observed in the SMA and M1, although not statistically significant. More importantly, the results showed that the functional connectivity between SMA and PMA was significantly higher in the PD group compared to controls ($p=0.016$) during motor tasks in the presence of sensory cues. These findings underscore the multifaceted influence of sensory cues in PD, highlighting their modulation effects on both regional neural activity and interregional functional connectivity within the motor network involved in movement planning and preparation.

Disclosures: A.K. Zadeh: None. **N. Sadeghbeigi:** None. **H. Safakheil:** None. **S.K. Setarehdan:** None. **L. Alibiglou:** None.

Poster

PSTR268: Parkinson’s Disease: Human Studies: Genetics and Diagnostic

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR268.06/B119

Topic: C.03. Parkinson's Disease

Support: WBI TR_2017

Title: Trehalase activity in duodenal samples collected from individuals with Parkinson's disease and age-matched controls.

Authors: *P. A. HOWSON[1], N. P. VISANJI[2], J. M. BROTCHIE[3];
[1]Atuka, Toronto, ON, Canada; [2]CRND, Univ. Toronto, Toronto, ON, Canada; [3]Atuka Inc., Toronto, ON, Canada

Abstract: Trehalose is being developed as a potential oral therapeutic for Parkinson's disease (PD). Following oral ingestion of trehalose, the majority is metabolised to glucose by trehalase in the wall of the jejunum and duodenum. It is thus important to know whether gut trehalase activity might be altered in PD and might affect the availability of orally administered trehalose. For instance, in inflammatory celiac disease, trehalase activity Is reduced by up to 90%. As gastrointestinal inflammation can occur in PD, we hypothesised that intestinal trehalase activity might be reduced in people with PD. To investigate this possibility, we collected duodenal biopsies from 10 individuals with advanced PD and 21 age-matched individuals undergoing routine, pre-planned endoscopies. Trehalase activity in the duodenal samples was assessed *in vitro* by assaying the ability of duodenal samples to convert trehalose to glucose. Individuals who smoked, were *Helicobacter pylori* positive, or who were assessed as having prodromal PD were excluded from the primary analysis. In total 9 individuals with PD and 17 age-matched controls were included in the primary analysis. There was no statistically significant difference in trehalase activity between individuals with PD (164.2 ± 95.2 U/g tissue, mean ± SD) and non-PD individuals (161.6 ± 94.1 U/g tissue). There was also no correlation between trehalase activity and individual age, length of storage before analysis or mass of duodenal sample collected. In summary, trehalase activity was unaltered in people with PD and so this potential confounder likely does not need to be considered when performing dose-selection for clinical trials using orally delivered trehalose in people with PD.

Disclosures: P.A. Howson: A. Employment/Salary (full or part-time):; Atuka. 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.; Weston Family Foundation. **N.P. Visanji:** None. **J.M. Brotchie:** A. Employment/Salary (full or part-time):; Atuka. 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.; Weston Family Foundation.

Poster

PSTR268: Parkinson's Disease: Human Studies: Genetics and Diagnostic

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR268.07/B120

Topic: C.03. Parkinson's Disease

Support: NIH/NINDS Grant NS118146
NIH/NIDNS Grant NS127211

Title: Integrative systems biology identifies consensus molecular patterns in Parkinson's disease

Authors: M.-Y. LAI, *M. ROSENE, S. CÁRDENAS ROMERO, A. LOPEZ CADAVOD, **B. A. BENITEZ**;
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Abstract: Background: The complex molecular alterations underlying cellular dysfunction phenotypic heterogeneity in PD remain unclear. We integrated different omics layers from participants of the Parkinson's Progression Markers Initiative cohort to uncover novel signatures and define different molecularly defined subtypes of PD.Methods: We selected PD patients (n = 608), including LRRK2 (n = 155) and GBA (n = 76) mutation carriers, sporadic PD (sPD) cases (n = 377), and controls (n = 186) with 5K cerebrospinal fluid (CSF) and urine proteomics, 20K whole blood transcriptomics, and plasma metabolomics (280 metabolites). We identified protein quantitative trait loci (pQTLs) and causal proteins in CSF and urine, leveraging Mendelian randomization and colocalization. Molecular signatures were curated by comparing multi-omics across different mutation types (GBA vs. sPD, LRRK2 vs. sPD, and sPD vs. controls) using R-limma, adjusted for age at baseline, sex, and the first four principal components. Integrative clustering across multi-omics was performed using iClusterBayes to cluster PD patients with consensus molecular patterns. We validated the differences in subtypes by comparing clinical features such as cognitive and motor scores, DaTscan data (caudate, putamen, and striatum), CSF synuclein (αSyn) levels, and αSyn seed amplification assay (αSyn-SAA).Results: We found 701 (153) cis-pQTLs and 420 (11) trans-pQTLs for CSF (urine) proteomics; 23 (4) causal proteins, and two proteins (HSP90B1 and HP) common to both CSF and urine; eight (zero) colocalizing with PD risk loci. In addition, we found a CSF αSyn-SAA genetic modifier (OR = 0.37, p = 1.4 x10-6). We also found that LRRK2 is a pleiotropic modifier that regulates four causal proteins in CSF. Genetically defined PD cases had a higher risk of PD than sPD cases (p = 6.4x10-8). We identified dysregulated CSF proteins associated with LRRK2 (281 proteins), GBA (132 proteins), sPD (54 proteins), and αSyn-SAA (3 proteins), altering proteostasis, neurodegeneration and lysosome, and chemokine signaling and axon guidance, respectively. We found 24 and five metabolites associated with LRRK2 and GBA, respectively. We identified two PD clusters with distinct patterns across multi-mic layers that exhibit different proportions of mutation types (p = 4.5x10-5), disease duration (p = 2.2x10-7), and UPDRS-II scores (p = 0.03). A model with αSyn-SAA and DaTscan can distinguish PD from controls precisely (AUC = 0.94).Conclusions: Our integrated consensus multi-omics approach provides insights into the molecular mechanisms and clinical significance within distinct PD subclusters.

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Poster

PSTR268: Parkinson's Disease: Human Studies: Genetics and Diagnostic

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Arizona Department of Health Services contract 211002
Arizona Biomedical Research Commission (contracts 4001, 0011, 05-901 and 1001

Title: Parkinson's disease is associated with shifts between DNA methylation and DNA nydroxymethylation in human brain

Authors: J. CHOZA[1], J. KOCHMANSKI[2], M. VIRANI[1], N. KUHN[2], M. ADAMS[3], *A. I. BERNSTEIN[1];
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Abstract: The majority of Parkinson's disease (PD) cases are due to a complex interaction between aging, genetics, and environmental factors and epigenetic mechanisms are thought to act as critical mediators of the complex interactions between these factors and disease. Evidence for a role of epigenetic regulation in PD has been building, particularly for DNA modifications, over the past 10-15 years. While 5-methylcytosine (5mC) is one of the most well-studied epigenetic marks, there is a growing recognition that 5-hydroxymethylcytosine (5hmC) also plays an important role in gene regulation as it has tissue-, cell-, and age-specific patterns across the genome distinct from 5mC. 5hmC occurs at its highest levels in the brain and is now thought to be particularly important in the central nervous system, particularly in the response to neurotoxicants. While multiple studies to date have explored the role of DNA modifications in PD, most studies have relied on bisulfite (BS) conversion, which does not distinguish between 5mC and 5hmC. Only a handful of existing studies focus specifically on 5hmC; these initial studies support a role for 5hmC in regulation of expression of genes important for PD pathogenesis and indicate that additional studies are warranted. Here, to identify changes in 5hmC in PD, we profiled 5hmC and 5mC simultaneously in an enriched neuronal population from PD post-mortem parietal cortex. This study expands on our previously published epigenome-wide association study (EWAS) performed on DNA isolated from neuron-enriched

nuclei from human postmortem parietal cortex from the Banner Sun Health Research Institute Brain Bank. Because we previously used BS conversion, we were unable to differentiate between 5mC and 5hmC. Here, we utilized additional DNA isolated from the same samples and performed oxidative BS (oxBS) conversion paired with the Illumina MethylationEPIC array to specifically measure 5mC and estimate 5hmC from paired BS-oxBS data. From this, we analyzed both 5hmC and 5mC as related measures using our recently proposed mixed effects model for reconciling base-pair resolution 5mC and 5hmC data. We identified 1030 differentially modified cytosines (DMCs) with paired changes in 5mC and 5hmC (FDR < 0.05). These sites are annotated to 695 unique genes, including the PD risk gene, DNAJC6 (PARK19), but largely in genes not previously implicated in PD or identified in our previous BS-based EWAS. These data suggest that there are significant shifts between 5mC and 5hmC associated with PD in genes relevant to PD pathogenesis that are not captured by analyzing BS-based data alone or by analyzing each mark as distinct datasets.

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Poster

PSTR268: Parkinson's Disease: Human Studies: Genetics and Diagnostic

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR268.09/B122

Topic: C.03. Parkinson's Disease

Support: RF1 MH121373
R01 NS097782

Title: A Deep Learning model for DTI-Based Classification of Parkinson's Disease using Brain Structural Connectivity Matrices

Authors: A. SHALABY[1], A. ALIJANPOUROTAGHSARA[2], K. CHITTA[1], K. MIRPOUR[2], J. CHOI[2], N. POURATIAN[2], ***J. LEE**[1];
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Abstract: Parkinson's Disease (PD) is a progressive neurodegenerative movement disorder, primarily characterized by bradykinesia and tremors. Leveraging a deep learning approach for PD diagnosis using Diffusion Tensor Imaging (DTI) is pivotal for its potential to efficiently analyze complex brain imaging data, with a particular emphasis on understanding the patterns of brain connectivity in PD. The goal of this study is to develop a novel deep learning model that utilizes the brain structural connectivity matrix (SCM), extracted from DTI, to characterize PD-related connectivity differences from healthy control and facilitate diagnosis. The study

comprised 150 participants, consisting of 70 PD patients who underwent deep brain stimulation surgery for moderate to severe motor symptoms, and 80 matched healthy subjects. All subjects underwent pre-treatment DTI with 64 directions, and the resulting images were preprocessed using FMRIB Software Library. The fundamental concept of structural connectivity analysis typically involves acquiring multiple images of each volume element, each sensitive to diffusion along a specific direction. By estimating the direction of water diffusion and the orientation of white matter fiber pathways within each voxel, we can generate a visual representation known as a tractogram. These tractograms offer insights into the connectivity between different regions, providing quantitative data on the number of structural connections within predefined brain regions as defined by the AAL3 Atlas, commonly referred to as the brain SCM. Following the extraction of SCM, we constructed a deep learning architecture employing convolutional neural network (CNN) to aid in the differentiation of PD and healthy subjects. CNN was thoroughly trained and validated using a 5-fold cross validation to ensure its robustness in accurately classifying subjects. Our model achieved an AUROC of 0.889 and a balanced accuracy of 84.2% in distinguishing PD patients from healthy subjects. Moreover, we explored deeper into the inner structure of the developed model by generating saliency maps (SM). These SMs highlight specific brain regions that play a critical role in the model's ability to differentiate between PD and healthy individuals. In conclusion, our study introduces a deep learning model for DTI-based classification of PD utilizing brain structural connectivity patterns. Our model demonstrates promising results by revealing insights into the intricate connectivity patterns of PD. With precise analysis, it has the potential to advance early detection strategies, aiding the development of more personalized treatment approaches enhancing PD care.

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Poster

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Location: MCP Hall A

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Topic: C.03. Parkinson’s Disease

Support: NIH-1R01NS129115-01

Title: Enabling remote assessments of Parkinson’s disease symptoms through augment reality

Authors: *A. BAZYK[1], R. KAYA[2], M. MILLER KOOP[3], A. ROSENFELDT[1], C. WALTZ[4], J. L. ALBERTS[5];
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Abstract: A gap in the medical management of Parkinson's disease (PD) is the lack of an objective, meaningful and patient-centered biomarker to precisely quantify motor symptoms and effectively track disease progression. An augmented reality (AR) headset offers the ability for patients to self-administer and collect data to quantify traditional biomechanical measures of performance in PD. The aim of this project is to develop a platform using AR technology, and its embedded motion sensors, to quantify all cardinal symptoms of PD, including postural instability and gait dysfunction (PIGD) for self-administered, remote assessments. One hundred and forty patients with PD (pwPD), (35 in HY stages I, II, III and IV) will be evaluated to ensure applicably across disease severity. Blinded MDS-UPDRS-III scores will be collected at each assessment. The Microsoft HoloLens 2 (HL2) will be used to deliver multiple upper extremity, lower extremity, and axial motor tasks to quantify PD symptoms, including a finger-tapping (FT) assessment and Timed Up and Go (TUG) task. IMU data from the headset can be used to segment the TUG into the individual tasks. Reconstruction of a hand model from RGB and depth images collected using the HL2 cameras provides 3D hand joint coordinates for upper extremity tasks. The RGB images are tracked using Mediapipe software and projected into 3D space using a custom Matlab implementation of Microsoft code to align RGB and depth images. FT metrics were calculated on the 3D distance between thumb and index finger. Comparison of preliminary data from two participants, one with mild PIGD (clinical gait score = 1; clinical freeze score = 0) and one with severe PIGD (clinical gait score = 3; clinical freeze score = 3) demonstrated that the individual with more severe PIGD, took 260.32% (36.32s vs 10.08s) longer to complete the TUG, with more time needed to complete all the individual tasks of the TUG (e.g. turning and gait). Furthermore, comparison of FT data from two participants, one with a mild clinical FT score (1) and one a severe score (3), demonstrated that the individual with worse clinical scores had a smaller average amplitude of 8.51cm vs 9.34cm, greater amplitude standard deviation (5.20cm vs 2.19cm) and exhibited amplitude decrement of -0.20 cm/s compared to -0.03 cm/s. Comparison of preliminary data suggest HL2 platform can be utilized to deliver motor tasks traditionally used to assess PD motor symptoms and detect performance difference between varying levels of PD disease severity. If successful, this platform will provide objective, accurate and quantitative measures of PD, in one platform, that can be assessed remotely to improve clinical care of pwPD.

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Poster

PSTR268: Parkinson's Disease: Human Studies: Genetics and Diagnostic

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR268.12/B124

Topic: C.03. Parkinson's Disease

Title: Can untargeted proteomics measures predict dopamine loss and motor and cognitive symptoms in patients with Parkinson's Disease(PD)?

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Abstract: The progressive loss of dopamine in Parkinson's Disease (PD) affects a broad spectrum of functions, from motor control to cognitive abilities. Early and accurate prediction of dopamine depletion could revolutionize PD treatment strategies by facilitating more personalized and timely interventions that may slow disease progression or alleviate symptoms. While current research primarily focuses on genetic markers, imaging techniques, and symptomatic treatments, the use of proteomics as a predictive tool remains underexplored.This study assesses the potential of untargeted proteomics as a predictive tool for dopamine depletion and correlates these molecular patterns with the severity of motor and cognitive symptoms. Traditional studies have typically employed targeted approaches that focus on specific proteins known to be associated with PD, potentially missing unknown proteins related to the disease's complex pathology. This study addresses this gap by utilizing an untargeted approach, which could reveal novel biological insights into PD and enhance understanding of its molecular underpinnings. This research integrated untargeted proteomics and AV-133 positron emission tomography (PET) scan data with clinical and demographic information to investigate predictors of dopamine loss and motor and cognitive symptoms in PD. Rigorous data preprocessing methods are employed to normalize and align the multi-modal datasets. Feature selection is conducted using statistical and machine learning techniques, including principal component analysis and logistic regression to identify key protein and imaging biomarkers. To find patterns that affect the rate of dopamine depletion, predictive models will be developed using algorithms such as logistic regression, random forests, and validated through cross-validation techniques to ensure their generalizability and effectiveness in clinical settings. It is anticipated that the integration of untargeted proteomics data and imaging data will enable more accurate predictions of dopamine depletion and symptom severity in PD patients. This approach is expected to uncover new biomarkers and enhance our understanding of PD, potentially leading to the development of non-invasive, early diagnostic tools and personalized therapeutic strategies, ultimately improving patient outcomes.

Disclosures: S. Samadi Tabrizi: None. **U.M. Ricoy:** None.

Poster

PSTR268: Parkinson’s Disease: Human Studies: Genetics and Diagnostic

Location: MCP Hall A

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Topic: C.03. Parkinson's Disease

Support: 1U24AG079685-02

Title: Seed Amplification of MSA alpha-synuclein aggregates preserves the biological and structural strain properties of brain derived aggregates

Authors: V. BANERJEE[1], F. WANG[1], C. BARRIA[1], S. RAMIREZ[1], M. PINHO[1], T. ALLISON[1], D. GORSKI[1], R. AL-LAHHAM[2], N. DE GREGORIO[1], ***D. HARRISON**[3,4], S. PRITZKOW[1], M. SHAHNAWAZ[1], M. L. BAKER[5], I. SERYSHEVA[6], C. A. SOTO[1]; [1]Dept Neurol, The Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX; [2]Pharmacol. and Toxicology, Univ. of Texas Med. Br., Galveston, TX; [3]UTHealth, HOUSTON, TX; [4]Dept Neurol, The University of Texas Health Science Center at Houston, Houston, TX; [5]Biochem., The Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX; [6]Structural Imagining Ctr. Dept. Biochem., The Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Synucleinopathies, such as Parkinson's disease (PD), Dementia with Lewy bodies (DLB), and multiple system atrophy (MSA) are insidious neurodegenerative diseases caused by the misfolding and aggregation of alpha-synuclein (αSyn) protein. Misfolded αSyn aggregates have the ability to spread pathological damage between cells and different brain regions following a similar seeding mechanism implicated in the propagation of infectious prions. Compelling evidence showed that αSyn aggregates can adopt a distinct conformational strain within different synucleinopathies. Recently, we reported that αSyn seed amplification assay (αSyn-SAA) enables to amplify various αSyn strains from biological samples, leading to different amplification products for PD and MSA patients. Here, we examined whether the MSA-seeded SAA-amplified αSyn fibrils maintain the biological and structural strain properties of the αSyn seeds present in the MSA patient's brain. We first analyzed the biological activities of both brain-derived and SAA-amplified αSyn aggregates in transgenic mice, followed by defining an atomic model of αSyn fibrils utilizing cryogenic electron microscopy (Cryo-EM). Our data shows that αSyn aggregates induced a neurological disease that is clinically and pathologically indistinguishable in mice inoculated with brain-derived and SAA-generated αSyn aggregates. We examined the clinical signs and disease durations, incubation period, neuropathological lesion profiles, and conformational properties of propagated αSyn aggregates. The structure of SAA amplified αSyn aggregates was resolved by Cryo-EM. The results indicated 3 prominent high propensity conformations present. MIII, with distinctive morphology and well-defined twisting, was reconstructed at 3.9 Å resolution. From the protofilament level, the SAA-amplified αSyn aggregates exhibited a similar structure of that previously reported for αSyn extracted from the brains of MSA patients. Therefore, our results suggest that SAA, under proper conditions, can amplify disease-specific αSyn conformations without modifying their strain properties, opening new avenues for individualized disease therapeutics and diagnostics.

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Poster

PSTR268: Parkinson's Disease: Human Studies: Genetics and Diagnostic

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR268.14/B126

Topic: C.03. Parkinson's Disease

Title: Changes of cortico-striatal effective connectivity during sentence processing and oral motor in Parkinson's disease

Authors: *E. HEMMATI[1], A. FALLAHI[2], M. R. NAZEM-ZADEH[3], **L. ALIBIGLOU**[4]; [1]Dept. of Neurosci., Iran Univ. of Med. Sci., Tehran, Iran, Islamic Republic of; [2]Sch. of Electrical and Computer Engin., Univ. of Tehran, Tehran, Iran, Islamic Republic of; [3]Dept. of Neurosci., Monash Univ., Melbourne, Australia; [4]Dept. of Physical Therapy, Indiana Univ. Indianapolis, Indianapolis, IN

Abstract: Parkinson's Disease (PD) is a progressive neurodegenerative disorder and the most prevalent movement disorder, often accompanied by language difficulties and speech impairments. Previous studies have demonstrated that sentence processing can be impaired in PD. Based on word order and complexity, sentences are categorized into canonical and non-canonical forms. Canonical sentences adhere to typical language structures like subject-verb-object, while non-canonical sentences deviate, posing more significant comprehension challenges. Research has shown that PD patients have more difficulty processing long sentences and complex syntactic patterns, particularly non-canonical ones. However, whether the underlying neural mechanisms of language impairments in PD are similar to motor symptoms and related to cortico-striatal pathway dysfunction remains unclear. This study investigated structural and functional changes in cortico-striatal language pathways affecting sentence comprehension and oral motor function in people with PD compared to neurologically healthy individuals. Using functional magnetic resonance imaging (*fMRI*), cortical-striatal pathways' activation levels and effective connectivity were assessed during sentence comprehension and oral diadochokinetic tasks. Twenty-three people with PD and twelve age and sex-matched healthy individuals participated in this study. In the fMRI results, comparing noncanonical to canonical sentence conditions showed decreased activation in the left inferior frontal gyrus and subcortical regions in PD compared to controls ($p<0.05$). Conversely, the control group exhibited significantly higher activity in frontostriatal pathway areas during noncanonical sentence comprehension ($p<0.05$). During the oral motor task, PD groups displayed comparable activity to controls in motor cortical areas but significantly less activity in subcortical regions, particularly in the caudate and putamen ($p<0.05$). Employing Dynamic Causal Modeling (DCM), we assessed effective connectivity between different pathways. For sentence comprehension, PD group exhibited significantly lower bilateral effective connectivity across cortico-cortical, cortico-subcortical, and subcortico-subcortical areas than controls ($p<0.05$). In the oral motor

task, PD group showed reduced bilateral connectivity between cortico-cortical and cortico-subcortical regions compared to controls ($p<0.05$). These findings suggest that decreased functional connectivity in cortico-cortical and cortico-subcortical pathways may contribute to impaired sentence comprehension and oral motor tasks in PD.

Disclosures: E. Hemmati: None. **A. Fallahi:** None. **M.R. Nazem-Zadeh:** None. **L. Alibiglou:** None.

Poster

PSTR268: Parkinson's Disease: Human Studies: Genetics and Diagnostic

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Topic: C.03. Parkinson's Disease

Support: Interagency cooperation Contract SRV00000081 University of Texas Rio Grande Valley/UT Health SA
Zachry Foundation Endowment
Greehey Family Foundation Endowment

Title: Fnirs reveals distinct cortical activation trajectories in response to deep brain stimulation for parkinson's disease and essential tremor

Authors: *L. PADILLA[1], M. LOZANO GARCIA[2], G. A. DE ERAUSQUIN[3], I. ZWIR[4]; [1]Biggs Inst. for Alzhaimers, Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX; [2]Neurosci., The Univ. of Texas Rio Grande Valley, Harlingen, TX; [3]The Glenn Biggs Inst. for Alzheimer and Neurodegenerative Dis., Univ. of Texas Hlth. Sci. Center, San Antonio, San Antonio, TX; [4]Neuroscience, Sch. of Med., Univ. of Texas Rio Grande Valley, Edimburg, TX

Abstract: Parkinson's Disease (PD) and Essential Tremor (ET) are neurological movement disorders characterized by tremors, rigidity, and bradykinesia. Deep brain stimulation (DBS) has emerged as a promising therapeutic approach for managing PD and ET symptoms by modulating brain activity and improving motor function. However, the precise mechanisms underlying its efficacy remain incompletely understood.In this study, we investigated cortical activation patterns in 14 PD/ET subjects with implanted DBS devices using functional near-infrared spectroscopy (fNIRS). Our experimental design included six sessions of inter- and intra-subject comparison before and after DBS stimulation. fNIRS recordings were conducted using the NIRx NIRScout X system, capturing data at 6.25 Hz with four different stimulation settings (C+0-, C+1-, C+2-, C+3-). Optodes were placed over the prefrontal cortex following the EEG 10/20 system. Data preprocessing involved filtering and trimming to 3 minutes per participant using NIRx Satori analysis software, with specific parameters applied for optimal data quality. Changes in oxyhemoglobin (HbO) and deoxyhemoglobin (HbR) concentrations were extracted

and co-clustered to assess measurement robustness. Clustering analysis using Ward's method with half-square Euclidean distance categorized subjects within each response-time and co-clustered across times. The coincidence rate between domains (co-clustering) was assessed using hypergeometric statistics (Fisher's Exact Test) to establish patient trajectories.Our analysis revealed distinct trajectories of cortical activation-response patterns across different DBS conditions, indicating differential neural responses to varying DBS parameters. These trajectories form an equifinality and multifinality temporal network, where similar patterns are shared before and after treatment or different patterns converge into a single post-treatment state. Integrating results with clinical data enables identification of patients best responding to person-centered treatments.Overall, our findings contribute to understanding the neural mechanisms underlying DBS efficacy in PD/ET subjects. By elucidating cortical activation patterns associated with different DBS settings, our results offer insights into individualized treatment optimization strategies. Further research is warranted to explore the functional implications of these activation patterns and refine treatment strategies for neurodegenerative movement disorders.

Disclosures: L. Padilla: None. **M. Lozano Garcia:** None. **G.A. de Erausquin:** None. **I. Zwir:** None.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.01/B128

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

Support: 5T32AG067952-03

Title: Analysis of Structural Heterogeneity of Brain-Derived Tau Oligomer Polymorphs Using Fluorescent Amyloid Multi Emission Spectra Microscopy

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Abstract: Several neurodegenerative disorders are characterized by the aggregation of misfolded proteins,including microtubule-associated tau protein. Although many of the tau strains' studies have beenperformed on tau fibrils, recent evidence has identified the soluble tau oligomers (TauO) as themore relevant and toxic species in the propagation of the disease due to their ability to seed taumisfolding. Tau oligomers are believed to be the primary neurotoxic species, and recent researchsuggests that these oligomers can also form prion-like strains with varying levels of neurotoxicity. While methods for isolating and characterizing tau aggregates have been established, there isstill a knowledge gap regarding the structural and biological characterization

of smaller, moredynamic tau polymorphs.To address this gap, we isolated brain-derived tau oligomers (BDTOs) from brain tissues ofAlzheimer's disease (AD), dementia with Lewy bodies (DLB), and progressive supranuclear palsy(PSP) patients. We characterized structures and morphologies of the BDTO strains and evaluatedtheir seeding potency in vitro model. We have applied Fluorescent Amyloid Multi Emission Spectra, known as FLAMES microscopy, that allows us to detect and profile different strains byusing commercially available amyloid fluorescent dyes. This methodology discriminates andreveals differences in the conformation of disease-relevant brain derived tau oligomers byexamining spectral shifts of these dyes.Our results indicate that BDTOs possess different structural and morphological features that aredistinguishable via principal component analysis. These findings suggest that the formation ofdistinct polymorphic tau oligomers may contribute to the development of multiple tauopathyphenotypes and shape the progression of neurodegenerative diseases. These results mayprovide insight for developing personalized therapy approaches to specifically target neurotoxictau species.

Disclosures: S.J. Park: None. **F. Lo Cascio:** None. **M. Montalbano:** None. **R. Kayed:** None.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.02/B129

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

Support: U24 AG072458
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R01 NS096344

Title: Isolation and Characterization of Tau Oligomers

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Abstract: Isolation and Characterization of Tau Oligomers

Cynthia Jerez[1,2]**,** Nikita Shchankin[1,2],Rhea Xavier[1,2], Urmi Sengupta[1,2], Nemil Bhatt[1,2], Rakez Kayed[1,2]
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Tau oligomers have been shown to be the main toxic tau species in a diverse group of age-related neurodegenerative diseases, collectively known as tauopathies, including Alzheimer's disease (AD). The characteristics of oligomers that exist in human disease, and that may associate most closely with disease propagation and toxicity, are not fully established because of the lack of standardization of the preparation of small oligomeric tau aggregates. Standardization of this can be achieved by isolating tau aggregates from authentic human tauopathy cases such as AD, as well as rigorously correlating their biophysical and biochemical properties with biological activity, developing probe sets for their selective detection, disseminating reliably examined samples, and lab-ready established protocols to the broader research community. To acquire biologically relevant tau oligomers for the study of tau aggregation and toxicity, we have designed protocols for the preparation and characterization of tau oligomers *in vitro* using other amyloid oligomeric seeds, as well as for the isolation of tau oligomers from biological samples using immunoprecipitation and sucrose gradient centrifugation. We have also created novel antibodies and optimized techniques for the detection of tau oligomers using common biochemical techniques including ELISA, dot blot, western blot, filter trap assay, as well as immunohistochemistry, Fluorescent Amyloid Multi Emission Spectra (FLAMES), and proteolytic digestion by proteinase K enzyme, were used to characterize oligomeric tau from recombinant protein and isolated from AD brain.

Disclosures: C. Jerez: A. Employment/Salary (full or part-time):; University of Texas Medical Branch. **R. Xavier:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch. **N.N. Bhatt:** None. **U. Sengupta:** None. **R. Kayed:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.03/B130

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

Support: NIH Grant RO1AG054025
NIH Grant RO1NS094557

Title: Tau mutants associated with familial tauopathies display variations in oligomeric conformations and aggregation propensities in-vitro

Authors: *R. J. XAVIER, A. A. BHOPATKAR, N. N. BHATT, N. PUANGMALAI, L. FUNG, M. HAQUE, C. JEREZ, R. KAYED;
Neurol., Univ. of Texas Med. Br., Galveston, TX

Abstract: The microtubule associated protein, Tau, is implicated in a multitude of neurodegenerative disorders that are collectively termed as Tauopathies. These disorders are characterized by the presence of tau aggregates within the brain of afflicted individuals. Mutations within the tau gene form the genetic backdrop for familial forms of tauopathies, such as frontotemporal dementia (FTD), but the molecular consequences of such alterations and their pathological effects are unclear. We sought to investigate the conformational properties of three mutants of tau 2N4R; A152T, P301L, and R406W, all implicated within FTD, and compare them to the native form (WT). We additionally wanted to probe the interaction between the mutant and WT protein to see if there exists any cross-seeding amongst them. Our immunochemical analysis reveals that mutant and WT oligomers exhibit similar affinity for conformation-specific antibodies but have distinct morphology and secondary structure. Additionally, these oligomers also possess different dye-binding properties and display varying sensitivity to proteolytic processing. These results point to conformational variety amongst them. We then tested the ability of the mutant oligomers to cross-seed the aggregation of WT-Tau monomer. Using similar array of experiments, we found that cross-seeding with mutant oligomers leads to the formation of conformationally unique WT oligomers compared to unseeded ones. Additionally, we see that such polymorphic properties are manifested in their biological effects on primary cortical neurons from mice. The results discussed in this paper provide a novel perspective on the structural properties of tau mutants and their interaction with the native form.

Disclosures: R.J. Xavier: A. Employment/Salary (full or part-time):; University of Texas Medical Branch. **A.A. Bhopatkar:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch, University of Mississippi Medical Center. **N.N. Bhatt:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch. **N. Puangmalai:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch. **L. Fung:** A. Employment/Salary (full or part-time):; University of Texas Southwestern Medical Center. **M. Haque:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch. **C. Jerez:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch. **R. Kayed:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.04/B131

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

Support: The Development and Promotion of Science and Technology Talents Project, Thailand
UK Dementia Research Institute

Title: Tau n-terminal splice variants exhibit different phosphorylation induced pathophysiology in hippocampal CA1 neurons

Authors: *C. SUNRAT[1], Y. DENG[1], S. M. MIZIELINSKA[1,2], S. J. MITCHELL[1], K. CHO[1,2]; [1]Basic and Clin. Neurosci., King's Col. London, London, United Kingdom; [2]UK Dementia Res. Institute, King's Col. London, London, United Kingdom

Abstract: Growing evidence suggests that the microtubule-associated protein tau (Tau) has important roles in postsynaptic function and long-term synaptic plasticity. Notably, we previously identified the GSK-3β phosphorylation sites 396/404 (PHF1E) to be important for long term depression (LTD). Importantly, in various neurodegenerative disorders the aberrant hyperphosphorylation of Tau can contribute to synapse dysfunction and elimination. Tau contains four domains: N-terminal projection region (NTR), proline-rich domain (PRD), microtubule-binding region (MBR) and carboxy-terminal region (CTR). Tau can undergo alternative splicing at the NTR (0N, 1N, 2N) or the MBR (3R/4R) giving rise to six isoforms. Much research has examined the role of the MBR splice variants, conversely, very little is known regarding the role of the NTR in pathophysiology. In this study, we investigate the effects of phosphomimic N-terminal splice variants (human Tau-2N4R-PHF1E, human Tau-1N4R-PHF1E) on synapse function in hippocampal CA1 neurons.

We found that Tau-2N4R-PHF1E exhibited higher amounts of Tau aggregation when compared with Tau-1N4R-PHF1E in HEK cells. Subsequently, in rat organotypic hippocampal slice culture, expression of Tau-2N4R-PHF1E reduced AMPA-receptor mediated synaptic currents ($EPSC_{AMPA}$) and significantly decreased synapse density in CA1 neurons. Moreover, Tau-2N4R-PHF1E impaired single dendritic spine plasticity in CA1 neurons induced by multiphoton-glutamate uncaging stimulation. Conversely, the expression of Tau-1N4R-PHF1E did not replicate the deficits induced by Tau-2N4R-PHF1E.

These findings highlight the distinct aggregation and pathophysiological properties resulting from the phosphorylation of 1N4R- and 2N4R-Tau. Notably, the 1N4R-Tau is the dominate isoform in the adult brain, and displays resilience to phosphorylation induced pathophysiology, potentially indicating that the lower abundant 2N4R-Tau isoform may initiate pathophysiology. These observations underscore the critical role of the N-terminal binding interactomes of Tau in driving the consequences of tau phosphorylation, highlighting their importance and merit for further investigation. Furthermore, advancing our understanding of how such phosphorylation events contribute to synapse weakening in the hippocampus.

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Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

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

Support: Alzheimer's Association (H.T.E)
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The Rainwater Foundation (H.T.E)
NIH Grant NS121786 (E.K)

Title: Altered translation in frontotemporal dementia tau-mutant human induced neurons

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Abstract: Protein synthesis is a vital biological process, important for multiple neuronal processes, such as, axon guidance and growth, synaptic plasticity, and learning and memory. Recently, studies have shown that dysregulated protein synthesis is a hallmark of neurodegenerative disorders, such as frontotemporal dementia (FTD). One of the causes of FTD is mutations in the gene for tau, a neuronally enriched microtubule binding protein. These FTD-causing tau mutations have been found to decrease global protein synthesis. Tau has also been shown to interact with ribosomes, the molecular machines responsible for translation (Evans *et al., Acta Neuropathoc Comms,* 2021; Koren *et al., Acta Neuropathologica,* 2019; Meier *et al*., *J. Neuro,* 2016).

Most of the studies on translation in FTD models have been conducted with tau overexpression models, such as transfected HEK cells or transgenic mice, presenting a clear confound. To overcome this confound, we investigated how FTD-mutant tau alters protein synthesis in human neurons, which have endogenous tau expression at normal physiological levels. We used various biochemical and molecular techniques, including puromycin labeling of de novo proteins, polysome profiling and harringtonine run-off assays to study the alterations in translation due to FTD-mutant tau in human neurons.

Utilizing these techniques, we show that FTD-mutant tau results in decreases in global translation. This data is recapitulated in our polysome profiling data, which shows a decrease in the polysome to monosome ratio in the FTD-mutant neurons. Our data suggests that these alterations in translation could be due to slowed elongation rates. We also detect tau co-sedimentation with ribosomes in human neurons as well as alterations in ribosomal protein localization.

Our results demonstrate that FTD-mutant tau results in alterations in translation and that these impairments occur at physiological levels of tau.

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Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

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

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Tau Consortium
JPB Foundation

Title: Human iPSC 4R tauopathy model uncovers modifiers of tau propagation

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Abstract: Tauopathies are age-associated neurodegenerative diseases whose mechanistic underpinnings remain elusive, partially due to lack of appropriate human models. Here, we engineered new human induced pluripotent stem cell (hiPSC)-derived neuronal lines to express 4R Tau and 4R Tau carrying the P301S *MAPT* mutation when differentiated into neurons. 4R-P301S neurons display progressive Tau inclusions upon seeding with Tau fibrils and recapitulate features of tauopathy phenotypes including shared transcriptomic signatures, autophagic body accumulation, and reduced neuronal activity. A CRISPRi screen of genes associated with Tau pathobiology identified over 500 genetic modifiers of seeding-induced Tau propagation. CROP-seq analysis of the top modifiers highlights both distinct transcriptomic phenotypes and shared gene co-expression between hit profiles. In progressive supranuclear palsy (PSP) and Alzheimer’s Disease (AD) brains, the UFMylation cascade is altered in neurofibrillary-tangle-bearing neurons. Inhibiting the UFMylation cascade in vitro and in vivo suppressed seeding-induced Tau propagation. This model provides a robust platform to identify novel therapeutic strategies for 4R tauopathy.

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Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.07/B134

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

Title: Leveraging a hiPSC derived 3D tri-culture model to accelerate neuronal maturation and induce microglial dysfunction in a model of tauopathy

Authors: C. FORMICA[1], I. ONOFRE[1], C. VAN BERKEL[1], L. SMIT[1], B. SAMSON-COUTERIE[2], ***S. JAIN**[1];
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Abstract: Human induced pluripotent stem cell (hiPSC) technology enables the derivation of several specific subtypes of neurons, astrocytes and microglia which are relevant to model important neurological disorders. However, the slow maturation rates of hiPSC-derived cells, particularly neurons, requires prolonged culture periods for neuronal maturation and the emergence of other disease-relevant phenotypes. To accelerate this process, at Ncardia, we developed two strategies based on a co-culture of hiPSCs-derived neurons and astrocytes. First, a 2D model combining an early treatment with maturation promoting compounds (Hergenreder, E 2024) with an extended culture period of up to 5 weeks. And second, a 3D neurospheroid culture maintained for up to 6 weeks. After 5 weeks, we confirmed the presence of mature neurons in the 2D model (NeuN and MAP2 positive), expressing pre-synaptic markers (synaptophysin-SYP) and discrete presence of post synaptic markers (PSD-95). In the neurospheroid model, after only one week in culture, we were able to confirm the presence of mature neurons (MAP2 positive) and astrocytes (GFAP positive), robust expression of synaptic markers, SYP and PSD-95 and mature synapses. All those markers continued increasing overtime. Additionally, compared to 2D cultures, 3D neurospheroids showed a more complex and structured firing pattern after two weeks on MEA, which is reminiscent of what is observed in mature cortical organoids and human electroencephalogram. Finally, we established a tri-culture system by adding microglia to the 2D and 3D co-culture models of Tauopathy (overexpressing Tau P301L and treated with Tau PFFs). We observed, for both systems, a robust integration of microglia within the co-cultures previously established and the development of hallmarks of tauopathy: expression of pTau and punctate Tau in neurites, release of pro-inflammatory cytokines and higher phagocytic activity of neurons expressing pTau and synaptic markers (predominantly observed in 3D vs 2D models). In conclusion, we generated a 3D tri-culture model that can

efficiently recapitulate morphology and function of the central nervous system *in vitro*, both in physiological and pathological conditions. This assay platform shows promise in modelling *in vitro* relevant neurodegenerative phenotypes specifically synaptic pruning in Alzheimer's Disease, difficult to obtain in 2D models. Moreover, our protocol can be easily adapted to high throughput screening platform and liquid handling robots, thus increasing its applicability to drug discovery.

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Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

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Program #/Poster #: PSTR269.08/B135

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

Support: NIH Grant R33NS115161
NIH Grant 1F31NS135935-01

Title: Depletion of TDP-43 sensitizes human cortical neurons to caspase 3-mediated cleavage of tau

Authors: *G. D. BURNS, A. CRUZ, M. S. BAGHEL, P. C. WONG;
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Abstract: Alzheimer's Disease (AD) and related dementias (ADRDs) are the leading causes of dementia and afflict millions of people every year. Clarification of disease mechanisms and target identification are critical unmet needs in the field. While the canonical amyloid-beta (Aß) and tau pathologies are implicated in AD cases, recent evidence revealed that non-canonical pathologies, including TDP-43 pathology, occur in the majority of cases. TDP-43 pathology is observed in 40-60% of all AD cases and about 50% of frontotemporal dementia (FTD) cases. Importantly, AD cases with TDP-43 pathology, compared to those without, exhibit steeper cognitive decline and more extensive brain atrophy. The underlying molecular mechanism by which TDP-43 drives neurodegeneration and cognitive impairments, however, remains elusive. Emerging evidence revealing that loss of TDP-43 splicing repression occurs in presymptomatic ALS individuals demonstrates that loss of TDP-43 function, as opposed to its cytoplasmic aggregation, underlies its disease pathology. Based on our previous finding that loss of TDP-43 leads to selective death of CA2/3 neurons and elevated caspase 3 activation as well as evidence in the human AD brain showing caspase 3-dependent cleavage of tau, we hypothesized that loss of TDP-43 is a key factor that promotes caspase 3-dependent tau cleavage and subsequent

pathological tau aggregation and neurodegeneration. Here, we show that depletion of TDP-43 in iPSC-derived cortical neurons elevates caspase 3-mediated cleavage of tau. These findings offer novel mechanistic and therapeutic insights for ADRDs harboring TDP-43 co-pathology.

Disclosures: G.D. Burns: None. **A. Cruz:** None. **M.S. Baghel:** None. **P.C. Wong:** None.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.09/B136

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

Support: NIH/NINDS:K23NS109284
Roy J Caver Charitable trust
Funding from CurePSP Foundation

Title: Phosphorylation impairs astrocytic tau uptake: implications for tauopathies

Authors: *N. FIROUZSHAHI[1,2], M. M. WEIS[3,1], M. R. SHIN[4,2], M. M. HEFTI[3,1];
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Abstract: Introduction: Astrocytic tau is a key pathologic feature of primary tauopathies such as progressive supranuclear palsy (PSP). We and others have shown that astrocytic tau is primarily of neuronal origin. However, the mechanisms by which astrocytes take up and process tau, and the effect of tau phosphorylation—a key event in disease pathogenesis—remain unclear. This study aims to investigate how phosphorylation affects the internalization of tau protein by astrocytes.
Methods: Human induced pluripotent stem cells (iPSCs) were differentiated into astrocytes using standard protocols from StemCell Technologies. The astrocytes were then treated with recombinant tau with or without phosphorylation. Tau will be phosphorylated by two distinct kinases, namely protein kinase A and src kinase, which belong to the serine/threonine and tyrosine kinase families, respectively. Successful phosphorylation was validated by dot blotting. Tau uptake was assessed using dot blotting and live-cell imaging.
Results: The serine/threonine phosphorylation group showed a significant decrease in uptake by live cell imaging (Repeated measures ANOVA, P<.001), while the tyrosine-phosphorylated group did not (unpaired t-test, P=.42). We confirmed our findings by fluorescently labeling recombinant tau (AF647) and serine/threonine-phosphorylated tau (AF555). We then added them simultaneously, with similar results (Repeated measures ANOVA, P<.001).
Conclusion: Our findings suggest that tau phosphorylation may *inhibit* astrocytic tau uptake and

possibly degradation, thus increasing levels of extracellular tau and potentially increasing rates of neuron-to-neuron propagation. Further research into the underlying mechanisms can provide valuable insights into the pathogenesis of tauopathies and potential therapeutic targets for these neurodegenerative disorders.

Disclosures: N. Firouzshahi: None. **M.M. Weis:** None. **M.R. Shin:** None. **M.M. Hefti:** None.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.10/B137

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

Support: Merit Review Grant I01BX005742
Career Development Award Level-2 BX004341
National Institutes of Health R01NS064131

Title: Sut-6 (NIPP1) mutant W292X suppresses tau-induced toxicity in a tau transgenic Caenorhabditis elegans model

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Abstract: Tau is a microtubule-associated protein that accumulates pathologically in multiple neurodegenerative diseases including Alzheimer's disease and frontotemporal dementias. To better understand mechanisms that modulate tau-induced toxicity, we use a transgenic *Caenorhabditis elegans* model where human 1N4R tau is expressed in all neurons. This causes significant phenotypes including tau-induced locomotor deficits, accumulation of highly phosphorylated and insoluble tau protein, progressive neuron loss, and reduced lifespan. Previously we performed forward mutagenesis screening in this transgenic *C. elegans* model and identified a W292X mutation in *sut-6.* The mammalian homolog of *sut-6* is *NIPP1* (Nuclear Inhibitor of Protein Phosphatase 1). NIPP1 inhibits protein phosphatase 1 (PP1), interacts with splicing factors, transcription factors, and binds RNA. The W292X mutation truncates the last 11 amino acids of the SUT-6 protein. We characterized the effect of this mutation as well as complete deletion of *sut-6* on tauopathy phenotypes in tau transgenic *C. elegans*. We found that deletion of *sut-6* or *sut-6(W292X)* ameliorated tau-induced locomotor behavior deficits, reduced

accumulation of tau protein, reduced neuron loss, but did not rescue lifespan shortening. Interestingly, *sut-6(W292X)* had a much stronger effect on tau-induced behavioral deficits compared to *sut-6(null),* but a similar effect on tau protein levels and neuron loss. In addition, neuronal overexpression of SUT-6 W292X protein suppressed tau-induced toxicity while wild type protein had no effect. To further understand how SUT-6 W292X suppresses tauopathy, we are determining the effects of SUT-6 W292X on various predicted SUT-6 functions. The identified W292X mutation truncates part of the RNA binding domain and PP1 inhibition domain. Using a splicing reporter, we found *sut-6(W292X)* had decreased rates of aberrant splicing while *sut-6(null)* had increased rates. Using co-immunoprecipitation assays, we found SUT-6 W292X was still able to pull down the PP1 homolog GSP-1 similarly to wildtype SUT-6. To try to further understand the possible importance of PP1 in the mechanism, we made a V177A mutation in *sut-6,* which is predicted to abrogate GSP-1 binding to SUT-6. *sut-6(V177A),* in contrast to *sut-6(W292X)* or *sut-6(null),* had no effect on tau-induced behavioral deficits. However, we need to confirm that V177A truly abrogates the SUT-6 and GSP-1 interaction before making firm conclusions. Nevertheless, the results altogether demonstrate that *sut-6/NIPP1* mutant W292X is a strong suppressor of tauopathy and that it may be due to changes in splicing/transcriptional regulation.

Disclosures: B.P. Henderson: None. **A.D. Beale:** None. **A.D. Saxton:** None. **B.C. Kraemer:** None. **R. Kow:** None.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.11/B138

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

Support: NIH Grant AG083630

Title: Tau protein proteolysis toxicity in Alzheimer's disease

Authors: N. M. FERNANDEZ[1], K. DOLEZAL[2], C. J. GORDON[1], J. STRAX[1], C. S. HOM[1], P. K. RYDER[3], A. E. DALE[1], ***C. J. HUSEBY**[1];
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[2]Midwestern Univ., Peoria, AZ; [3]Pacific Univ., Forest Grove, OR

Abstract: Tau protein normally functions as an intrinsically disordered monomer to stabilize and promote growth of microtubules, a key component of the neuronal cytoskeleton involved in intracellular transport. In Alzheimer’s disease (AD), however, tau dissociates from microtubules and enters misfolding pathways that yield aggregates having cross-β-sheet structure and

filamentous morphology. In addition to AD, Tau aggregation is a defining feature of several human neurodegenerative diseases collectively termed tauopathies in which the initial formation of aggregates is followed by spread among brain regions. Microscopic events of neurofibrillary formation are first detected as nonfibrillar Tau deposits in neuronal axons and cytoplasm marking the beginning of aberrant processes. Although some Tau protein monomer peptide fragments aggregate spontaneously, recombinant full-length Tau *in vitro* or expressed in mammalian cells does not aggregate spontaneously even at supersaturation. Inducing a Tau nucleus can be accomplished by a heterogenous association such as with a membrane, small molecules, or peptides stabilizing a misfolded species for subsequent elongation steps. Tau is subject to a variety of post-translational modifications (PTM) including proteolytic cleavage generating peptide fragments varying in length. Proteolysis is a normal process of protein degradation and cellular function control, but it also has been implicated in tau aggregate formation. For example, Tau peptides spanning the microtubule-binding repeat region overcome kinetic barriers to aggregate spontaneously. However, tau peptides also have been demonstrated to directly induce misfolding and aggregation of full-length Tau *in vitro* and in mammalian cells. Proteolytic cleavage sites along the longest human brain Tau isoform where used. Tau-derived peptides were ordered by commercial synthesis or made in-house with greater than 95% purity. Thioflavin dye fluorescent assays were used to quickly test for aggregate formation alone as well as ability to induce aggregation and incorporate with full-length human Tau protein. Our analysis of 200 Tau derived peptides to date show that truncated Tau protein species can form aggregate structures in vitro while others do not. We also found at longer incubation times some short peptides can act as inducers of full-length tau aggregation indicating that some proteolytic cleavage sites can be toxic while others are protective preventing aggregate formation. Toxic and protective peptides derived from Tau protein provide evidence for implicating specific proteases and their associated pathways in disease.

Disclosures: N.M. Fernandez: None. **K. Dolezal:** None. **C.J. Gordon:** None. **J. Strax:** None. **C.S. Hom:** None. **P.K. Ryder:** None. **A.E. Dale:** None. **C.J. Huseby:** None.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

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Program #/Poster #: PSTR269.12/B139

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Chan-Zuckerberg Initiative (CZI) grant# 222111

Title: Probe-dependent Proximity Profiling (ProPPr) uncovers similarities and differences in phospho-Tau-associated proteomes between tauopathies.

Authors: D. MORDERER[1], M. C. WREN[1], F. LIU[1], ***A. MAISTRENKO**[2], N. KOURI[1], B. KHALIL[1], N. POBITZER[3], D. W. DICKSON[4], M. E. MURRAY[1], W. ROSSOLL[5]; [1]Neurosci., Mayo Clin., Jacksonville, FL; [2]Mayo Clin., Jacksonville, FL; [3]Neurosciencs, Mayo Clin., Jacksonville, FL; [4]Pathology & Neurosci., Mayo Clin., Jacksonville, FL; [5]Dept. of Neurosci., Mayo Clin., Jacksonville, FL

Abstract: Background: Neuropathological inclusions formed by hyperphosphorylated protein tau in the brain are a hallmark of neurodegenerative disorders commonly referred to as tauopathies that represent a number of cognitive and motor syndromes in people of older age. Different tauopathies are characterized by disease-specific morphological presentations of tau lesions, affected brain regions and cell types, tau isoforms present in the inclusions, and structures of tau filaments. The factors that govern the formation of disease-specific tau lesions and distinct patterns of neurodegeneration and clinical presentations are unknown. To identify potential modifiers and effectors of tau pathology, we performed profiling of phospho-tau associated proteins in 4 major tauopathies. **Methods:** Here we established the Probe-dependent Proximity Profiling (ProPPr) method combined with DIA-LC/MS/MS in FFPE frontal cortex tissue sections for proteomic profiling of AT8-positive phospho-tau aggregates. The study cohort includes cases from 4 major tauopathies and unaffected controls: Alzheimer's disease (AD, N=6), corticobasal degeneration (CBD, N=6), Pick's disease (PiD, N=4), progressive supranuclear palsy (PSP, N=2) and control cases (CTL, N=8). Selected findings were validated by fluorescent co-immunostaining of frontal cortex sections from the validation cohort cases (AD, N=3; CBD, N=3; PiD, N=3; PSP, N=3; CTL, N=4). **Results:** We identified a total of 1318 phospho-tau-associated proteins, including 229 proteins identified in all tauopathies. GO molecular function terms enriched in the latter protein set included chaperone activity, transmembrane transporter activity, and guanyl nucleotide binding. Fluorescent immunostaining confirmed association of the retromer complex protein VPS35 with specific tau lesions in all the studied tauopathies. Statistical comparison of protein abundances between diseases revealed 31 differentially expressed proteins. Immunostaining confirmed disease-specific associations of the ferritin light chain protein (FTL) and the neurosecretory protein VGF within distinct pathological lesions. Furthermore, closer examination of FTL-positive microglia in CBD astrocytic plaques suggested a potential role of this microglial subpopulation in CBD pathogenesis.**Conclusions:** We established a new approach to profile proteins that are associated with tau lesions in different tauopathies. Proteins found in all the tauopathies likely distinguish disease-independent cellular response mechanisms to tau pathology. We also identified proteins that associate with distinct disease-specific tau lesions.

Disclosures: D. Morderer: None. **M.C. Wren:** None. **F. Liu:** None. **A. Maistrenko:** None. **N. Kouri:** None. **B. Khalil:** None. **N. Pobitzer:** None. **D.W. Dickson:** None. **M.E. Murray:** None. **W. Rossoll:** None.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.13/B140

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R21AG065606
R01AG041274
R01AG070761

Title: Tau-pt271-mediated sex-dependent postoperative delirium-like behavior in aged mice

Authors: *J. ZHANG[1], P. YU[2], Y. DONG[2], F. LIANG[2], Z. XIE[2], Y. ZHANG[2];
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Abstract: Tau-PT271-mediated sex-dependent postoperative delirium-like behavior in aged mice
Jing Zhang, Peng Yu, Yuanlin Dong, Feng Liang, Zhongcong Xie and Yiying Zhang
Introduction: Postoperative delirium (POD) is a significant complication following surgery, yet its underlying mechanisms remain largely unclear. Evidence has shown that changes in sex hormones can influence Alzheimer's disease pathogenesis and dementia . Recent research indicates that elevated levels of Tau phosphorylated at threonine 217 (Tau-pT217) could be a pathway through which anesthesia/surgery induces POD-like symptoms in aged mice . The present study aims to examine whether the sex-dependent expression of Tau-pT217 might explain the gender-specific occurrence of POD-like behavior in aged mice. **Methods**: The study involved 18-month-old wild-type (WT) C57BL/6J female and male mice. The anesthesia/surgery group underwent a simple laparotomy under 2 hours of 1.4% isoflurane anesthesia supplemented with 40% oxygen. Behavioral tests, enzyme-linked immunosorbent assay (ELISA), nanoneedle technology, western blotting, and other methods were used to assess the outcomes. Additionally, testosterone inhalation therapy was given to aged female mice, while orchiectomy or the androgen receptor (AR) inhibitor enzalutamide was used on aged male mice to explore the role of AR signaling in Tau-pT217 levels and POD-like behavior. **Results**: Anesthesia/surgery triggered POD-like behavior and significantly elevated Tau-pT217 levels in the blood and brain only in female, but not male, aged mice. The AR expression in the lungs was notably higher in male aged mice than in females. Testosterone inhalation in aged female mice activated AR

signaling, reducing Tau-pT217 levels in blood, lungs, and brain tissues, and subsequently alleviating POD-like behavior. Conversely, orchiectomy or AR inhibitor treatment in aged male mice resulted in reduced AR activity, elevated Tau-pT217 levels, and increased POD-like behavior. **Conclusions**: These findings suggest that gender differences in postoperative outcomes, including POD-like behavior, might be driven by sex hormone regulation of Tau-pT217 through AR signaling. The results indicate that testosterone inhalation could reduce Tau-pT217 levels via AR activation, offering a potential therapeutic strategy for mitigating POD-like symptoms in aged female mice. This study provides a foundation for further exploration of sex-dependent mechanisms in POD and potential gender-specific interventions.

Disclosures: J. zhang: None. **P. yu:** None. **Y. Dong:** None. **F. liang:** None. **Z. Xie:** None. **Y. Zhang:** None.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.14/B141

Topic: C.02. Alzheimer"s Disease and Other Dementias

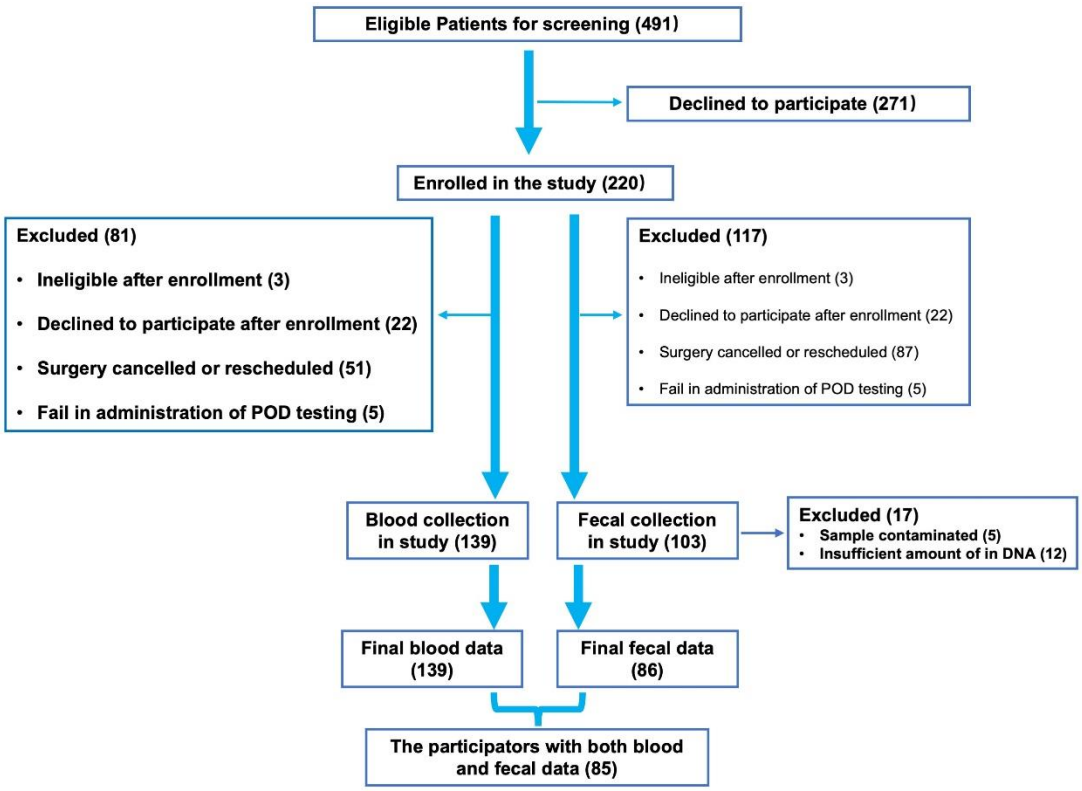
Support: R21AG065606

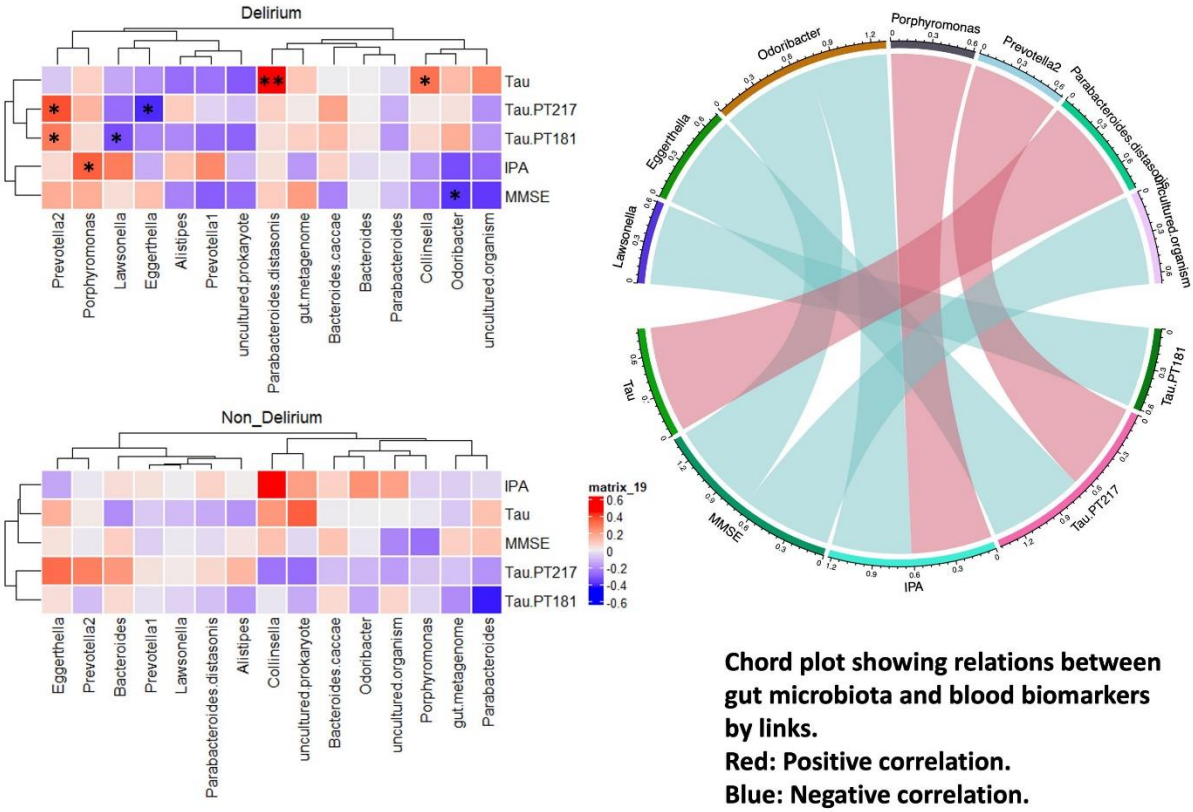
Title: Tau Protein and Gut Microbiota Correlation in Patients with Postoperative Delirium

Authors: S. SINGH[1], W. XIANG[2], F. LIANG[1], Y. DONG[1], Z. XIE[1], ***Y. ZHANG**[3]; [1]Anesthesia, Critical Care and Pain Med., Massachusetts Gen. Hospital/Harvard Med. Sch., Charlestown, MA; [2]Biol. of aging, USC, Los Angeles, CA; [3]Massachusetts Gen. Hospital/Harvard Med. Sch., Charlestown, MA

Abstract: Introduction: Postoperative delirium (POD) is a prevalent and costly complication in elderly surgical patients, often resulting in adverse outcomes. The underlying mechanisms and reliable biomarkers of POD remain poorly understood. This study at Massachusetts General Hospital included patients over 65 undergoing surgeries like laminectomy, knee, or hip replacements under general or spinal anesthesia. We examined the potential relationships between gut microbiota profiles and plasma Tau protein levels (Tau-PT217 and Tau-PT181) in patients with POD.
Method: We employed 16S rRNA gene sequencing to analyze gut microbiota and Nanoneedle technology to measure plasma Tau protein levels.
Results: Among 491 patients, 12% developed POD. Notably, Parabacteroides distasonis was linked with POD occurrence. There was a strong positive correlation (r = 0.807) between Tau protein levels and gut bacteria Parabacteroides distasonis, and negative

correlations were observed between Tau-PT217 and the gut bacteria Eggerthella, as well as between Tau-PT181 and Lawsonella.
Conclusions: These findings highlight significant correlations between specific gut microbiota and plasma Tau protein and phosphorylation Tau protein levels in patients with POD, emphasizing the potential involvement of the gut-brain axis in this condition.





Chord plot showing relations between gut microbiota and blood biomarkers by links.
Red: Positive correlation.
Blue: Negative correlation.

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Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.15/B142

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG079145-01
AG061190-01
P30AG066508
AG RF1AG070761
MH113257

Title: Nanoscale imaging of pT217-tau in aged rhesus macaque: Trans-synaptic propagation and seeding of tau pathology in entorhinal and dorsolateral prefrontal cortex

Authors: *D. DATTA[1], I. PERONE[1], D. WIJEGUNAWARDANA[2], F. LIANG[3], Y. M. MOROZOV[4], J. I. ARELLANO[5], A. DUQUE[6], Z. XIE[7], C. H. VAN DYCK[8], M. P. JOYCE[5], A. F. ARNSTEN[9];
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Abstract: Progress in Alzheimer's disease (AD) have revealed a novel fluid biomarker, tau phosphorylated at T217 (pT217-tau), in CSF and plasma, that predicts AD prior to cognitive deficits. Understanding the role of pT217-tau is important in assessing efficacy of novel treatments aimed at early-stage disease. However, it is unknown why pT217-tau is effective in predicting brain pathology, as little is known about early, soluble pT217-tau brain expression. These questions are difficult to address in humans, as soluble p-tau is rapidly dephosphorylated postmortem, and PET scans detect late-stage, fibrillated tau. However, the etiology of pT217-tau in aging brains can be probed in rhesus macaques, where perfusion fixation allows capture of phosphorylated proteins in their native state. Aging macaques naturally develop tau pathology with the same qualitative pattern and sequence as humans, including initial cortical pathology in layer II of the entorhinal cortex (ERC) evident early in aging, and later in layer III of the dorsolateral prefrontal cortex (dlPFC). We utilized multi-label immunofluorescence and immunoelectron-microscopy to examine the subcellular localization of early-stage pT217-tau in ERC and dlPFC of aged macaques (18-34y) with naturally occurring tau pathology and assayed pT217-tau levels in plasma. Our results show that pT217-tau labeling is primarily observed in postsynaptic compartments, accumulating in: 1) dendritic spines on the calcium-storing smooth endoplasmic reticulum spine apparatus near asymmetric glutamatergic-like synapses, and 2) in dendritic shafts, where it aggregated on microtubules, often "trapping" endosomes associated with Aβ42. The dendrites expressing pT217-tau were associated with autophagic vacuoles and dysmorphic mitochondria, indicative of early neurite degeneration. We observed trans-synaptic pT217-tau trafficking between neurons within omega-shaped bodies and endosomes, specifically near excitatory, but not inhibitory synapses. We also examined pT217-tau in blood plasma in macaques across age-span and observed a statistically significant age-related increase in pT217-tau. These data provide the first direct evidence of pT217-tau trafficking between neurons near synapses to "seed" tau pathology in higher brain circuits, interfacing with the extracellular space to become accessible to CSF and blood. The expression of pT217-tau in dendrites with early signs of degeneration may help to explain why this tau species can herald future disease.

Disclosures: D. Datta: None. **I. Perone:** None. **D. Wijegunawardana:** None. **F. liang:** None. **Y.M. Morozov:** None. **J.I. Arellano:** None. **A. Duque:** None. **Z. Xie:** None. **C.H. van Dyck:** None. **M.P. Joyce:** None. **A.F. Arnsten:** None.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.16/C1

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

Support: NIH R01AG074570
NIH RF1NS110437
NIH K99AG078500
NSF CHE2109008

Title: Minimalist seed-competent mimics of pathological tau strains

Authors: *J. DEL VALLE;
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Abstract: The accumulation of tau protein deposits is characteristic of several neurodegenerative diseases that manifest with cognitive decline, movement disorders, and dementia. The conformational plasticity of tau allows it to play a role in many important cellular processes; however, its misfolding and self-assembly into pathological filaments results in both loss of normal function and gain of toxic function. Disease-associated filamentous tau exhibits cross-beta architecture, wherein protein monomers adopt beta-arch folds that stack in register. These filaments can seed the misfolding of physiological tau and propagate across neurons in a prion-like manner. Recent cryo-EM structural data demonstrate that the conformations of tau protomers within pathological protofilaments can vary by disease, even when they are comprised of the same isoform and sequence. This raises the intriguing possibility of a link between conformational fold, seeding activity, and disease progression. Current models of tau propagation based on co-factor-induced aggregation fail to capture the structural diversity of pathological tau folds. Given the scarcity and variability of seed-competent patient-derived extracts, efforts to recapitulate pathological tau folds are urgently needed. Here, we describe the structure-based design of peptide derivatives that mimic the form and function of tauopathic filaments. Diversity-oriented covalent stapling of core motifs observed in 4R tau folds from idiopathic disease affords cyclic beta-arch peptides that self-assemble into amyloid filaments. A subset of these “mini-tau” fibrils potently seed endogenous tau in engineered biosensor cells and primary neuronal cell cultures. Structural elucidation of a mini-tau filament by cryo-EM reveals several conformational features congruent with those in pathological tau folds. Our studies provide a framework for the minimization of functional, disease-associated epitopes of amyloidogenic proteins that could find broad application in the development of vaccines and therapeutic antibodies.

Disclosures: J. Del Valle: None.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.17/C2

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

Support: LF Experiment - Lundbeckfonden
Torben og Alice Frimodts Fond
Grosserer Foghts fond
Lizzie og Mogens Staal fonden

Title: Spatiotemporal map of tau seeds in progressive supranuclear palsy

Authors: *M. RASMUSSEN[1], L. KNECHT[1], M. HUANG[2], W. MCEWAN[2], S. KAALUND[1]; [1]Bispebjerg Hosp. - Ctr. for Neurosci. and Stereology, Copenhagen, Denmark; [2]Dept. of Clin. Neurosciences, UK Dementia Res. Inst., Cambridge, United Kingdom

Abstract: Progressive supranuclear palsy (PSP) is a severe neurodegenerative disorder. Pathologically, PSP is characterized as a four microtube-binding-repeat (4R) tauopathy, with abundant tau inclusions in various cell types (glial and neuronal cells) and regions of the brain. In PSP native tau proteins convert into ‘seed competent’ tau species that generate fibrils which assembles into aggregates. While neuropathological stages suggest a stereotypic pattern of the progression of tau aggregates throughout the brain, the rate of fibrilization and spatial progression of tau is still unclear. Our aim is to determine PSP-tau seeding kinetics across the human brain hemisphere by detecting seed-competent species, testing the hypotheses that seeding kinetics differ between 1) early and late-stage pathology, and 2) regions with predominantly astrocytic and neuronal/oligodendroglial tau. Resolving this is important for understanding how different regions and cell-types may contribute to progression of tau accumulation. In order to study the temporal (early vs. late) and regional seeding capacity, we have collected frozen brain tissue from 16 regions from 10 donated PSP brains reflecting high to low tau pathology burden. The tau seeding kinetics are studied using the seeding amplification assay (SAA). So far, we have established a Tau SAA that can monitor seeding derived from 10 µg brain homogenate. The seeding was considered positive if 2 out of 4 replicates per brain regions showed a plateau higher than the negative control. The SAA showed seeding in all 16 regions from high and low pathology burden brains. We observed individual seeding kinetics, suggesting that our seeding assay reflects differences in tau seed concentration and potency. Our pre-liminary analyses show no systematic differences in seeding kinetics between high and low pathology stages, nor between regions. Though this assay can detect tau seeds and thus PSP tau

pathology in regions yet not positive for tau aggregates. This allows us to add detailed information about the tau seed's localization in the human PSP brain.

Disclosures: M. Rasmussen: None. **L. Knecht:** None. **M. Huang:** None. **W. McEwan:** None. **S. kaalund:** None.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.18/C3

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

Title: Electrophysiological characterization of ipsc-derived excitatory neurons, including a comparison to genetically modified frontotemporal dementia neuron model, using automated patch clamp

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Abstract: Induced pluripotent stem cells (iPSCs) show great potential for the generation and characterization of neuronal subtypes as well as the investigation of neurological disease models. However, in practice, the intercellular variability in a population of iPSC-derived neurons in combination with the low-throughput nature of manual electrophysiological experiments, have made such studies challenging.Here we use automated patch clamp (APC) for high-throughput characterization and comparison of commercially available healthy normal (WT) and frontotemporal dementia (FTD; genetically engineered Granulin R493X heterozygous knockout) iPSC-derived excitatory neurons (iCell Induced Excitatory Neurons). WT and FTD iPSC-derived excitatory neurons were generated using neurogenin-2 overexpression resulting in consistent iPSC differentiation into a highly pure glutamatergic neurons population (>90%), with similar gene expression levels for of synaptic (PSD95, Synapsin 1) and glutamatergic receptors (vGlut2, GRIA1, GRIA4). We first optimized cell suspension preparation for APC analysis in order to retain cell viability and function from neurons cultured for 16, 23, 30 or 35 days in culture. These cells were then analyzedfor voltage-gated (Kv and Nav) and ligand-gated (AMPA) ion channel currents and how they develop over time. NaV currents increased in both WT and FTD neurons, with more dramatic increases in WT over time. KV currents were generally lower in WT compared to FTD neurons, with little change in current amplitude over time. AMPAR current development was different between WT and FTD - WT increased over time while FTD decreased resulting in roughly equivalent AMPA currents between WT and FTD neurons by 30

days in vitro. We also recorded action potentials in both WT and FTD model iPSC-derived excitatory neurons, and compared action potential parameters (such as spike frequency, spike threshold and action potential amplitude). The number of cells firing action potentials increased for both WT and FTD excitatory neurons over time in culture and our preliminary data suggest that FTD neurons start firing at lower input currents and at higher frequencies than WT.

Disclosures: S. Karatsiompani: None. **P. Scaduto:** A. Employment/Salary (full or part-time):; Sophion Bioscience. **S. Schachtele:** None. **K.R. Rosholm:** None.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.19/C4

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

Support: Research Grants Council HK General Research Fund (14107118)

Title: Mutant huntingtin induces neuronal apoptosis via derepressing the poly(A) tail-modifying enzyme

Authors: *E. CHAN, *E. CHAN;
The Chinese Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: MicroRNAs (miRNAs) are small non-coding RNAs responsible for RNA silencing and the posttranscriptional regulation of gene expression. Poly(A)-modifying enzyme catalyzes the addition of adenosine to the 3′ end of miRNAs, which promotes their subsequent degradation. In this study, we demonstrated that a transcriptional repressor was recruited to both RNA foci and protein aggregates, which caused gene upregulation in Huntington's disease (HD). We further identified a subset of corresponding miRNAs were downregulated in levels in HD models. We further showed that this pathway was activated in HD patient induced pluripotent stem cell-derived neurons. This study highlights the importance of miRNA dysfunction in the pathogenesis of HD and as a potential therapeutic direction for the disease.

Disclosures: E. Chan: None. **E. Chan:** None.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.20/C5

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

Support: NTUH Grant MS329
NTUCCS-113-13
Taiwan Health Foundation

Title: Notch1 mRNA expression and oligodendrocyte differentiation in Multiple System Atrophy

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Abstract: Multiple system atrophy (MSA) is a complex and rare neurodegenerative movement disease. The distinction between parkinsonism-predominant (MSA-P) and cerebellar-predominant (MSA-C) forms reflects the clinical diversity within the disorder. The presence of glial cytoplasmic inclusions (GCIs) and the aberrant accumulation of α-synuclein in oligodendrocytes (OLs) are the hallmark of the disease and play a significant role in its pathogenesis. The widespread myelin loss preceding neuronal death underscores the profound impact of MSA on both the central and autonomic nervous systems. Understanding these underlying mechanisms is crucial for the development of effective therapies. Accumulation of synuclein has been reported to be associated with impaired survival of neural precursor cells (NPCs) through interference with the Notch signaling pathway. Several studies have shown that inhibition of NOTCH1 signaling promotes OLs differentiation and myelination, but its specific role in synuclein aggregation of matured OLs remains elusive. Here, we investigated the mRNA expression of Notch1 and its relationship with the differentiation status of oligodendrocyte derived from human induced pluripotent stem cell (iPSC) lines from MSA patients (both MSA-P and MSA-C subtypes) as well as healthy controls (HC). Results revealed a diminished differentiation propensity in MSA-P compared to MSA-C and HC. Immunofluorescence staining showed heightened phosphorylated α-synuclein expression investigated from the early stages (the germ layer cells) of MSA-iPSC induced OLs. Notch1 mRNA was overexpressed in MSA-induced OLs relative to HC from 12th day of iPSC-derived OLs. These data suggest Notch1's potential involvement in MSA pathogenesis and OLs differentiation, linking α-synuclein pathology with the OLs lineage in MSA. These findings provide insights into the molecular mechanisms underlying OL dysfunction in MSA, with implications for therapeutic development.

Disclosures: M. Chen: None. **M. Kuo:** None. **R. Wu:** None.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.21/C6

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

Support: Parkinson's Foundation Grant
Nu Rho Psi Grant
ASBMB Grant

Title: Synucleinopathies: Comparative Evaluation of α-, β-, and γ-Synuclein Toxicity in Different Yeast Strains

Authors: *S. GACEK, L. CASARES, F. BERTOLOTTI, T. NASSUNA, R. OSSELBORN, S. DEBBURMAN;
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Abstract: Synucleinopathies are a group of neurodegenerative disorders linked with the misfolding and aggregation of α-synuclein, the most well-known among them being Parkinson's disease (PD). α-Synuclein belongs to a larger family of proteins that include β- and γ-synuclein. Mutant forms (P123H and V70M) of β-synuclein have been shown to cause Dementia with Lewy Bodies (DLB), however, the extent to which β- and γ-synuclein are neurotoxic is still highly understudied compared to α-synuclein. While specific alterations in cellular environments (including nitrative stress, lysosomal degradation, mitochondrial dysfunction) and post-translational modifications alter the toxicity and aggregation of α-synuclein, less is known of their impacts on β- and γ-synuclein. Here, we used our lab's budding yeast (Saccharomyces cerevisiae) model to comparatively evaluate these three wildtype synucleins and explore their pathological potential through the assessment of toxicity, localization, and expression. We report that: 1) Wildtype and mutant β-synuclein is toxic, whereas γ-synuclein is non-toxic; 2) β-synuclein toxicity is related to expression level, strain, and extent of aggregation; 3) β-synuclein displays a higher molecular weight than expected in yeast; 4) Toxicity potential of all three synucleins, including γ-synuclein, is impacted to varying degrees by specific synucleinopathy-related genetically modified yeast strains that alter nitrative stress, glycation, acetylation, triglycerides, and SUMOylation. This study highlights the relevance of evaluating a fuller range of synucleinopathy-related processes to better understand what drives the toxicity of all three synucleins.

Disclosures: S. Gacek: None. **L. Casares:** None. **F. Bertolotti:** None. **T. Nassuna:** None. **R. Osselborn:** None. **S. DebBurman:** None.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.22/C7

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

Title: A scalable platform, based on physiologically-relevant cellular models which can be leveraged for the high-throughput screening of drug candidates targeting pathological accumulation and aggregation of TAU, αSyn and TDP-43

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Abstract: Neurodegenerative disorders are marked by the accumulation of misfolded proteins, a pathological feature. This aggregation is so critical to the understanding of these diseases that they are now categorized under the umbrella of proteinopathies. These categories are based on the type of protein that is most commonly found aggregated in each disorder. Although protein misfolding, accumulation and aggregation is a major cause of neurodegeneration; there remains a major unmet need of physiologically-relevant models to both study the mechanism of propagation and aggregation, and to screen potential therapeutics. At Ncardia, we have developed models for tauopathies, synucleinopathies and TDP43 aggregation using human iPSC-derived stem cell technology (hiPSC). All these models have been miniaturized to 384-well format to make them suitable for high-throughput compound screening. For tauopathies using high content imaging (HCI) we demonstrated quantifiable presence of phosphorylated TAU species in Ncyte® Neural Mix (co-culture of hiPSC-derived neurons and astrocytes) cultures compared to untreated control cultures. In our synucleinopathy model, we demonstrated that treatment with α-synuclein preformed fibril (PFF) on three different cultures, cortical and dopaminergic neurons (derived from hiPSCs) and Ncyte Neural Mix, displayed statistically significant increases in both α-syn and pS129 by HCI as compared to untreated control cultures. Finally, our TDP-43 proteinopathy model demonstrated electrophysiological deficits by MEA, mislocalization of TDP-43 to the cytoplasm, reduction in STMN2 protein levels and increase in neurofilament-L (NF-L) secretion as compared to controls. In summary, we have developed a scalable platform, based on physiologically-relevant cellular models which can be leveraged for the high-throughput screening of drug candidates targeting the reduction of pathological propagation and accumulation of proteins in tauopathies, synucleinopathies and TDP43 aggregation.

Disclosures: K. Riegman: A. Employment/Salary (full or part-time):; Ncardia Services BV. **I. Onofre:** A. Employment/Salary (full or part-time):; Ncardia Services BV. **C. Formica:** A. Employment/Salary (full or part-time):; Ncardia Services BV. **J. Koepke:** A. Employment/Salary (full or part-time):; Ncardia Services BV. **C. van Berkel:** A. Employment/Salary (full or part-time):; Ncardia Services BV. **L. Smit:** A. Employment/Salary (full or part-time):; Ncardia Services BV. **B. Samson-Couterie:** A. Employment/Salary (full or

part-time):; Ncardia Services BV. **S. Jain:** A. Employment/Salary (full or part-time):; Ncardia Services BV.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.23/C8

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

Support: FONDECYT-1210375.
ANID-Subdirección de Capital Humano/Doctorado Nacional/2022-21231149

Title: Activation of pannexin-1 channels evoked by α-synuclein: possible repercussions for neuronal mitochondrial dysfunction.

Authors: *T. ALVEAR[1], J. A. ORELLANA[2], A. SILVA[1];
[1]Pontificia Univ. Católica de Chile, Santiago, Chile; [2]Dept. de Neurología, Pontificia Univ. Católica de Chile, Santiago, Chile

Abstract: The abnormal intracellular aggregation of α-synuclein is a key histopathological feature in Parkinson's disease. Our recent research revealed that α-synuclein heightens the opening of pannexin-1 (Panx1) channels in astrocytes, disrupting $[Ca^{2+}]_i$ balance, mitochondrial morphology, and cell survival. Panx1 channels are plasma membrane channels that facilitate the molecular and ionic exchange between the cytoplasm and the extracellular space. Under pathological conditions, the persistent opening of these channels leads to cell dysfunction and death. The impact of α-synuclein on Panx1 channel activity in hippocampal neurons has not been established. To address this, we conducted ethidium (Etd) uptake experiments in primary hippocampal neurons, acute brain slices, or mice exposed to α-synuclein (0.1 to 100 nM). In addition, Panx1 protein levels were evaluated by immunofluorescence and western blotting. Additionally, different mitochondrial parameters, including morphology, membrane potential, and superoxide production, were assessed using MitoGreen, Mitored CMXROS, and Mitosox labeling, respectively. Our findings revealed heightened activity and levels of neuronal Panx1 channels in cultured hippocampal neurons and acute brain slices when stimulated with α-synuclein. Similarly, brain slices from adult mice intrahippocampally injected with α-synuclein exhibited increased Panx1 channel activity. Notably, the α-synuclein-induced activity of Panx1 channels altered mitochondrial superoxide production and membrane potential, as well as the morphology of mitochondria. We speculate that α-synuclein-induced activation of neuronal Panx1 channels may be a novel mechanism in the pathogenesis and progression of α-synucleinopathies.

Disclosures: T. Alvear: None. **J.A. Orellana:** None. **A. Silva:** None.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.24/C9

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

Title: A role for casein kinase-2 dysregulation in cortical synaptic dysfunction associated with synucleinopathy disorders

Authors: *A. SCOTT[1], S. DUTTA[2], H. KHAN[3], L.-A. ROSSITTO[4], K. JAYANT[3], X. CHEN[5], J.-C. ROCHET[1];
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Abstract: Cortical dysfunction is thought to contribute to the non-motor symptoms associated with Parkinson's disease (PD) and other synucleinopathies. Recent studies have revealed functional changes in cortical circuitry in pre-clinical models of alpha-synuclein (aSyn) propagation, but with limited mechanistic insight. We hypothesized that seeded aSyn aggregation leads to cell signaling perturbations, potentially reflected by alterations of the phosphoproteome, in mouse brain. To address this hypothesis, we utilized a mouse model of aSyn aggregation involving the injection of aSyn preformed fibrils (PFFs) in the cortex or striatum to study the downstream effects of seeded aSyn aggregation. We showed via immunohistochemical staining that PFF injection in either region leads to the presence of aSyn aggregates that stain positive for the phosphoserine-129 form of the protein (pSer129-aSyn) in the sensorimotor cortex and other anatomically connected brain regions. Phosphoproteomic analysis of homogenates prepared from the sensorimotor cortex revealed significant differences between the PFF- and monomer-injected mice 3 months post-injection. Gene ontology analysis of the phosphoproteomic changes revealed a link between aSyn PFF administration and perturbations of synaptic signaling. Further, motif enrichment analysis coupled with kinase prediction indicated that the majority of motifs up-regulated in the brains of PFF-treated animals consisted of predicted casein kinase-2 (CK2) phosphorylation sites. CK2, a constitutively active kinase that is dysregulated in multiple disorders, is involved in phosphorylating aSyn at Ser129. Additional immunohistochemical data indicated that the regulatory beta and catalytic alpha subunits of CK2 were co-localized with pSer129-aSyn^{+} aggregates in the cortices of PFF-injected mice. We also observed punctate CK2-alpha subunit staining in the nuclei of cortical neurons, suggesting that PFF treatment alters CK2 subunit dynamics, in turn leading to the

dysregulation of CK2 activity. Collectively, these results suggest that synaptic dysfunction in the sensorimotor cortex of PFF-injected mice is mediated at least in part by CK2 dysregulation. These findings deepen our understanding of the molecular underpinnings of synucleinopathy disorders, laying the groundwork for developing well-tailored intervention strategies.

Disclosures: A. Scott: None. **S. Dutta:** None. **H. Khan:** None. **L. Rossitto:** None. **K. Jayant:** None. **X. Chen:** None. **J. Rochet:** None.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.25/C10

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

Support: 2023 Whitcome Undergraduate Research Fellowship, UCLA

Title: The Functional Role of microRNAs in the Pathogenesis of Synucleinopathies in Alzheimer's Disease

Authors: *Y. SUN[1], S. ZHANG[1], C. PENG[1,2,3,4];
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Abstract: The discovery of alpha-Synuclein (α-Syn) in the brains of Alzheimer's Disease (AD) patients marks a significant advancement in our understanding of this complex neurodegenerative disorder, traditionally characterized by amyloid-beta plaques and neurofibrillary tangles. This finding raises pivotal questions about the role of α-Syn, a protein previously associated primarily with Parkinson's Disease, in the pathogenesis of AD. Motivated by this breakthrough, our study explores the influence of microRNAs (miRNAs) on α-Syn accumulation in AD, focusing on the analysis of 47 miRNAs that are homologous between humans and mice and their regulatory effects on α-Syn in primary hippocampal neuron models. Through methodologies including the generation of α-Syn preformed fibrils (PFFs), cultivation of primary neuron cultures, and miRNA synthesis and application, this research endeavors to uncover the mechanistic relationships between miRNAs and α-Syn pathology in AD. Our results suggest the promising potential of miRNA modulation in controlling α-Syn aggregation, offering a novel perspective on therapeutic strategies for AD. This study not only highlights the newly identified significance of α-Syn in AD but also initiates a foundational inquiry into miRNA-based therapeutic approaches for mitigating synucleinopathies in AD patients.

Disclosures: Y. Sun: A. Employment/Salary (full or part-time):; Dept. of Neurol., David Geffen Sch. of Medicine, Univ. of California—Los Angeles, Los Angeles, CA. **S. Zhang:** A. Employment/Salary (full or part-time):; Department of Neurology, David Geffen School of Medicine, University of California—Los Angeles, Los Angeles, CA, USA. **C. Peng:** A. Employment/Salary (full or part-time):; Department of Neurology, David Geffen School of Medicine, University of California—Los Angeles, Los Angeles, CA, USA.

Poster

PSTR269: Cellular and Molecular Mechanisms of Tauopathies, Synucleinopathies, and Other Degenerative Diseases

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR269.26/C11

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

Support: NIH Grant NS086074
NIH Grant NS092093
Aligning Science Across Parkinson's/MJFF ASAP-024407

Title: Internalized a-synuclein fibrils are rapidly truncated and resist degradation in neurons while glial cells rapidly degrade a-synuclein fibrils

Authors: *M. KARIM, E. GASPARINI, E. TIEGS, R. SCHLICHTE, S. C. VERMILYEA, M. K. LEE;
Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Parkinson's disease (PD) and other α-synucleinopathies are characterized by the accumulation of α-synuclein (αS) pathology that can spread via the cell-to-cell transmission of αS aggregates. To better understand how various brain cells contribute to the spreading of αS pathology, we examined the metabolism of αS aggreges or pre-formed fibrils (PFFs) in neuronal and glial cells (microglia, astrocytes, and oligodendrocytes). Neurons internalize αS monomers and rapidly degrade the αS monomers within 6 hours. In contrast, following the internalization of αS PFFs by neurons, the full-length αS rapidly disappears with a stable accumulation of truncated αS with a half-life of over 48 hours. Epitope mapping and fractionation studies indicate that αS PFF was truncated at the C-terminal region following uptake and remained insoluble/aggregated. However, microglia and astrocytes rapidly metabolized αS PFFs with half-lives <6 hours in these glial cells. Differential processing of αS by neurons was recapitulated in cell lines as differentiated CLU198 and SH-SY5Y neuronal cell lines stably accumulate truncated αS from internalized αS PFFs but the undifferentiated cells rapidly metabolize αS PFFs. Immunolocalization and subcellular fractionation studies show that internalized αS PFF is initially localized to endosomes followed by lysosomes. Colocalization of αS PFF to other organelle shows some αS PFF in endoplasmic reticulum marker but no significant localization of

αS PFF with the markers of Golgi or autophagosomes. In neurons, truncated αS PFF accumulates in the lysosomes, indicating that the truncation happens in the late endosomes or lysosomes. Lysosome is largely responsible for the degradation of internalized αS PFFs, particularly in glial cells, as only the lysosomal inhibition leads to a consistent increase in the accumulation of internalized αS PFFs in all cell types. However, inhibition of lysosomes or proteasomes do not inhibit αS truncation in neurons. Significantly, internalized αS PFF causes lysosomal dysfunction in neurons, as indicated by decreased Magic Red Cathepsin B staining and increased Galectin-3 staining in αS PFF treated neurons. Our results show that neurons do not efficiently metabolize internalized αS aggregates and generate potentially aggregation-prone truncated αS. In contrast, glial cells may protect neurons from αS aggregates by rapidly clearing αS aggregates.

Disclosures: M. Karim: None. **E. Gasparini:** None. **E. Tiegs:** None. **R. Schlichte:** None. **S.C. Vermilyea:** None. **M.K. Lee:** None.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.01/C12

Topic: C.06. Neuromuscular Diseases

Support: CNPq/MCTI/CT-Saúde (408910/2022-4)

Title: Electroneuromyography insights and clinical evaluation of Long COVID patients with previous hospitalization

Authors: M. REIS PRADO[1], F. SOUZA[1], A. PACHECO[1], L. ANDERSON[2], Ê. BASSI[3], G. D. SOUZA[4], A. C. SILVA[4], F. D. SILVA[5], I. S. MELO[1], S. FIGUEIREDO[1], K. B. OLIVEIRA[1], *O. W. CASTRO[1], J. BRANDÃO[1], A. S. JÚNIOR[6], ***O. W. CASTRO**[7];
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Abstract: Long COVID (LC) is a chronic condition developed by some people after SARS-CoV-2 acute infection, including a range of complications affecting the respiratory, cardiac, neurological, and digestive systems. In this scenario, our study entails a clinical-epidemiological analysis of LC patients previously hospitalized with moderate to severe COVID-19, with a focus on evaluating the peripheral nervous system via electroneuromyography (ENMG). This study received ethical approval from the Institutional Review Board of the Federal University of

Alagoas (CAAE: 68344823.2.0000.0155). Participants were individuals who tested positive for SARS-CoV-2 and were hospitalized between March 2020 and December 2022. After providing written informed consent, participants completed a clinical-epidemiological questionnaire and were submitted to ENMG. Currently, there are 24 enrolled individuals, with a majority of them being male (52.38%) and aged 50 to 59 years (42.11%). Persistent LC symptoms reported include fatigue (85.71%), dyspnea (52.38%), headache (57.14%), hypertension (52.38%), cardiac arrhythmias (57.14%), memory impairment (61.90%), sensory alterations (71.43%), mental disorders (33.33%), and sleep disturbances (52.38%). Of the seventeen patients analyzed by ENMG, only three did not show any abnormalities. The other fourteen participants displayed pathologies such as polyneuropathy, peripheral sensory-motor axonal polyneuropathy, neuropathies of the median, radial, peroneal, cutaneous, and/or ulnar nerves, neuromyopathy, radiculopathy, and multiradiculopathy. ENMG data are being analyzed using artificial intelligence to identify LC-associated patterns, along with potential blood biomarkers linked to neuromuscular and neuroinflammatory disorders. Taken together, our preliminary findings suggest long-term effects of COVID-19 post-infection, considerably affecting the quality of life for patients with LC.

Disclosures: M. Reis Prado: None. **F. Souza:** None. **A. Pacheco:** None. **L. Anderson:** None. **Ê. Bassi:** None. **G.D. Souza:** None. **A.C. Silva:** None. **F.D. Silva:** None. **I.S. Melo:** None. **S. Figueiredo:** None. **K.B. Oliveira:** None. **O.W. Castro:** None. **J. Brandão:** None. **A.S. Júnior:** None. **O.W. Castro:** None.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.02/C13

Topic: C.06. Neuromuscular Diseases

Support: CMT Research Foundation

Title: Evaluation of inosine as a potential treatment for X-linked Charcot-Marie-Tooth disease

Authors: M. M. FREIDIN[1], S. ESPINOZA[2], D. GONG[2], *C. K. ABRAMS[2];
[1]Neurol. & Rehabil., Univ. of Illinois At Chicago, Chicago, IL; [2]Neurol., Univ. of Illinois at Chicago, Chicago, IL

Abstract: Mutations in the gene GJB1, coding for the protein connexin 32 (Cx32), have been shown to cause the X-linked form of Charcot-Marie-Tooth disease (CMT1X), an inherited peripheral neuropathy. The genetic cause of CMT1X has been known for over 25 years, but the mechanism by which mutant Cx32 protein in Schwann cells (SCs) leads to peripheral neuropathy is poorly understood. Macrophage numbers are significantly increased in Cx32KO nerve and

appears to significantly contribute to severity of Cx32 related neuropathy. Other published studies and metabolomic findings from our lab show that inosine and other by-products of extracellular ATP metabolism are reduced in Cx32KO peripheral nerve compared to WT mice; suggesting that loss of Cx32 leads to an inability of ATP to exit the SC into the extracellular space. A mechanistic hypothesis for the pathogenesis of CMT1X proposes that defective Cx32 hemichannel function causes dysregulation of mitochondrial SC ATP production as well as release into the extracellular space. Low extracellular levels of anti-inflammatory ATP metabolites, particularly the stable inosine, lead to a chronic inflammatory state in nerve, increased macrophage infiltration to impact myelin and axon pathology. Thus, increases in macrophages may be due to reduced levels of inosine, which asserts its anti-inflammatory effects at cellular A2 receptors. We used transcriptional, metabolomic, and pathologic approaches to test the hypothesis that supplementation of inosine can ameliorate CMT1X neuropathy. Following preliminary dose response determinations, Cx32KO mice were treated with inosine (280mg/kg) or PBS daily for 7days, 1-month, and 4-months. Sciatic and femoral nerve samples were harvested and assayed for inflammatory gene expression and macrophage counts, respectively. Nerve conduction studies and motor and strength behaviors were also conducted on the 4-month treatment group. Iba1 staining shows a significant reduction in macrophages in femoral motor nerve following 7 and 30days of Inosine. We also find significant reductions in inflammatory and repair SC genes TNF, Timp1, Trem2, and CD4 by qPCR after 30days of inosine. Finally, significant increases in grip strength and some features of NCS were observed following 4 months of inosine. Gene expression and histology studies of the 4-month cohort are currently in process. More definitive determinations of whether long term treatment with inosine reduces or ameliorates peripheral neuropathy are ongoing. Taken together, these results strongly suggest that treatment with inosine may provide a therapeutic effect for CMT1X and other peripheral neuropathies.

Disclosures: M.M. Freidin: None. **S. Espinoza:** None. **D. Gong:** None. **C.K. Abrams:** None.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.03/C14

Topic: B.09. Glial Mechanisms

Support: UIC Honors College Winter 2023
UIC Honors College Spring 2024

Title: Age-related changes in inflammatory and AMPK-stress pathway genes at 2-, 4-, and 6-months of age in wild-type, Cx32-knockout, and mutant mouse models of CMT1X

Authors: E. JIN[1], *M. M. FREIDIN[2], C. K. ABRAMS[3];
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Abstract: Mutations in GJB1, the gene encoding Connexin 32 (Cx32), cause the X-linked Charcot-Marie-Tooth disease (CMT1X), a delayed-onset inherited demyelinating peripheral neuropathy. While the genetic cause of CMT1X has been known for over 25 years, the mechanism by which mutant Cx32 protein in Schwann cells (SCs) leads to peripheral neuropathy is poorly understood. .Recent studies in our lab have demonstrated that mutant genotypes displayed loss of function in behavioral tests and by histological measures. These deficits became more evident as the mice aged. Additionally, using microarray and metabolomic approaches to compare wild-type and Cx32-knockout (Cx32KO) sciatic nerve samples, we have identified significant regulation inflammatory/stress pathway genes as well as robust changes in ATP and metabolites associated with the AMP-activated protein kinase (AMPK) metabolic/stress pathways. We and others have also found significant increases in macrophage numbers in peripheral nerves from Cx32KO mice, which appears to significantly contribute to severity of the Cx32-related neuropathy. The lowered extracellular levels of anti-inflammatory ATP and metabolites may lead to increased macrophage infiltration helping to establish a chronic inflammatory state in CMT1X nerves and, ultimately, impact myelin and axon pathology. This study uses semi-quantitative Real Time PCR (RT-qPCR) to examine the regulation of several genes associated with these pathways in WT, Cx32KO, and three targeted CMTX1 Crispr mutant mouse models at different times during disease progression. Sciatic nerve samples from wild-type (WT), Cx32KO, p.T55I, p.R75W, and p.E102G Crispr mutants were collected from 2-, 4-, and 6-month old mice and assayed by RT-qPCR for relative expression levels of GPNMB, PVMN, TIMP1, and TREM2. No significant differences were noted at 2 months of age for any group. However, TIMP1, and TREM2, genes associated involved in AMPK-mediated and inflammatory pathways and are highly regulated in models of peripheral nerve injury and regeneration, showed increases at 4 and 6 months, with significant upregulation in 6-month mutant mice compared to wild type mice. These changes correspond to previously observed increases in nerve pathology and decreases in motor and strength behaviors.

Disclosures: E. Jin: None. **M.M. Freidin:** None. **C.K. Abrams:** None.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.04/C15

Topic: C.06. Neuromuscular Diseases

Title: Homozygous mutations in GDAP1 and MFN2 genes resulted in autosomal recessive forms of Charcot-Marie-Tooth disease in consanguineous Pakistani families

Authors: *D. HUSSAIN, MFH;
Zoology, Govt. Grad. Col. Muzaffargarh, Affiliate with Bahauddin Zakariya University, Multan, Muzaffargarh, Pakistan

Abstract: Background: Charcot-Marie-Tooth disorders (CMT) is a heritable neurodegenerative disease of peripheral nervous system diseases with which more than 100 genes and their mutations has been associated.. Methods: Two consanguineous families (PAK-CMT1-DG KHAN) and Layyah (PAK-CMT2-LAYYAH) with multiple CMT affected subjects were enrolled from Punjab province in Pakistan. Basic epidemiological data were collected for the subjects. Nerve conduction study (NCS) and electromyography (EMG) was performed for patients. Whole exome sequencing (WES) followed by Sanger sequencing was applied to report the genetic basic of CMT. Results: NCS findings revealed that sensory and motor nerve conduction velocities for both families were less than 38m/s. EMG presented denervation, neuropathic motor unit potential and reduced interference pattern of peripheral nerves. WES identified a novel non sense mutation (226 (c. 226 G > T) in GADP1 gene and a previously reported missense mutation in MFN2 gene (c. 334 G > A) that is causing CMT4A in PAK-CMT1-DG KHAN and CMT2A in family PAK-CMT2-LAYYAH family respectively. Mutations followed Mendalian pattern with autosomal recessive inheritance mode. Multiple sequence alignment by Clustal Omega indicated that mutation containing domain in both genes is highly conserved and in situ analysis revealed that both mutations are likely to be pathogenic. Conclusion: We are reporting a novel non sense and a previously known missense mutation in GAPD1and MFN2 gene, respectively, caused CMT in consanguineous Pakistani families.

Disclosures: D. Hussain: None.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.05/C16

Topic: C.06. Neuromuscular Diseases

Title: Characterizing Neuromuscular Deficits in the C3-PMP-22 Mouse Model of Charcot-Marie-Tooth Disease Type 1A.

Authors: *P. J. MOORE, J. VITERI, N. R. KERR, S. AYYAGARI, A. ROSHANI DASHTMIAN, F. B. DARVISHI, W. ARNOLD;
NextGen Precision Hlth. Initiative, Univ. of Missouri, Columbia, MO

Abstract: Charcot Marie Tooth disease (CMT) type 1A is the most common form of CMT related to a duplication of the *PMP22* gene that causes an autosomal dominant neuropathy associated abnormal peripheral nerve myelination, axonal dysfunction/loss, and motor unit degeneration. Patients with CMT1a experience progressive symptoms of weakness, muscle atrophy, and sensory loss. The C3-PMP-22 mouse (C3) is a mouse model of CMT1a with 3 copies of the PMP22 gene. Prior work has demonstrated phenotypic features of disrupted motor coordination, altered myelination, but absent signs of overt axonal loss. To investigate the phenotypic features of the C3 mouse model more fully, we performed a battery of assessments including behavioral assessments of motor coordination and strength as well as in vivo gastrocnemius motor unit electrophysiology to assess summated muscle excitation (compound muscle action potential, CMAP), motor unit size (single motor unit potential, SMUP), motor unit number (motor unit number estimation, MUNE), and neuromuscular junction (NMJ) transmission (repetitive nerve stimulation, RNS). 11 C3 and 13 wildtype C57BL6 control mice were assessed between the ages 2-6 months. Experimenters were blinded to genotype. Motor coordination, as assessed by rotarod latency to fall, and motor strength, as assessed by all limb grip, both showed significant reduction in C3 mice versus controls (rotarod: time X genotype, mixed effects, $p = 0.0306$, grip: time X genotype, mixed effects, $p = 0.0023$). CMAP, a measure of summated muscle excitation following supramaximal nerve stimulation, and MUNE, an estimation of motor unit connectivity, were both significantly reduced in C3 versus control mice (CMAP: time X genotype, mixed effects, $p = 0.0001$; MUNE: time X genotype, mixed effects, $p = 0.0446$). In contrast, SMUP, a measure of motor unit size, demonstrated significant differences for time ($p<0.0001$) and genotype ($p=0.0275$) but no interaction between time and genotype ($p=0.4395$). Longitudinal RNS demonstrated no differences (mixed effect analysis: age, genotype or age X genotype), but on multiple comparisons at 5.5 months revealed significantly greater decrement (NMJ transmission failure) in C3 versus control mice at 5.5 months (C3: --28% vs control: -11%, Sidaks multiple comparisons). Together these data indicate that the C3 mouse model of CMT1a reproduce clinical features of motor dysfunction associated with electrophysiological deficits in motor unit number, muscle excitation, and possibly NMJ transmission deficits. The phenotypic characterizations of this pre-clinical model of CMT1A can be used to test impacts of preclinical therapeutics.

Disclosures: P.J. Moore: None. **J. Viteri:** None. **N.R. Kerr:** None. **S. Ayyagari:** None. **A. Roshani Dashtmian:** None. **F. B. Darvishi:** None. **W. Arnold:** None.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.06/C17

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

Support: Grant # RO1NS127781

Title: Development of an in-vitro model for Charcot-Marie-Tooth disease

Authors: *J. KOENIG, D. SUMMERS;
Univ. of Iowa, Iowa City, IA

Abstract: Maintenance of proteostasis is critical for preserving cell integrity and appropriate responses to a changing environment. Impairments in proteostasis can lead to a deleterious imbalance in protein synthesis, localization, and degradation. Such impairments have important consequences for neurons, which must function for a human's entire lifespan. Charcot-Marie-Tooth disease (CMT) describes a group of inherited peripheral neuropathies. A common feature in certain instances of these neuropathies is mutations in aminoacyl-tRNA synthetases (ARS). This suggests that a defect in the protein synthesis aspect of proteostasis may underly the unclear etiology of CMT. However, the mechanism(s) through which ARS mutations may cause the sensory and muscular phenotypes observed in CMT patients remains unknown. To address this gap in knowledge, I developed an in-vitro model for the effects of mutant ARSs in sensory neurons. This model utilizes neurons cultured from the dorsal root ganglion (DRG) of embryonic day 14 (E14.5) CD10 mice and genetically encoded fluorescent reporters to visualize newly synthesized proteins in live neurons. Once grown, DRGs undergo chemical axotomy to remove axons while maintaining cell integrity. The axons are subsequently regenerated. This approach allows me to assess axon regeneration dynamics in a mutant ARS background. In this DRG culture model, lentiviral expression of a CMT associated mutant tyrosyl-tRNA synthetase (mYARS) induces dose-dependent axon degeneration and cell death when compared to wildtype and empty vector controls. Further, I observed a significant decline in neuronal protein synthesis with mYARS expression compared to controls. Axon outgrowth is heavily reliant on protein synthesis; unexpectedly, however, axon regeneration is unaffected in the mYARS condition. These findings demonstrate that expression of mYARS in DRG cultures induces axon degeneration, mirroring a prominent event in many CMT neuropathies. Furthermore, these findings help elucidate CMT's etiology, which may indeed involve a synthesis defect. As mYARS does not seem to impact axon regrowth dynamics, this raises the possibility of the existence of certain factors present in neurons during the initial axon growth stages. Factors that confer resistance to the synthesis dampening effect. Future identification of these factors through genetic and pharmacological screens could explain the delayed onset of CMT in humans. Beyond CMT, this model system can be applied to identify key nodes in neuronal proteostasis networks, which have broad relevance to the growing list of neurological disorders characterized by loss of proteostasis.

Disclosures: J. Koenig: None. **D. Summers:** None.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.07/C18

Topic: C.06. Neuromuscular Diseases

Title: Development and validation of a functional, human induced pluripotent stem cell (hiPSC)-derived Organ on a Chip model of autoimmune polyneuropathy

Authors: *B. ROBAINA-CAICEDO[1], K. AUTAR[1], A. AMALFITANO[1], Z. LIU[1], J. J. HICKMAN[1,2];
[1]Hesperos Inc., Orlando, FL; [2]University of Central Florida, Orlando, FL

Abstract: Autoimmune polyneuropathies, such as Chronic Inflammatory Demyelinating Polyneuropathy (CIDP) and Multifocal Motor Neuropathy (MMN), affect an estimated 20 million individuals across the US. This heterogenous class of neurodegenerative disorders is characterized by a variety of clinical manifestations, including demyelination, sensorimotor deficits, and the presence of serum autoantibodies against peripheral nervous system (PNS) antigens. Notably, serum autoantibodies vary widely across patients, and are hypothesized to drive distinct clinical manifestations. Thus, there remains a need for clinically-translatable models capable of recapitulating PNS biology while preserving unique, patient-specific mechanisms of disease progressions. Such models encourage the identification of novel pathways and mechanisms for disease progression driven by patient-specific serum autoantibodies, while also providing a platform to screen novel therapeutics for their efficacy in stratified patient populations. The present work describes the development of a functional, human-based PNS model consisting of induced-pluripotent stem cell (iPSC)-derived motoneurons (MN) and Schwann cells (SC) cultured over microelectrode arrays (MEAs). SC cultured on the distal side tunneled chambers drive axonal growth through the secretion of trophic factors that encourage MN development and maturation. The average conduction velocity (CV) of axons growing through the tunnels and over microelectrodes is calculated as a function of electrical signal and time using our proprietary CV platform. The identification and segmentation of true biological signal from background was validated using lidocaine, a sodium channel blocker that inhibits neuronal action potential. This model has been successfully used to recapitulate the clinical features of CIDP and MMN using clinically-confirmed patient serum samples. Upon administration of patient sera, our model demonstrated a statistically-significant drop in CV, as well as the presence of complement proteins C3 and C5b-9, suggestive of autoantibody-driven, classical complement pathway activation. In line with this, the administration of complement-inhibiting therapeutics restored deficits in CV and complement protein deposition, highlighting the capability and utility of this model to assess the efficacy of therapeutic compounds designated for autoimmune polyneuropathies. In summary, this work provides evidence for the utility and rationale design of human-based, functional models for the assessment of novel therapeutics and delineation of complex neurodegenerative disease mechanisms.

Disclosures: B. Robaina-Caicedo: A. Employment/Salary (full or part-time):; Hesperos, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent

holder, excluding diversified mutual funds); Hesperos, Inc. **K. Autar:** A. Employment/Salary (full or part-time):; Hesperos Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Hesperos Inc. **A. Amalfitano:** A. Employment/Salary (full or part-time):; Hesperos Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Hesperos Inc. **Z. Liu:** A. Employment/Salary (full or part-time):; Hesperos Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Hesperos Inc. **J.J. Hickman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Hesperos, Inc..

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.08/C19

Topic: C.06. Neuromuscular Diseases

Support: Progetto National Center for Gene Therapy and Drugs based on RNA Technology, CUP J33C22001130001
EPNRRCN303

Title: Anti NKCC1 amiR gene therapy to treat mouse model of hydrocephalus

Authors: *F. GHANDOUR[1], F. PICCARDI[2], F. PICCARDI[1], A. CONTESTABILE[1], L. CANCEDDA[1];
[1]Brain Develop. and Dis., [2]Italian Inst. of Technol., Genova, Italy

Abstract: Hydrocephalus is a life-threatening neurodegenerative disease resulting from excess accumulation of cerebrospinal fluid (CSF) in the cerebral ventricles. The increased CSF volume causes the ventricles to enlarge, and the consequent rise in intracranial pressure can compress and damage the brain. In addition to general neurological symptoms (nausea, vomiting, headaches, drowsiness, etc.), the main symptoms of hydrocephalus are dementia, gait disturbances, and urinary incontinence. To date, treatment options for relieving hydrocephalus have been limited to surgical CSF drainage techniques, as no effective pharmacological treatments have yet been introduced. From a molecular standpoint, one of the key players in CSF production under physiological conditions is the sodium-potassium-chloride cotransporter isoform 1 (NKCC-1), which is highly expressed at the choroid plexus in the brain. NKCC1 contributes to approximately half of the CSF production, and it is dysregulated in diverse animal models of hydrocephalus. In the kaolin-induced model of hydrocephalus, we demonstrated that the level of functional (phosphorylated) NKCC1 (pNKCC1) is significantly upregulated in the choroid plexus luminal membrane. Thus, we hypothesized that reducing the expression of

NKCC1 could limit the excessive accumulation of CSF in hydrocephalus and consequently rescue the cognitive and motor symptoms in the kaolin-induced model of hydrocephalus. Here, we tested the potential therapeutic application of NKCC1 gene knockdown by intracerebroventricular injection of adeno-associated viral vector (AAV1/2) expressing artificial microRNA (amiR) targeting NKCC1 in the kaolin-induced hydrocephalus mouse model. Our data provide evidence that NKCC1 may be implicated in hydrocephalus pathogenesis and that NKCC1 knockdown gene therapy could become a new therapeutic strategy for people with hydrocephalus in the future.

Disclosures: F. Ghandour: None. **F. Piccardi:** None. **F. Piccardi:** None. **A. Contestabile:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); co-inventors on granted patent US 9822368, EP 3083959, and JP 6490077; patent application WO 2018/ 189225; and patent application IT 102019000004929. **L. Cancedda:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); co-inventors on granted patent US 9822368, EP 3083959, and JP 6490077; patent application WO 2018/ 189225; and patent application IT 102019000004929. Other; co-founder of IAMA Therapeutics.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.09/C20

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R01NS127204
NIH Grant R01NS082351

Title: Giant Axonal Neuropathy - Disruption of autophagy due to neurofilament accumulation

Authors: *C. PANJA;
Neurol., Northwestern Univ., Chicago, IL

Abstract: Neurofilament accumulation is a marker of several neurodegenerative diseases, but it is the primary pathology in Giant Axonal Neuropathy (GAN). This childhood onset autosomal recessive disease is caused by loss-of-function mutations in gigaxonin, the E3 adaptor protein that is essential for neurofilament degradation. Using a combination of genetic and RNA interference (RNAi) approaches, we found that dorsal root ganglia from mice lacking gigaxonin have impaired autophagy and lysosomal degradation through two mechanisms. First, neurofilament accumulations interfere with the distribution of autophagic organelles, impairing their maturation and fusion with lysosomes. Second, the accumulations sequester the chaperone 14-3-3, a protein responsible for the localization of the transcription factor EB (TFEB), a key

regulator of autophagy. This dual disruption of autophagy likely contributes to the pathogenesis of other neurodegenerative diseases with neurofilament accumulations.

Disclosures: C. Panja: None.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.10/C21

Topic: B.09. Glial Mechanisms

Support: NIH-NIDCR T32 grant DE007057
NIH 1R15NS128837
Ball State University ASPiRE Junior Faculty Award
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Title: Sarm1 regulates schwann cell reprogramming and timely nerve regeneration.

Authors: *L. B. SCHMITD[1], H. HAFNER[1], A. WARD[2], N. P. BISCOLA[3], L. A. HAVTON[4], A. L. KALINSKI[5], R. J. GIGER[6];
[1]Univ. of Michigan, Ann Arbor, MI; [2]Univ. of Michigan, Ann Arbor, Ann Arbor, MI; [3]Neurol., [4]Dept. of Neurol., Icahn Sch. of Med. at Mount Sinai, New York, NY; [5]Biol., Ball State Univ., Muncie, IN; [6]Neurology/Cell and Developmental Biol., Univ. of Mchigan, Ann Arbor, MI

Abstract: Upon peripheral nervous system trauma, the distal part of severed axons undergoes sterile alpha and TIR motif-containing 1 (SARM1)-regulated disintegration, a process known as Wallerian degeneration (WD). Denervated Schwann cells (SCs) execute an elaborate repair response marked by elevated expression of c-Jun and $p75^{NTR}$ and downregulation of myelin genes. Similar to Wallerian degeneration slow (*WldS*) mice, axon regeneration in the sciatic nerve is greatly delayed in *Sarm1-/-* mutants. Bulk RNA sequencing of axotomized DRGs showed that *Sarm1-/-* is not necessary for the activation of neuron intrinsic growth programs or conditioning-lesion enhanced neurite outgrowth *in vitro*, contrasting previous studies with *WldS* mice. *In vivo*, SCG10+ sensory axons in injured *Sarm1-/-* mice rapidly extend into the lesion area but stall at the transition to the distal nerve. Commensurate with this, a longitudinal spatial transcriptomics study revealed similar transcriptional changes at the nerve injury site in WT and *Sarm1-/-* mice. Along the distal nerve, we find few gene products that are upregulated in the *Sarm1-/-* nerve prior to WD; however, canonical markers of the SC repair response, including *Jun* and *Ngfr* ($p75^{NTR}$), are greatly delayed in mutants. Regenerated axons in the *Sarm1-/-* show long-lasting defects in nerve conduction and reduced caliber at the ultrastructural level. Behavioral studies revealed defects in hindfoot function. To demonstrate the non-permissive

nature of *Sarm1-/-* distal nerve tissue, we grafted *Sarm1-/-* sciatic nerve segments into WT recipient mice and observed reduced regeneration compared to parallel processed mice that received WT grafts. Primary SCs prepared from WT and *Sarm1-/-* sciatic nerve support comparable neurite outgrowth of primary DRG neurons. *Ex vivo*, however, *Sarm1-/-* nerves show greatly delayed reprogramming of SCs into repair SCs, suggesting that signals from WD-resistant axons block activation of the SC repair response. This observation provides an opportunity for pharmacological screening of pathways that suppress the induction of the SC repair response. Together, our studies show that the *Sarm1-/-* distal nerve microenvironment is not conducive to the regeneration of WT neurons. These findings highlight the importance of WD for timely SC reprogramming and nerve regeneration. Understanding SC plasticity following nerve injury remains crucial for advancing PNS regeneration.

Disclosures: L.B. Schmitd: None. **H. Hafner:** None. **A. Ward:** None. **N.P. Biscola:** None. **L.A. Havton:** None. **A.L. Kalinski:** None. **R.J. Giger:** None.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.11/Web Only

Topic: B.09. Glial Mechanisms

Support: JSPS 16K10990, 19J40281, 23K10498, 22K19275, JP22H04676, 23H00372, 21H05292, 23H02394
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Title: Hypoxia-inducible factor 1α promotes Schwann cell peripheral nerve myelination

Authors: Y. KOBAYASHI-UJIIE[1], S. WAKATSUKI[2], ***T. ARAKI**[3];
[1]Peripheral Nervous Syst. Res., Natl. Inst. of Neurosci, NCNP, Tokyo, Japan; [2]Peripheral Nervous Syst. Res., Natl. Inst. of Neurosci., Tokyo, Japan; [3]Peripheral Nervous Syst. Res., Natl. Inst. Neurosci, NCNP, Tokyo, Japan

Abstract: Schwann cells are essential for supporting the metabolic activity of neurons and myelination in the peripheral nervous system. Hypoxia plays a role during development of aerobic animals, and has recently been demonstrated to regulate oligodendrocyte differentiation in the central nervous system. Here we demonstrate that hypoxia-inducible factor 1α (HIF1α) in Schwann cells promotes myelination. HIF1α protein expression is post-transcriptionally

regulated and highly induced in myelinating Schwann cells during development and after injury. We also demonstrated that peripheral nerve tissue experiences hypoxic conditions during physiological development and repair after injury. Stabilization or overexpression of HIF1α in Schwann cells promotes myelination in culture. Analysis of HIF1α targets revealed that HIF1α induces genes implicated in Schwann cell myelination and repair. Furthermore, we found that conditional deletion of HIF1α in Schwann cell results in delayed functional and morphological recovery from peripheral nerve injury. These results suggest that HIF1α is a novel myelination regulator of Schwann cells.

Disclosures: Y. Kobayashi-Ujiie: None. **S. Wakatsuki:** None. **T. Araki:** None.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.12/C22

Topic: B.09. Glial Mechanisms

Support: NIH Grant 1R01NS127442

Title: Plexin-b1 mediated schwann cell mobilization and alignment enhance regeneration and functional recovery in the peripheral nervous

Authors: *J. LI, H. NI, D. HALAWANI, R. H. FRIEDEL, H. ZOU;
Dept. of Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Schwann cell plasticity is central to the remarkable regenerative capacity of the peripheral nervous system (PNS). Despite this, the role of the transmembrane receptor Plexin-B1 (PB1), which plays a crucial role in nerve development and cell-cell recognition, in activating Schwann cells(SCs) and driving the detailed process of nerve regeneration remains poorly understood. Here, we employed a sciatic nerve transection model, coupled with X-gal reporter system and single-cell sequencing analysis, to track the specific high expression of PB1 in SCs post-injury, revealing its role in accelerating axonal regeneration and sensory-motor function recovery. Further, through in vitro primary SCs aggregation and dispersion experiments, we discovered that PB1 mediates the perception of mechanical stress post-injury, rapidly mobilizing SCs migration and dispersion. Additionally, PB1 enhances the recognition of SCs and their radial alignment with nerve fibers, supporting organized target nerve regeneration. By accelerating the formation of nerve bridges, improving the extracellular matrix at the injury core, and physically remodeling macrophages, PB1 creates a favorable microenvironment for nerve regeneration. Lastly, we observed that PB1 alters the endocytic capabilities of SCs, resulting in phenomena akin to membrane leakage, although this does not significantly affect the ability of SCs to clean up myelin debris and remyelinate. In summary, our data reveal that PB1

meticulously regulates the spatial configuration and biological functions of SCs, significantly promoting the rebirth and proper alignment of nerve fibers, and providing new insights into the high plasticity of SCs in nerve regeneration.

Disclosures: J. Li: None. **H. Ni:** None. **D. Halawani:** None. **R.H. Friedel:** None. **H. Zou:** None.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

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Topic: B.09. Glial Mechanisms

Support: ASTROLIGHT FA9550-20-1-0386
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GrapheneCore3881603-Graphene Flagship
PRIN-PNRR-2022 (P2022Z27NS-NANODYN)

Title: Graphene oxide-based interfaces for the modulation of glia-neuron calcium signalling

Authors: *R. FABBRI[1], G. CONTE[1], A. SCIDÀ[1], A. CANDINI[1], D. SPENNATO[1], A. KONSTANTOULAKI[1], M. CAPRINI[2], G. P. NICCHIA[3], E. TREOSSI[1], R. ZAMBONI[1], D. C. SPRAY[4], V. PALERMO[1], V. BENFENATI[1];
[1]ISOF-CNR, Bologna, Italy; [2]Pharm. and Biotech., Univ. of Bologna, Bologna, Italy; [3]Dept. of Biosci., Biotech. and Envrn., Univ. of Bari, Bari, Italy; [4]Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Satellite glial cells (SGCs) in sensory ganglia share several properties with astrocytes of the central nervous system, but the communication between SGCs and sensory neurons has not been fully explored (Hanani & Spray, Nat Rev Neurosci., 2020). Due to its favourable combination of biocompatibility, electrical, and mechanical features, graphene is being effectively employed as a neural interface (Fabbri et al., Nanoscale, 2021). We recently demonstrated that graphene can be used as a glial interface to selectively modulate the functionality of brain astrocytes via electrical stimulation, with no detrimental gliotic reactivity (Fabbri et al., Nature Nanotechnology, in press, 2024). In this study, we exploit the unique properties of graphene-oxide (GO) and reduced GO (rGO)-coated electrodes for controlling Ca^{2+} signalling in SGCs/neurons of Dorsal Root Ganglia (DRG) *in vitro* by electrical stimulation. We tested the impact of GO and rGO on cell viability and morphological properties of the DRG primary co-culture from post natal rats. Biocompatibility, immunostaining and RT qPCR data indicated that GO and rGO substrates are biocompatible interfaces, promoting the growth of

Cx43 positive SGCs and GAP43 positive neurons co-cultures seeded on their surface, without the need for any additional adhesion treatment. We performed Ca^{2+} imaging on DRG co-cultures grown on indium tin oxide (ITO) coated with GO or rGO films. We discovered that electrical stimulation elicits distinct intracellular Ca^{2+} responses in DRG neurons and SGCs *in vitro*, depending on the electrical properties of rGO/GO interfaces. SGCs and neurons stimulated by insulating GO electrodes show a slow and sustained Ca^{2+} response. Conversely, SGCs/neurons stimulated by conductive rGO electrodes exhibit a more oscillatory Ca^{2+} response. Pharmacology revealed that distinct pathways are activated by rGO/GO-electrodes, with intracellular Ca^{2+} release via IP_3, extracellular Ca^{2+} influx by TRPV channels and intercellular Ca^{2+} signalling trough gap junctions differentially involved in the effect. Importantly, although the amplitude of the Ca^{2+} response of SGCs and neurons are comparable among them in both co-cultures on GO and rGO, the electrical stimulation response is prompter on SGCs then on surrounding neurons. Collectively these results confirm that GO/rGO-coated electrodes could be used to selectively modulate neuron and glia function also in the Peripheral Nervous System or sensory circuits, as a basis for future applications in neuroscience investigation and bioelectronic medicine (Fabbri et al., Pharmacol Ther. 2023).

Disclosures: R. Fabbri: None. **G. Conte:** None. **A. Scidà:** None. **A. Candini:** None. **D. Spennato:** None. **A. Konstantoulaki:** None. **M. Caprini:** None. **G.P. Nicchia:** None. **E. Treossi:** None. **R. Zamboni:** None. **D.C. Spray:** None. **V. palermo:** None. **V. Benfenati:** None.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.14/C25

Topic: C.06. Neuromuscular Diseases

Support: Beca Doctoral CONICET (NNG)
PICT-2018-00684 (NU)
IBRO Early Career Award (NU)
ISN Career Development Grant (NU)

Title: Characterizing the transversal organization of the membrane-associated periodic skeleton in rodent nerves using 3D-dSTORM microscopy

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Abstract: A decade ago, cortical cytoskeleton of axons and dendrites has been described to have a 1-D periodical arrangement, referred to as the Membrane-associated Periodic Skeleton (MPS). The MPS is a periodic protein structure consisting of actin "rings" located transversely to the axon and separated every 190 nm by α/β-spectrin tetramers "spacers". Most of published studies describe the MPS *in vitro* (in cultured neurons) and postulates that its presence is important in the organization of membrane components, in the regulation of axon diameter, in the control of microtubule dynamics, the physical resistance of the axon and during axonal pruning. We aim to elucidate the transversal organization of β2-spectrin tetramers within the MPS and to learn whether this organization is a conserved feature of the MPS. Although there are reconstructions showing the transversal localization in cultured neurons, these have never been analyzed quantitatively, and, more importantly, nor have they been analyzed in axons within nerve tissue. The MPS can only be described using super-resolution (SR) microscopy approaches, since its spatial features lay below the diffraction limit of light. Therefore, we started a project to investigate this in rodent nerve slices, i.e. *in situ*, using two-color 3D-dSTORM microscopy. We have first established a protocol for the examination of transcardially perfused 1 month-old c57 mouse sciatic nerves: cryosectioning and immunohistochemistry, to make them suitable for 3D-dSTORM. We have registered β2-spectrin signal and analyzed the data with a clustering method. In the sciatic nerves of WT mice, we have found that β2-spectrin tetramers are arranged in each period at regular and fixed distances irrespective of axonal identity and that scales with axon diameter. We have also evidenced that the localization of a β2-spectrin tetramer in one period is correlated with the position of tetramers in neighboring periods, suggesting a structural constrain for their interaction with actin rings. Being able to describe the ground truth of β2-spectrin organization, would lead us to understand its modifications in other situations such as peripheral trauma, degeneration or mutations that could lead to cytoskeletal disorders.

Disclosures: N.G. Gazal: None. **L. Lopez:** None. **G. Escalante:** None. **A.M. Szalai:** None. **E.A. Gorostiza:** None. **F. Stefani:** None. **N. Unsain:** None.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.15/C26

Topic: C.06. Neuromuscular Diseases

Title: Comparative Transcriptome Profiling of Multiple Human Induced Pluripotent Stem Cell Derived Schwann Cell Populations

Authors: *G. MCCABE, M. DAU, V. TRUONG, P. WALSH;
Anatomic Inc., Minneapolis, MN

Abstract: Schwann cells are integral to the peripheral nervous system with pivotal roles in myelinating and supporting neurons, thus contributing to proper nerve function. Dysfunctions in Schwann cells have been implicated in a spectrum of neurological disorders, including diabetic peripheral neuropathy, Charcot-Marie-Tooth disease, and neuropathic pain syndromes. However, procuring consistent and reliable Schwann cells for drug discovery and disease modeling has posed significant challenges, primarily due to the limited availability of human-derived sources and the inherent variability in isolation methods. Our group has previously developed an efficient, scalable method for generating Schwann cell precursors (SCPs) from human induced pluripotent stem cells (hiPSCs). This is accomplished through directed differentiation under precisely defined media conditions within 9 days - without the need for genetic manipulation. In this study, we matured the Schwann cells over a period of a week and performed bulk RNA sequencing to thoroughly characterize these schwann cell precursors transcriptomically. We compare the data set with publicly available primary human Schwann cell (hSC) tissues and other hiPSC derived Schwann cell data repositories. Analysis revealed that this SCP population (RealSCP) has a consistent molecular phenotype that is similar to primary hSC in important ways that are highly relevant for drug screens and mechanism-based studies. To further explore how this cell type can be used in screening applications, we developed co-cultures with hiPSC-derived motor neurons and sensory neurons to look at axon alignment and myelination. We see that co-cultures undergo rapid SCP-axon alignment within 48 hours, and myelination was observed via transmission electron microscopy and expression of the myelin-associated proteins-myelin basic protein (MBP) and myelin protein zero (MPZ) as early as 5 weeks in culture. In summation, SCPs can be efficiently and rapidly generated from hiPSCs and cultured with neurons to form myelinating models of the peripheral nervous system. These co-culture systems will lay the groundwork for future experimentation modeling neuropathology in a patient-specific manner.

Disclosures: G. McCabe: A. Employment/Salary (full or part-time):; Anatomic Incorporated. **M. Dau:** A. Employment/Salary (full or part-time):; Anatomic Incorporated. **V. Truong:** A. Employment/Salary (full or part-time):; Anatomic Incorporated. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Anatomic Incorporated. **P. Walsh:** A. Employment/Salary (full or part-time):; Anatomic Incorporated. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Anatomic Incorporated.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.16/C27

Topic: B.09. Glial Mechanisms

Support: CIHR bridge grant
CIHR grant
FRQS Postdoctoral Fellowship

Title: Glial Cannabinoid type-1 (CB1) receptors: a key player in neuromuscular junction repair?

Authors: *R. PIOVESANA[1], S. CHARRON[2], D. ARBOUR[2], L. BELLOCCHIO[3], G. MARSICANO[4], R. ROBITAILLE[2];
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Abstract: Following nerve injury, extensive morphological and functional changes reshape synaptic elements at neuromuscular junctions (NMJs) to promote its reinnervation. This process is in part mediated by Perisynaptic Schwann cells (PSCs), glial cells at the NMJ, essential for its maintenance and repair. Cannabinoids are frequently used in the treatment of neuropathic pain related to nerve injury. However, despite evidence for their roles in the regulation of axonal guidance and synapse formation during development of the central nervous system and management of pain in the peripheral sensory system, their possible contribution in response to peripheral nerve injury remains unclear. Cannabinoid type 1 receptors (CB1R) may be involved in repair owing to their presence in the peripheral nervous system and at the NMJ. Here we present a novel role of glial CB1Rs in motor recovery following nerve injury. CB1R absence, through treatment with the antagonist AM251 or in CB1R-KO, accelerated NMJ denervation with an increased expression of the phagocytic marker MAC-2. Also, their absence delayed the NMJ reinnervation following nerve injury. Importantly, these results were completely replicated when CB1R were knocked down only in glial cells using conditional GFAP-CB1R-KO mice. Reflecting the repair mode of PSCs, a reduced muscarinic activation of PSCs is normally observed at reinnervating NMJs. However, at reinnervating NMJs of CB1-KO animals, PSC Ca^{2+} responses induced by local application of muscarine were significantly higher than the ones at the contralateral leg, a phenotype incompatible with NMJ repair. Lastly, CB1R activation enhanced reinnervation after nerve injury, as revealed by a faster and more complete reinnervation with a significant increase of mono-innervated NMJs, suggesting a positive role of this receptor in nerve repair processes. Our results highlight a novel role of (endo)cannabinoids in NMJ repair after peripheral nerve injury, opening a possible therapeutic strategy for facilitating nerve repair following injury or to address inadequate NMJ maintenance observed in motor neuron-related neurodegenerative diseases.

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Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.17/C28

Topic: C.06. Neuromuscular Diseases

Support: CIHR

Title: Motor-unit-dependence and functional innervation tailor neuromuscular recovery from nerve injury and in ALS

Authors: *S. CHARRON, E. TREMBLAY, R. ROBITAILLE;
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Abstract: Nerve injury leads to neuromuscular junction (NMJ) denervation and motor impairment. While the morphological repair of the NMJ in the peripheral nervous system post-injury is well known, the functional recovery in various motor unit (MU) types and their correlation with NMJ innervation is understudied. Moreover, the contribution of partial reinnervation to muscle function remains ill-defined. In addition, neurodegenerative disorders like amyotrophic lateral sclerosis (ALS) leads to nerve damage and NMJ denervation, in a motor-unit dependant manner, which may present distinct functional alterations. In this work, we characterized muscle function in parallel to NMJ innervation in WT mice following nerve injury, as well as in $SOD1^{G37R}$ mice, a model for ALS. We studied two muscles with different motor units: the *Soleus* (SOL, slow and fatigue resistant MUs) and the EDL (fast fatigable MUs). Neuromuscular function was assessed using a muscle force transducer to measure contractile force induced by nerve stimulation to test the neuromuscular contribution or by direct muscle stimulation. NMJ innervation was determined using a triple immunostaining of the NMJ: pre, postsynaptic and glial components. NMJ function and innervation were monitored at 8, 11, 14, 16 and 21 days postinjury (dpi). At 8dpi, both muscles were still fully denervated and no muscle contraction were evoked by nerve stimulation. The neuromuscular contractile capacity improved markedly to 80% at 11 dpi in the SOL with only 5% of fully innervated NMJs and 80% of partially innervated ones (15% denervation). However, the EDL contractile capacity was only 35% with 2% of fully innervated NMJs and still 40% of fully denervated NMJs. Interestingly, while contractile capacity was similar between the two muscles at 14, 16 and 21 dpi, the SOL surprisingly presented significantly more partial and less fully innervated NMJs than the EDL. In ALS, however, the EDL presented profound NMJ alterations and progressive functional deficits, which paralleled disease stages. As expected, the SOL was highly resistant with delayed NMJ alterations and muscle function largely preserved until advanced disease stage. Our results highlight different dynamics of repair and functional recovery following nerve injury: the SOL is initially faster than the EDL, but the EDL improves more consistently its innervation. In ALS, however, the EDL is selectively vulnerable compared to the SOL, both structurally and functionally. Our data also indicate that partially innervated NMJs contribute to muscle contraction and function. Altogether, our findings reveal a strong link between structure and functionality of the NMJ.

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Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.18/C29

Topic: B.09. Glial Mechanisms

Title: Involvement of IFN-γ in Trigeminal Spinal Subnucleus Caudalis on Orofacial Neuropathic Pain associated with Infraorbital Nerve Injury

Authors: *S. ASANO[1], *S. ASANO[3], A. OKADA-OGAWA[4], K. IWATA[4], K. MIZUTA[2], M. SHINODA[5];
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Abstract: [Background and aims]It is crucial to understand the mechanisms underlying neuropathic pain to develop the appropriate treatment for these patients. Recently, many researchers have focused on the interferon-gamma (IFN-γ) signaling involved in persistent pain mechanisms. However, the exact mechanism is still unknown. We examined the involvement of IFN-γ-signaling in the trigeminal spinal subnucleus caudalis (Vc) on orofacial mechanical hypersensitivity associated with trigeminal nerve injury.
[Methods]Male SD rats were used in this study. Infraorbital nerve injury (IONI) was established by partial ION ligation. The head-withdrawal threshold (HWT) to mechanical stimulation of the whisker pad skin was measured before and on day 3 after IONI or sham treatment. The HWTs were also measured on day 3 following continuous intra-cisterna magna (i.c.m) administration of IFN-γ antagonist (10 μg/3 days) in IONI rats or IFN-γ (10 μg/3 days) in naïve rats. Moreover, localization of IFN-γ receptor and quantification of IFN-γ in Vc were evaluated on day 3 after IONI or sham treatment.
[Results]The HWT was decreased on day 3 after IONI. The expression of the IFNγ receptor was significantly higher in astrocytes in IONI rats. IFN-γ antagonism in Vc recovered the decrement of the HWT in IONI rats, and i.c.m administration of IFN-γ decreased the HWT in naïve rats. In Vc, the IFN-γ receptor was expressed in astrocytes, and its expression level increased on day 3 after IONI.
[Conclusions]The present findings suggest that IFN-γ is involved in astrocyte activation and causes the hyperactivation of the TG ganglion neurons following trigeminal nerve injury, resulting in orofacial neuropathic pain.

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Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.19/C30

Topic: B.09. Glial Mechanisms

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NSF IOS 2227963
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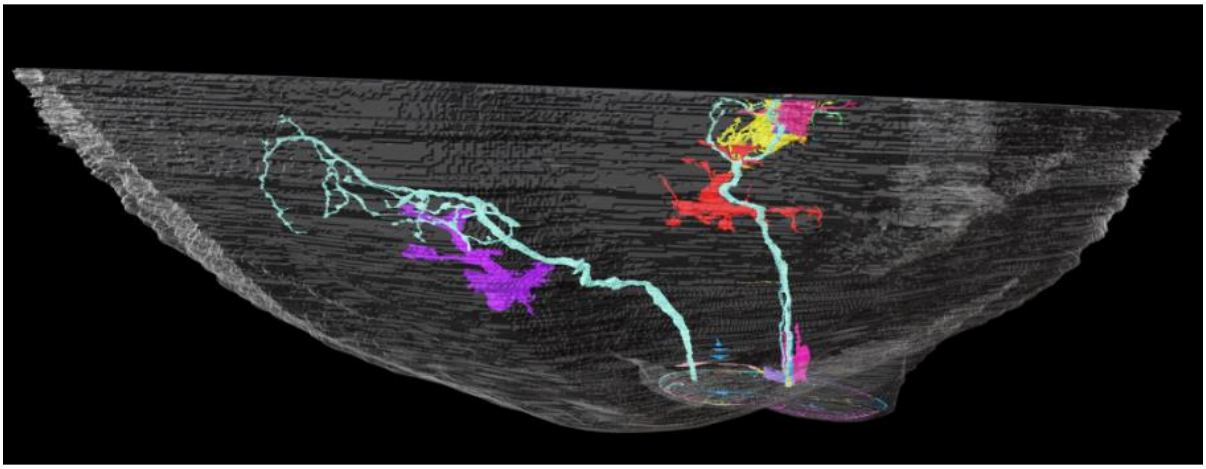
Title: Identification of glial cell types in the mollusc Berghia stephanieae using gene expression and volume electron microscopy

Authors: *H. SANT[1], A. COOK[2], M. RAMIREZ[2], R. SCHALEK[3], J. W. LICHTMAN[3], P. S. KATZ[1];
[1]Biol., Univ. of Massachusetts, Amherst, Amherst, MA; [2]Biol., Univ. of Massachusetts Amherst, Amherst, MA; [3]Mol. and Cell. Biol., Harvard Univ., Cambridge, MA

Abstract: Glial cells play an important role in nervous system development and function. However, little is known about glial cell types in molluscs. We identified marker genes for glial subtypes using single-cell transcriptomics (scRNA-seq) and visualized their expression using in-situ hybridization chain reaction (HCR) in the gastropod mollusc Berghia stephanieae. Apolipophorin, a known marker for Drosophila astrocytes, and recently identified as a glial cell marker in Octopus vulgaris, was also differentially expressed in scRNA-seq by glia in Berghia. Apolipophorin HCR labeling revealed abundant glia, including some giant glia in Berghia's nervous system, suggesting its use as a pan-glial marker. Glial cell types were also identified in a volume electron microscopy dataset of the rhinophore ganglion based on ultrastructural features. Glial cells have distinct ultrastructural features and diverse morphologies. There are large glia that encase numerous neuronal soma. Some glial cells partitioned axon bundles or synaptic areas of neuropil. Others bordered the sheath and vasculature. Membrane-to-membrane junctions between glia suggest a potential glial signaling network. Glial cells identified in Berghia appear analogous to wrapping glia, astrocytes, cortex glia, and ensheathing glia described in other animals. The 3D structures of various glial cells were reconstructed. Modern molecular and imaging techniques have provided unprecedented access to studying glia in molluscan nervous systems. Future research on molluscan glia will offer insights into the function and evolution of glia across animals.



Disclosures: H. Sant: None. **A. Cook:** None. **M. Ramirez:** None. **R. Schalek:** None. **J.W. Lichtman:** None. **P.S. Katz:** None.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.20/C31

Topic: E.09. Motor Neurons and Muscle

Support: JSPS-KAKENHI grant number 23K20363

Title: Excitation of aortic baroreceptor reduces muscle contractility by inhibiting contraction-induced reflex of sympathetic nerve synapsing at neuromuscular junction in rats

Authors: *H. HOTTA[1], N. WATANABE[2], K. TAKENO[3], M. MORIYA[2], H. NISHIMUNE[3]; [1]Tokyo Metropolitan Inst. for Geriatrics and Gerontology, Tokyo, Japan; [2]Dept. of Autonomic Neurosci., Tokyo Metropolitan Inst. for Geriatrics and Gerontology, Tokyo, Japan; [3]Lab. of Neurobio. of Aging, Tokyo Metropolitan Inst. for Geriatrics and Gerontology, Tokyo, Japan

Abstract: We hypothesized that muscle sympathetic nerve modulates function of neuromuscular junction (NMJ) in conjunction with arterial regulation. This possibility was investigated by physiological and histological analysis on hindlimb muscle of mature male rats. First, we examined the effects of baroreceptor activation on muscle contraction-induced muscle-lumbar sympathetic reflex discharges and muscle contractility in anesthetized rats. Tibial motor fibers were stimulated to elicit tetanic contraction of the gastrocnemius and soleus muscles and reflex discharges in lumbar sympathetic postganglionic nerves. When the aortic depressor nerves (ADNs) were intact, a rapid increase in mean arterial pressure to approximately 150 mmHg by intravenous injection of phenylephrine decreased the reflex discharges by 57 $\pm$ 16 % (mean $\pm$

SD) of the control values, resulting in reduction of the tetanic force (TF) by 7.8 ± 4.2%. However, bilateral denervation of the ADNs, almost completely abolished the hypertension-induced decrease of both reflex discharges and TF. Second, to histologically examine connection between sympathetic nerve innervating artery and NMJ in gastrocnemius muscle tissue, nicotinic receptors, motor nerves, adrenergic nerves, and arteries were fluorescently labeled. We observed in whole mount preparations that an extension of adrenergic nerve around an artery connected to a NMJ. Further, a pre- and a postsynaptic marker of adrenergic nerve was both detected at NMJs. The results indicate that the baroreceptor afferent signal from the ADN decreases contractility of hindlimb muscles by inhibiting contraction-induced reflex of the sympathetic nerve synapsing at NMJ. This mechanism presumably has physiological significance in adaptation to gravity.

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Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.21/C32

Topic: C.06. Neuromuscular Diseases

Support: This work was partially supported by the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation.

Title: Lack of mRNA Methylation in Schwann Cells Results in Demyelination & Regenerative Failure

Authors: *M. SARI, A. JOHNSON, A. YU, R. MI, X. HU, A. HOKE;
Dept. of Neurol. Div. of Neuromuscular Med., Johns Hopkins Univ., Baltimore, MD

Abstract: A) Introduction
N6-methyladenosine (m6A) RNA methylation is the most common modification of mRNA and is a critical regulator of RNA function. The m6A methyltransferase complex, consisting of several subunits including writer, eraser, and reader components catalyzes the regulation of m6A marks. Mettl14 is an important writer component. Here, we investigate the developmental and regenerative role of mRNA methylation in Schwann cells by specific deletion of Mettl14 (P0-Cre crossed with $Mettl14^{floxed/floxed}$).
B) Method
Neuromuscular SHIRPA was performed as an overall assessment of neuromuscular function. Accelerating rotarod assay was used to evaluate motor coordination and balance. Forelimb and hindlimb grip strength tests were performed to evaluate skeletal muscle strength. Histological studies were performed on intact and regenerative sciatic nerves. EdU Cell Proliferation Assay

was performed and EdU-positive cells were quantified using fluorescence microscopy in neonatal cultured Schwann cells. Bulk RNA sequencing analysis was also performed on the sciatic nerve.
C) Results
In behavioral assessment, Mettl14 cKO mice have progressively reduced NM SHIRPA scores starting at 4 months, and significantly reduced performance on rotarod and grip strength assays starting at 3 months of age. In histological studies, Mettl14 cKO mice had normal developmental myelination, but showed progressive demyelination, increased g-ratio, and decreased axon density starting at 4 months old. These findings parallel the phenotypic results. Following sciatic nerve crush, Mettl14 cKO mice show impaired regeneration, which was associated with reduced recruitment of macrophages and decreased MCP (monocyte chemoattractant protein-1) levels. In Schwann cells cultured from the Mettl14cKO mice, there was a cell proliferation defect as measured by EdU incorporation. Moreover, the loss of Mettl14 in Schwann cells resulted in the downregulation of key myelin protein genes including Pmp22, Mbp, and Mpz, and the cell cycle regulator gene (CyclinD1).
D) Conclusion
Together, our findings indicate that mRNA methylation plays an important regulatory role in Schwann cell maintenance. Although initially, they develop normally, Mettl14 cKO mice have progressive demyelination, regenerative failure, and Schwann cell proliferation defect.

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Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.22/C33

Topic: B.09. Glial Mechanisms

Support: F31NS130956

Title: The diverse roles of basement membrane on the form and function of Schwann cells

Authors: *M. LILLIS[1], D. GOULD[2], J. R. CHAN[3];
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Abstract: During development, Schwann cells (SCs) secrete a basement membrane that is necessary for radial sorting large-caliber axons prior to myelination. After large-caliber axons are sorted, SCs ensheathe small-caliber axons in Remak bundles by intercalating their membrane around multiple axons. Remak bundle formation is poorly understood and this project aims to

identify the relevant mechanism. Given that laminin, a primary basement membrane component, is necessary for radial sorting, we assessed whether another basement membrane component, collagen-IV, could regulate Remak bundle formation. In mice deficient in the secretion of collagen-IV from SCs we see a defect in Remak bundle formation in 2-month-old mice but normal myelination. Specifically, the Remak bundles are significantly larger and SCs are unable to fully intercalate axons, leading to 'naked' axons exposed to the nerve environment. This finding suggests that Remak bundle formation may require a distinct mechanism from radial sorting. Therefore, we explored potential collagen-IV binding partners. Two collagen-IV receptors, Itga1 and Itga2, are expressed in adult Remak SCs but not myelinating SCs. We analyzed global Itga1 knockouts and conditional Itga2 knockouts but found no defects in Remak bundle formation. Therefore, we hypothesized that collagen-IV may be acting as a scaffold to organize other basement membrane proteins. Based on preliminary data, we are exploring the possibility that laminin expression is altered in the absence of collagen-IV. This would indicate that Remak bundle formation utilizes the same mechanisms as radial sorting. We are examining the effects of conditionally ablating the laminin receptor Itgb1 at various time points—specifically after sorting and prior to Remak bundle formation. We hypothesize this will lead to Remak bundles where axons are not fully intercalated, phenocopying the collagen-IV mutant mice. These findings will provide insight into the relevant mechanisms for Remak bundle formation and may indicate that there is a continuum between radial sorting and the intercalation of small caliber axons.

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Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.23/C34

Topic: B.10. Multiple Sclerosis and Other Demyelinating Diseases

Support: Midwestern University start-up funds to MT & ME
Midwestern University Research Core facility outsourcing fund to MT & ME

Title: Auditory and Peripheral Neuropathy Upon Schwann Cell ablation in Adult and Aging DTA Mice

Authors: N. ZOGHBY[1], J. CRONBERG[1], S. SAWANT[1], E. MARKUSON[1], S. LING[1], H. ZHANG[1], *M. EBEID[1,2,3], M. TRAKA[1,2,3];
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Abstract: Demyelinating diseases of the peripheral nervous system (PNS) affect an increasing number of people, and can be either inherited, such as Charcot-Marie-Tooth (CMT), or acquired from an autoimmunological insult, trauma or nerve injury. In these diseases there is substantial damage to Schwann cells (SchCs) leading to loss of the myelin sheath and subsequent impaired peripheral nerve conduction. As a result, affected individuals suffer from sensorimotor peripheral neuropathy and auditory neuropathy. Enhancing the endogenous remyelination potential of the PNS is a promising therapeutic approach for promoting functional recovery and axonal survival in these demyelinating diseases. *PLP-CreER^{T};ROSA26-eGFP-DTA* (*DTA*) mouse is an established animal model for cell-specific ablation of *Plp1*-expressing glial cells in the nervous system upon induction with tamoxifen administration. Here we studied the process of glial cell regeneration and ultimately remyelination in auditory system and sciatic nerve of mature adult (4-6 month-old) and middle-aged (8-10 month-old) *DTA* mice upon induction of glial cell ablation. Recovering mice are collected at different time points post-induction (at 1, 3 & 5 weeks) to analyze glial cell regeneration within the auditory system and the sciatic nerve. Inner ears and sciatic nerves are fixed, then spiral ganglion and auditory nerve fibers, as well as sciatic nerve fibers are dissected and immunostained either as whole mount or sectioned to label different types of glial cells, as well as myelin, axonal and node of Ranvier markers. Transmission electron microscopy is used to identify myelin and neuronal ultrastructure. Auditory brain stem responses (ABR) are used to assess hearing function. Grip strength testing is used to assess motor function. In mature adult *DTA* mice, robust SchC and satellite cell regeneration occurs by 5 weeks post-induction in auditory system that relies mainly on cell proliferation. Myelin didn't show significant loss in auditory nerve fibers, however, ABR thresholds were elevated in mature adult *DTA* mice at 5 weeks post-induction compared to pre-induction indicating auditory neuropathy in this model. Forelimb and hindlimb grip strength values were significantly decreased in both mature adult and middle-aged *DTA* mice at 5 weeks post-induction and normal nodes of Ranvier were less frequently observed in sciatic nerve fibers, indicating peripheral neuropathy in these mice.

Disclosures: N. Zoghby: None. **J. Cronberg:** None. **S. Sawant:** None. **E. Markuson:** None. **S. Ling:** None. **H. Zhang:** None. **M. Ebeid:** None. **M. Traka:** None.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.24/C35

Topic: E.09. Motor Neurons and Muscle

Support: Fore Batten Foundation
Children's Brain Diseases Foundation

Dept of Pediatrics, Washington University in St Louis
NINDS R21 126907

Title: Identifying and treating CLN3 disease outside the central nervous system

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Abstract: Neurodegenerative lysosomal storage disorders such as CLN3 disease have primarily been thought of as affecting the central nervous system (CNS), with effects upon both neurons and glia within the brain and spinal cord. However, children with this disorder also have peripheral sensory-motor and gastrointestinal problems. We hypothesized that in addition to central nervous system (CNS) degeneration, CLN3 deficiency may also directly affect neuronal and/or glial cell populations in the rest of the body. We have explored both functional and pathological changes in the peripheral nervous system (PNS) and the enteric nervous system (ENS) in *Cln3*$^{\Delta ex7/8}$ mice at different stages of disease progression. There was no sciatic nerve axon loss or demyelination in *Cln3*$^{\Delta ex7/8}$ mice, but significant loss of terminal Schwann cells (tSCs) at lower limb neuromuscular junctions (NMJ), and progressive NMJ denervation. This was accompanied by pronounced myofiber atrophy, fewer displaced myofibril nuclei. Atrophy was also evident in bowel smooth muscle with *Cln3*$^{\Delta ex7/8}$ mice. Moreover, the small intestine and cecum of *Cln3*$^{\Delta ex7/8}$ mice were frequently distended with fecal material. As *Cln3*$^{\Delta ex7/8}$ mice aged we saw a progressive slowing of bowel transit, that was also apparent to a lesser degree in age-matched WT mice. Subsequently there was a significant and profound loss of HuC/D-stained enteric neurons and S100B-stained enteric glia within the myenteric plexus, which varied between bowel regions and worsened with age. Neonatal administration of intravenous gene therapy using an AAV9-hCLN3 vector completely prevented tSCs and NMJ pathology, atrophy of both skeletal and smooth muscle, positively impacted bowel transit and largely prevented the loss of enteric neurons and glia. These findings reveal an underappreciated, but profound, impact of CLN3 disease outside the CNS and these novel aspects of disease may be treatable using gene therapy.

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Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.25/C36

Topic: B.10. Multiple Sclerosis and Other Demyelinating Diseases

Title: Lipid accumulation in PIEZO2 positive sensory neurons causes disrupted mechanical sensation

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Abstract: PIEZO2 is a major mechanosensitive ion channel present on dorsal root ganglion (DRG) neurons that prove to be affected by lipid perturbations. X-linked adrenoleukodystrophy (x-ALD) is the most prevalent peroxisome disorder caused by ABCD1 gene mutation. ABCD1 gene encodes an ATP binding cassette protein that is responsible for transport of acylated very long-chain fatty acids (VLCFAs) into the peroxisomes for degradation. Mutations in the ABCD1 gene lead to disrupted lipid metabolism, VLCFA accumulation, spinal cord and peripheral nerve degeneration. The frequent occurrence of pain and sensory changes is poorly understood. Abundant ABCD1 expression was detected in human DRG neurons, while in mouse DRG, higher ABCD1 expression was found in surrounding satellite glial cell but barely in DRG neurons. As the major VLCFA elongase, ELOVL1 overexpression also leads to VLCFA accumulation. Here, we created a new model by crossing mouse carrying PIEZO2 driven cre with conditional human ELOVL1(hELOVL1) overexpression, thus causing VLCFA accumulation in PIEZO2(+) sensory neurons. PIEZO2 has demonstrated functional correlation with the mechanical hypersensitivity in Abcd1-/- mice. In the current model, immunostaining of PIEZO2confirmed DRG neuron localization. High hELOVL1 mRNA detected in DRG tissues of PIEZO2cre-hELOVL1 mice suggested successful PIEZO2cre driven overexpression. Significantly increased VLCFA in DRG tissue from PIEZO2cre-hELOVL1 mice compared to PIEZO2cre-WT mice further validated functional change. Interestingly, we saw a significant reduction of plasmalogens and sphingomyelin in PIEZO2cre-hELOVL1 DRG, suggesting changes in membrane composition resulting from disrupted VLCFA metabolism. Most importantly, the PIEZO2cre-ELOVL1 mice presented significant changes in mechanical sensation, particularly in female mice. These results establish a functional correlation between

dysregulated lipid metabolism in DRG neurons and altered sensation, pointing to a potential mechanism of lipid induced peripheral neuropathy.

Disclosures: N. Golovanov: None. **Y. Gong:** None. **A. Glover:** None. **F. Eichler:** None.

Poster

PSTR270: Peripheral Nerve Glia, Nerves, and Neuropathy

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR270.26/C37

Topic: I.04. Physiological Methods

Support: NIH R21 (Grant no. 1R21NS114982-01A1)

Title: Functional composition of the pudendal nerve in the female Yucatan Minipig.

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Abstract: Functional composition of the pudendal nerve in the female Yucatan Minipig
The complex network of nerves that control sensory, motor, and autonomic functions in the urogenital and colorectal systems stem from nerve roots located in the lower thoracic, lumbar, and sacral regions. Any damage to these neural structures, whether it's central or peripheral, can significantly affect a person's quality of life. Over the past few decades, scientists have developed a range of procedures based on electrical neuromodulation, with the pudendal nerve being a key area of focus. Animal models, such as the Yucatan Minipig, are crucial in helping us understand the anatomical pathways related to both the normal and abnormal functioning of pelvic organs. These models also aid in the development of new and more effective treatments. This study provides a functional overview of the pudendal nerve in female Yucatan Minipigs. The pudendal nerve is made up of an upper cord, formed by anastomotic branches S1 and S2, and a lower cord formed by contributions from S2 and S3. These cords come together to form a small network known as the pudendal plexus before it re-enters the pelvis. To gain insight into the neural mechanisms behind scES-induced effects on the lower urinary tract, the Hubscher laboratory has developed a model of neural stimulation of the upper and lower cords of the pudendal nerves in female Yucatan minipigs with moderate-severe spinal cord injury under anesthesia while the intra-anal pressure was measured. Preliminary data suggests that electrical stimulation of the pudendal nerve's upper cord leads to the contraction of the external anal

sphincter muscle. However, similar stimulation of the lower cord does not elicit any contractile response. These findings collectively suggest that the fibers in the upper cord have a motor function, while it can be inferred that the fibers in the lower cord likely have a sensory function.

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Poster

PSTR271: Understanding and Treating Stroke

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR271.01/C38

Topic: C.08. Ischemia

Support: NIH/NINDS Grant R01NS100947
NIH/NINDS Grant R61NS130199

Title: Targeting inflammatory prostaglandin signaling for ischemic stroke

Authors: *Z. JIN[1,2], C. JIANG[1,2], E. CHO[1,2], S. BAHRAMINEJAD[1,2], Y. YU[1,2], J. JIANG[1,2]; [1]The Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; [2]Pharmaceutical Sciences, The University of Tennessee Health Science Center, Memphis, TN

Abstract: Brain ischemia accounts for the most acute stroke cases and constitutes a leading cause of deaths and disabilities, particularly among the older adults. Intravenous thrombolysis by recombinant tissue plasminogen activator (rtPA) is the only available pharmacotherapy for acute ischemic stroke, and mechanical thrombectomy provides another treatment option for patients with large artery occlusion. However, the overall narrow treatment windows and potential risks in hemorrhagic transformation and damage to blood vessels limit the patient eligibility. New drugs with extended therapeutic window to treat ischemic stroke are urgent needed. Cyclooxygenase 2 (COX-2) has long been implicated in stroke-triggered brain injury and inflammation, but targeting COX enzymes can cause broad complications in microvascular systems. As such, modulating a specific downstream pathway has been increasingly considered. As a major enzymatic product of COX-2 in the brain, prostaglandin E2 (PGE_2) is elevated by various brain insults and, in turn, aggravates neurotoxicity largely via $G\alpha_s$-coupled receptor EP2. Recent studies using postnatal and cell type-specific ablation of EP2 redefined the pathogenic roles of PGE_2/EP2 signaling in the adult ischemic brain. We and others have previously validated the feasibility of pharmacologically targeting EP2 for ischemic stroke in mouse models of middle cerebral artery occlusion (MCAO). Herein, we evaluated the therapeutic effects of a brain-penetrant EP2-selective small-molecule antagonist in a mouse model of photothrombotic ischemia, where ischemic damage is induced within a given cortical area

by photo-activation of a previously administered light-sensitive dye Rose Bengal (100 mg/kg, i.p.). We found that pharmacological inhibition of EP2 by the test lead compound (5 mg/kg or 10 mg/kg, i.p.) administered 4.5 hr after ischemic stroke substantially reduced the sensorimotor cortical infarct volume in a dose-dependent manner. In addition, post-stroke treatment with the EP2 antagonist in these mice accelerated the body weigh recovery and reduced neurological impairments in sensorimotor, locomotor, and coordination functions, revealed by a panel of behavioral tests including open field test, pole test, and corner test. The beneficial effects of EP2 inhibition in this proof-of-concept study reinforce the feasibility of targeting the PGE_2/EP2 axis-mediated inflammatory signaling as a new therapeutic strategy to reduce ischemic injuries in the brain and improve post-stroke recovery.

Disclosures: Z. Jin: None. **C. Jiang:** None. **E. Cho:** None. **S. Bahraminejad:** None. **Y. Yu:** None. **J. Jiang:** None.

Poster

PSTR271: Understanding and Treating Stroke

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR271.02/C39

Topic: C.08. Ischemia

Support: AHA 24SCEFIA 1255866
5R01NS126273-03 NIH/NINDS
AHA 23TPA1069224

Title: Long-chain Acyl-CoA Synthetases 6 in Sickle Cell Disease: A Promising Target to Improve Functional Outcomes

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Abstract: Background: Sickle cell disease (SCD) is the most common severe monogenic disorder affecting 300,000 individuals worldwide annually. The main challenges in the treatment of SCD are the sickle-shaped red blood cells that tend to aggregate along the vessel walls leading to inflammation and vascular occlusions. Therefore, patients with SCD suffer from ischemic stroke, transient ischemic attacks, and multiple organ dysfunction. One of the major hallmarks of SCD is the low levels of omega-3 fatty acids (ω3-FA) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in the blood. Thus, identifying the regulatory elements that control ω3-FA metabolism holds high therapeutic potential. As such, biosynthesis of ω3-FA through long-chain acyl-CoA synthetases (ACSLs) is essential for cell member function and survival. It

has been reported that downregulation of ACSL6, which is predominately expressed in the brain, can cause ω3-FA deficiency, as a result of cardiovascular diseases. The impact of ACSL6 on the central nervous system in pathological conditions (e.g., SCD), however, remains unknown. The goal of the study is to determine the role of ACSL6 in brain circulation and neurological outcomes in SCD. **Method:** A transgenic mouse model of SCD (Townes mice, 5-6 weeks old) and repetitive mild hypoxia-ischemia (rmHI) were utilized to mimic sickle cell anemia conditions. To manipulate ACSL6 levels in the brain following SCD, mice received intraperitoneal bolus injections of the ACSL6 activator (GW3965, 20mg/kg/day) and retro-orbital injection of AAV-ACSL6. Cerebral blood flow (CBF) before/after rmHI was measured by Two-photon laser Scanning Microscopy (TPLSM) and Laser Speckle Contrast Imaging (LSCI). The impact of ACSL6 on neuroinflammation and mitochondrial function was studied by protein chip assay and Seahorse respirometry. The novel objection recognition test and T-maze were implemented to evaluate functional learning/memory after rmHI. **Results:** ACSL6 protein levels in the hippocampus were significantly decreased concurrently with cerebral blood flow hypoperfusion, neuroinflammation, mitochondrial dysfunction, and learning/memory deficits following rmHl. Intriguingly, treatment with GW3965 or AAV-ACSL6 can increase ACSL6 levels, thereby mitigating hypoperfusion, neuroinflammation, and mitochondrial dysfunction. This further leads to favorable neuronal survival and improved neurological outcomes. **Conclusion:** Our findings suggest that targeting ACSL6 signaling is a potential therapeutic approach in the treatment against SCD-induced brain injury and associated learning/memory impairments.

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Poster

PSTR271: Understanding and Treating Stroke

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR271.03/C40

Topic: C.08. Ischemia

Support: AHA grant 24POST1198296
NIH grant NS122808

Title: Role of cofilin in recurrent hypoglycemia exposure-linked stroke risk in insulin-treated diabetic rats

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Abstract: Diabetes is one of the most significant risk factors for stroke. Stroke is one of the major causes of death and permanent disability. People with diabetes have a 1.5-2 times higher stroke risk. Correlational clinical observations indicate that hypoglycemia exposure is linked to an increased risk of stroke and worsened post-stroke outcomes in diabetes patients. Antidiabetic therapies increase the risk of hypoglycemia in diabetes patients. Acute hypoglycemia, ischemic stroke, and cardiac arrest increase platelet activation. In earlier studies, we observed that the ITD (insulin-treated diabetic) rats exposed to mild/moderate recurrent hypoglycemia (RH) had a 20% shorter clotting time than the ITD+RH+Glucose control rats. Additionally, in the in vivo thrombosis model, the clot weight in the ITD+RH rats was 46% higher than the control rats. Furthermore, the rate of ADP and collagen-induced platelet aggregation was higher in the ITD+RH rats when compared to the control rats. Moreover, cofilin is known to regulate platelet activation by regulating actin dynamics, which is vital for adhesion, spreading, aggregation during hemostasis, and thrombosis. Since the role of cofilin in RH exposure-induced increased platelet sensitivity is not known, we examined the effect of RH exposure on platelet cofilin (both phospho and total) levels. Young male rats were made diabetic using streptozotocin and insulin pellets were implanted to correct hyperglycemia. Insulin and insulin+glucose was given once a day for 5 consecutive days to induce RH (or euglycemia) in ITD+RH and ITD+RH+Glucose rats, respectively. One day after the last hypoglycemia/euglycemia exposure, blood was drawn from the rat, and platelets were isolated. The p-cofilin and total cofilin protein levels in platelets were then determined using western blotting, and p-cofilin levels were normalized with total cofilin levels. Levels of p-cofilin were significantly higher by 80% (180±20, n=12, $p<0.05$) in the ITD+RH rats as compared to the ITD+RH+Glucose rats (100±24, n=7). Our findings, so far, suggest that cofilin hyperphosphorylation in RH-exposed ITD rats may be responsible for platelet activation and increased stroke risk. Next, we are planning to determine how RH exposure affects platelet function and stroke risk by increasing cofilin phosphorylation.
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Poster

PSTR271: Understanding and Treating Stroke

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR271.04/C41

Topic: E.04. Voluntary Movements

Support: NIH Grant R01NS053606

Title: Error Fields: A personalized three-dimensional robotic motor re-learning treatment

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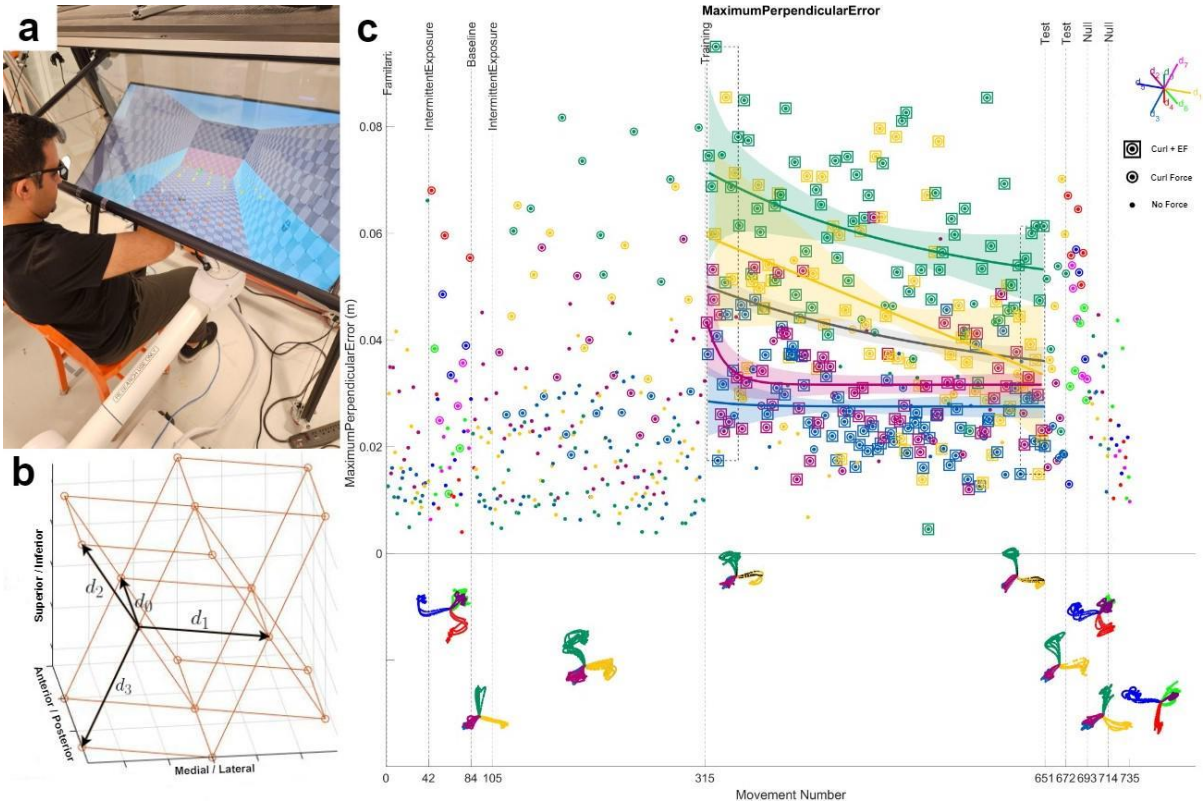
Abstract: Guided upper extremity movement training lacks conclusive benefits in stroke patients (Hornby et al., 2010). Conversely, training techniques that allow errors can enhance learning, adaptation, or neuroplasticity (Abdollahi et al., 2014). Error augmentation (EA), which amplifies error visually or haptically, improves motor relearning across tasks (Lin et al, 2018). However, varying responses necessitate training that is tailored to the individual's error *sensitivity* (Patton et al, 2013). Error Fields (EF) method builds a statistical model of movement errors, then inverts it to design customized therapy, augmenting those errors that are made more often. In a planar reaching experiment(Aghamohammadi et al, 2022), EF showed superior error reduction compared to EA ($p=0.029$) and Controls ($p=0.00008$). However, this planar experiment lacked full real-world 3D actions. We present an immersive 3D training environment that encourages natural arm movement variability with an unobstructed visual display eye-hand coordination experiments and therapy. We paired the burt robot (Barrett Medical) (Townsend et al, 2019) for real-time 3D haptics with our LookinGlass (Patton et al, 2013) augmented reality display (Fig. 1a). Unity software provided a rich, real-time graphical environment with several monocular cues to depth. A single session study on 24 neurologically intact participants, each who randomly received either EF, classic EA, or null treatment. All participants performed 735 targeted reaching movements in 4 possible directions in space, randomly presented (Fig. 1b) subjected to direction-dependent haptic curl force fields which emulated movement challenges similar to a stroke, necessitating learning to compensate. Early results suggest that EF treatment significantly reduced error (Fig. 1c, fitted lines) and that learning was direction-dependent. Our work addresses a significant need for more knowledge on how personalized robotic interventions can enhance motor learning.



Disclosures: N. Aghamohammadi: None. **B. Borghi:** None. **V. Wojcik:** None. **A. Cancrini:** None. **A. Ramirez:** None. **A. Mostofinejad:** None. **C. Celian:** None. **J.L. Patton:** F. Consulting Fees (e.g., advisory boards); Barrett Medical Co..

Poster

PSTR271: Understanding and Treating Stroke

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR271.05/C42

Topic: E.04. Voluntary Movements

Support: NIDILRR Grant 90REGE0017

Title: A Systematic Approach for the Development of a Passive ExoNET for Forearm Supination

Authors: *V. WILSON[1,2], C. OKONMA[1,2], P. RYALI[1,2], C. CELIAN[2], J. L. PATTON[2,1]; [1]Univ. of Illinois at Chicago, Chicago, IL; [2]Shirley Ryan AbilityLab, Chicago, IL

Abstract: Impairments caused by stroke can adversely affect the ability to position and orient the hand, thereby compromising completion of daily tasks. Forearm supination may be

particularly compromised, making it difficult to manipulate or grasp objects. Despite the potential for technology to enhance function, the field of rehabilitation technology has not given equal emphasis to hand orientation as it has to hand location. Practical supination-supporting devices are scarce, particularly those that are suitable for diverse environments, aesthetically pleasing, and user-friendly.
We present a forearm ExoNET: a novel wearable device designed to both assist and improve supination for individuals post stroke and others with movement deficits. The ExoNET uses a network of passive elastic actuators to generate a variety of clinician-specified patterns of multijoint torque. The ExoNET is configurable, both as 1) assistive orthotic facilitating forearm supination and hand positioning for everyday tasks, and 2) as a therapeutic device to strengthen supinator muscles and foster motor coordination. The device can uniquely combine these functions, providing resistance throughout the user's active range of motion and transitioning to assistance upon reaching their movement limit, thus ensuring completion of the full range of supination. A custom-designed tool measures a user's unique torque deficits, determined by measuring static supination torque at several positions, active supination range of motion, and grip strength. We then tailor the arrangement of the elastic actuators to meet each user's needs through the usage of a mathematical optimization model. Each tension element generates a sinusoidal torque profile that functions mathematically as basis functions, which can be linearly combined to achieve any desired torque profile through tuning via optimization.
The graphical user interface of the mathematical model allows clinicians, care partners, and patients to adjust the device's design and structure in real-time. The use of passive elastic actuators ensures the device is lightweight, portable, and safe to operate. Because it is passive, it is low cost and non-intimidating while offering wide functionality, lowering the barrier to access technology that is often seen with complex devices, hence has significant potential to improve patients' quality of life.

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Poster

PSTR271: Understanding and Treating Stroke

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR271.06/C43

Topic: E.04. Voluntary Movements

Support: NIDILRR RERC COMET 90REGE0005-01-00

Title: Cortical patterns associated with grasp and release movements in individuals with stroke: An EEG study

Authors: *K. KINNERK[1], A. MOSTOFINEJAD[2], C. CELIAN[1], D. KIM[3], D. G. KAMPER[4], J. L. PATTON[5];
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Abstract: As the leading cause of serious long-term disability in the United States, stroke can severely hinder upper limb motor function, such as object manipulation. Grasp and release of objects are crucial for successful completion of activities of daily living (e.g., drinking water, grabbing one's cell phone). Despite a plethora of research on novel neurorehabilitation paradigms, the neurological mechanism(s) underlying impaired grasp and release in stroke are not clearly understood. Desynchronization patterns from electroencephalography (EEG) affords possibilities, but it is currently unknown whether release provides a clear signal that may be used in neurorehabilitation. Toward this end, we recorded EEG signals with the Unicorn Hybrid Black headset in 10 individuals with chronic hemiparesis subsequent to stroke. Participants performed a grasp-and-release task of a tennis ball with the paretic and the non-paretic hands. Analyses focused on activity in the sensorimotor cortices in the lesioned and non-lesioned hemispheres (electrodes C3 & C4). Event-related desynchronization (ERD; decreased power in mu and theta bands during movement preparation and execution). During grasp preparation we observed alpha band ERD in the contralesional cortex of the non-paretic hand. In contrast, we observed beta band ERD in both cortices for both hands. Interestingly, average ERD was stronger for the paretic than the non-paretic hands over the ipsilesional cortex, although significance was not reached. Moreover, the release movement of both hands was associated with a stronger beta band synchronization (ERS) over the ipsilesional cortex (the so-called beta rebound). Relationships between stroke-induced motor impairment and ERD/S offer insights into the underlying neural mechanisms and hold promise for informing therapeutic interventions aimed at restoring hand function in stroke survivors (e.g., motor-imagery based BCI systems).

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Poster

PSTR271: Understanding and Treating Stroke

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR271.07/C44

Topic: E.04. Voluntary Movements

Support: NIH grant R01NS 053606

Title: Upper extremity spasticity as perturbation in reaching

Authors: *A. RAMIREZ[1,2], A. CANCRINI[1,2], B. BORGHI[1,2], N. AGHAMOHAMMADI[3,4], J. L. PATTON[3,4], C. CELIAN[3];
[1]Univ. of Illinois Chicago, Chicago, IL; [2]Center for neuroplasticity, Shirley Ryan AbilityLab, Chicago, IL; [3]Ctr. for neuroplasticity, Shirley Ryan AbilityLab, Chicago, IL; [4]University of Illinois Chicago, Chicago, IL

Abstract: In numerous studies exploring novel therapeutic approaches, a neurotypical control group serves as a benchmark to assess performance of participants with impairments. These control groups undergo perturbations aimed at simulating impairments, which participants must adapt to and learn from. These perturbations can range from simple (visual shifts distortion), to more dynamic variations (velocity-dependent curl fields that apply a force proportional to the user's speed). While these perturbations effectively showcase learning and adaptation among neurotypical participants, they often fall short as analogues to the real impairments because they do not represent the underlying neural mechanisms of spasticity and unwanted synergies. Here, we present a robotic perturbation that mimics impairments associated with post-stroke spasticity. By leveraging existing models of upper extremity spasticity in the elbow and shoulder joints, these models generate joint torques based on joint angle and angular velocity, simulating the pre-catch slow stretch region and the fast stretch region seen in spasticity studies. Rather than using this model for assessment, our approach uses them as a perturbation - a force field. We convert the torques to end effector forces using the jacobian and utilize the b.u.r.t. robot (Barrett Medical, Watertown, MA) to apply these forces during a targeted-reaching task.We believe this force serves as a more accurate analog of spasticity and are evaluating the learnability of this perturbation compared to common robot adaptation perturbations, such as velocity-dependent curl field. Simulated spasticity as a perturbation has wide potential to yield deeper insights into the mechanisms of spasticity, enhancing our understanding of therapy effectiveness and demonstrate what spasticity might feel like to patients' families and training clinicians.

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Poster

PSTR271: Understanding and Treating Stroke

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR271.08/C45

Topic: E.04. Voluntary Movements

Support: NIH Grant R01NS053606

Title: Synthetic data for identification of sensory-motor control in reaching

Authors: *A. CANCRINI[1,2], N. AGHAMOHAMMADI[2,1], B. BORGHI[2,1], A. RAMIREZ[1,2], C. CELIAN[2], J. L. PATTON[2,1];
[1]Univ. of Illinois Chicago, Chicago, IL; [2]Shirley Ryan AbilityLab, Chicago, IL

Abstract: Sensory inputs such as vision, proprioception, and touch critically influence post-stroke recovery (*Ginsburg and Willard, Translational Research, 2009; Hamburg and Collins, New England Journal of Medicine, 2010; Schork, Nature, 2015*). A comprehensive assessment of a patient's sensory contributions is crucial for initiating effective, personalized therapy. Enhanced sensory augmentation, tailored to the individual, can substantially improve learning outcomes and decrease errors. We present a mathematical model to understand the identification process and address sensory roles in training environments, potentially leading to advanced motor control strategies and effective movement task execution. Our long-term objective is to characterize the contribution of individual sensory modalities in motor learning.
Here, we employ synthetic data in simulo to first create known data, then use it to understand the mathematical inversion methods seen in system identification. We simulate techniques such as shifted and blanked vision and haptic distortions introduced by the robot to excite the system. Synthetic dynamic simulations incorporate muscular, visual, and proprioceptive components. Then, we invert to identify important model parameters such as sensory feedback gains. Our efforts show that we can refine our identification methods by testing various sensory perturbations and tasks that can best identify the model parameters. Here we determine the most effective family of disturbances and motions that should be applied. These strategies are designed to be empirically reproducible with perturbations that are practically applicable. Ultimately, the insights gained from this research will provide valuable principles and guidelines for optimizing sensory stimuli to improve precision neurorehabilitation.

Disclosures: A. Cancrini: None. **N. Aghamohammadi:** None. **B. Borghi:** None. **A. Ramirez:** None. **C. Celian:** None. **J.L. Patton:** F. Consulting Fees (e.g., advisory boards); Barrett Medical.

Poster

PSTR271: Understanding and Treating Stroke

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR271.09/C46

Topic: E.04. Voluntary Movements

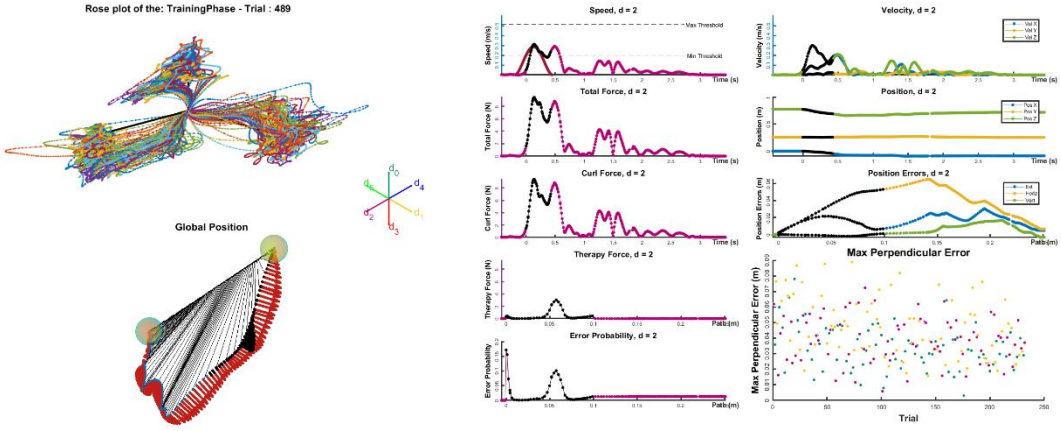
Support: NIH Grant R01NS053606

Title: Personalized robotic training on a planar reaching task with simulated stroke

Authors: *B. BORGHI[1,3], N. AGHAMOHAMMADI[5,2], A. RAMIREZ[2,4], C. CELIAN[6], J. L. PATTON[7,2], A. CANCRINI[2], V. WOJCIK[8,2], A. MOSTOFINEJAD[9];

[1]Richard and Loan Hill Dept. of Biomed. Engin., [2]Univ. of Illinois Chicago, Chicago, IL; [3]Arms and Hands, [4]Shirley Ryan Abilitylab, Chicago, IL; [5]Richard and Loan Hill Bioengineering Dept., [6]Shirley Ryan AbilityLab, Chicago, IL; [7]bioengineering (UIC); Ctr. for neuroplasticity (SRALab), Shirley Ryan AbilityLab, Winnetka, IL; [8]Shirley Ryan AbilityLab, Hanover Park, IL; [9]Univ. of Toronto, Newmarket, ON, Canada

Abstract: Personalized Robotic Training on a Planar Reaching Task withSimulated Stroke Bruno Borghi[12], Naveed Reza Aghamohammadi[12], Victoria Wojcik[1,2] Adriana Cancrini[12], ArturoRamirez[12], Courtney Celian[2], Mohammad Amin Mostofinejad[3], James L. Patton[121] *Universityof IllinoisChicago, Chicago, IL*[2] *ShirleyRyan AbilityLab, Chicago, IL*[3] *Universityof Toronto, Toronto, ON, Canada* Based on recent advancements inneuro-adaptivecontrol duringreaching[1],we evaluateda novel algorithmfor generatingcustomizedtrainingforces called *ErrorFields*(EF),appliedby a roboticarm system *burt*(BarrettMedical)interfacedwithour augmented realitysystem*Looking Glass*. EF Was a personalizedforce, proportionalto the instantaneoustendency oferror, across 560 movements in3 possiblemovement directions.The aimof thisprojectistoexplore motor learningof robot-generated “curl”force fields.Learningwas enhanced by ourcustom Error Fields.We hypothesizedthat participantswho receivedcustomizedEF wouldshow a higherand faster decrease inerror across trialscompared to participantswho receivedonly the curl force field.In a trainingperiodwithrobot-generated forces, trajectoriesunderwent modificationsdue to internalmodel adaptationof the neuro-muscular system,leadingto improvedperformance measured interms of decreased positionerror. These resultsshowcase the potentialof such error-enhancingtechniquesto facilitatemotor improvement,individualizedfor activitiesand neurorehabilitationpurposes.Figure1: Representationof the data collected duringthe late-trainingphase from one subjectperformingthe experimentwiththe robot.[1]J. L. PattonandF. A. Mussa-Ivaldi, “Robot-assistedadaptivetraining: custom forcefields for teaching movementpatterns,”IEEETransactions onBiomedical Engineering, vol. 51, no. 4, p. 636–646,2004Supported by NIH grant R01 NS 053606



Disclosures: B. Borghi: None. **N. Aghamohammadi:** None. **A. Ramirez:** None. **C. Celian:** None. **J.L. Patton:** F. Consulting Fees (e.g., advisory boards); Barrett Medical Co.. **A. Cancrini:** None. **V. Wojcik:** None. **A. Mostofinejad:** None.

Poster

PSTR271: Understanding and Treating Stroke

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR271.10/C47

Topic: E.04. Voluntary Movements

Support: NIH Grant 2R15HD093086

Title: Three-dimensional vibrotactile feedback training promotes reach-to-grasp improvement after stroke: a proof-of-concept case series

Authors: *R. N. MAZOROW[1], R. K. RAYES[2], M. E. GUERRERO[1], J. FLORES[1], K. D. BASSINDALE[1], R. A. SCHEIDT[1];
[1]Biomed. Engin., Marquette Univ. & Med. Col. of Wisconsin, Milwaukee, WI; [2]Med. Sch., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: About half of stroke survivors experience decreased sensation of limb position and movement, leading to a decrease in upper extremity motor control. Daily tasks are complicated by this diminution of hand position sense and subsequent attenuation of reach feedback. Although visual feedback of the arm and hand can partly compensate, this reliance results in slow, jerky movements and reduction in awareness of the surrounding environment. An emerging body of literature suggests that supplemental vibrotactile feedback (VTF) may provide a useful compensatory aide via sensory augmentation of impaired proprioception. This case series examines the acquisition of 3-dimensional VTF for stroke survivors with extended training. We hypothesize that 1) survivors of stroke can learn to utilize 3-dimensional VTF post-training without concurrent vision, yielding improvements from baseline to final testing in reaching accuracy with VTF, and 2) VTF will have greater accuracy than proprioception alone post-training. To test these hypotheses, we are recruiting survivors of stroke greater than three months post-stroke, with Upper Extremity Fugl-Meyer (UE-FM) scores between 15 and 60 (inclusive), and with preserved tactile sensation in the ipsilesional arm. Participants with diseases interfering with neuromuscular function or fixed contractures of the contralesional limb are excluded. Participants undergo 9 hours of training with 3-dimensional VTF about hand position during reach-to-grasp actions performed with their contralesional arm. VTF is applied to the stationary ipsilesional arm. Kinesthetic testing occurs before training and after every subsequent 3 hours of training. Kinematic data are analyzed to determine the average target capture error. Paired t-tests between the proprioception and VTF test tasks are run within and between test sessions. To accommodate the unique workspace size for each participant, comparisons of error are performed in units of percent workspace. Preliminary results with one participant (53-year-old female, 8 years post-stroke, UE-FM score=60) showed that 9 hours of training with VTF reduced target capture error by half (Baseline: 82.93%; Final: 41.51%), supporting our first

hypothesis. However, final testing showed less reach error with proprioception alone (34.13%) compared to VTF, not supporting our second hypothesis. These results demonstrate that 3-dimensional VTF can be learned by survivors of stroke and may be a viable long-term solution for kinesthetic augmentation. Ultimately, this study will determine the extent to which vibrotactile training may help mitigate sensorimotor deficits post-stroke.

Disclosures: R.N. Mazorow: None. **R.K. Rayes:** None. **M.E. Guerrero:** None. **J. Flores:** None. **K.D. Bassindale:** None. **R.A. Scheidt:** None.

Poster

PSTR271: Understanding and Treating Stroke

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR271.11/C48

Topic: C.08. Ischemia

Support: LundbeckFonden pre-graduate Scholarstipend

Title: Establishing a large animal model of focal stroke in the motor cortex of pigs

Authors: *V. H. KUANG[1], D. ORLOWSKI[1], L. M. FITTING[1], K. R. DRASBEK[2], B. HANSEN[2], C. SKOVEN[2], J. H. SORENSEN[1];
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Abstract: Stroke is a leading cause of death and long-term disability globally. Within stroke patients, ischemic stroke constitutes 85-90% of all cases. Yet, post-ischemia response mechanisms remain understudied. Animal models are a promising avenue to bridge this gap. However, despite the high number of ischemic stroke models in rodents, the high rate of failed human clinical trials suggests a low translational value. We have done the initial work to establish a Rose Bengal photothrombotic model in pigs. Two postmortem heads from female Danish Landrace pigs, aged 6 months and weighing up to 60 kg, were used for a microanatomical exploration of the surgical access to the pig motor cortex. As in the Goettingen minipig, we found that the motor cortex occupies the dorsoposterior part of the frontal lobe, adjacent to the transition to the somatosensory cortex of the parietal lobe, and frontal to the visual cortex of the occipital lobe. Our postmortem experiment confirms the feasibility of surgical access to the M1 cortex and the installation of equipment for light exposure needed for Rose Bengal photoactivation. This includes a 3D-printed ring mounted in the cranial burr hole to control the light beam for photothrombosis. We hypothesize that a Rose Bengal lesion in the motor cortex of pigs will result in motor behavioral changes corresponding to the somatotopic representation lesioned. Expected changes include impairments in spatiotemporal gait parameters

and limb paresis. We expect the procedure to produce both vascular and cytotoxic edemas, and the mean lesion volume to be around 1 cm^3. We predict that an MRI brain scan will reveal decreased ipsilateral diffusivity, increased kurtosis, and reduced white matter integrity which will be confirmed by histology. A reliable large-animal Rose Bengal model of stroke can help test the efficacy and safety of therapeutic interventions. Moreover, a controlled lesion in the motor cortex can help explore the detailed pig motor cortex anatomy and the relationship to motor function in order to elucidate if a 'pig homunculus' or a 'porculus' exists.

Disclosures: V.H. Kuang: None. **D. Orlowski:** None. **L.M. Fitting:** None. **K.R. Drasbek:** None. **B. Hansen:** None. **C. Skoven:** None. **J.H. Sorensen:** None.

Poster

PSTR271: Understanding and Treating Stroke

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR271.12/C49

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH R01

Title: Changes in Dopamine Modulation Under Global Versus Focal Ischemia

Authors: *C. E. WITT[1], A. E. ROSS[2];
[1]Univ. of Cincinnati, Cincinnati, OH; [2]Chem., Univ. of Cincinnati, Cincinnati, OH

Abstract: Strokes (ischemic and hemorrhagic) are the 5th leading cause of death in United States thus leading to a national health burden. Volumes of work have gone into understanding the behavior of neurochemicals during global ischemic events; however, there is a large shift within the neuroscience community to study these events at the site of injury (focal ischemia). Focal ischemia is of particular interest because of its confounding effects in localized regions. Over the past 5 years, the Ross lab has worked on crafting microfluidic platforms to study localized and sustained ischemic events in tissue. In this work, these technologies were used to compare standard tissue interrogation methods for analysis (i.e. perfusion chamber for ex vivo work) to unravel how dopamine (DA) dysregulation is handled in the brain. This work focuses on the CA1 region of the hippocampus due to the high levels of glutamate and DA that accumulate in the extracellular space during ischemic events. Herein, signaling patterns of dopamine were investigated under both ischemic conditions using fast-scan cyclic voltammetry (FSCV). Transient DA was monitored in hippocampal slices for 45 minutes and the amount released, extracellular duration, and event frequency were analyzed. Overall, this work goes to establish baseline signaling profiles for DA in healthy CA1 regions of hippocampi while also uncovering the nuanced changes in DA response to global vs focal ischemia.

Disclosures: C.E. Witt: None. **A.E. Ross:** None.

Poster

PSTR272: Brain Injury and Therapeutic Strategies

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR272.01/C50

Topic: C.10. Brain Injury and Trauma

Support: Foundation "Gueules Cassées" [grant numbers 70-2019, 23-2019, 24-2020, 24-2021, 55-2021, and 22-2022]
National Research Agency (ANR) [Grant number ANR-19-ASTR-0027]

Title: Biocompatibility and regenerative potential assessment of 3D bioprintable biomaterials for brain regenerative implants

Authors: *M. COMBEAU[1], J. CLAUZEL[1], N. COLITTI[1], L. ROBERT[1], A. BRILHAULT[1], F. DESMOULIN[1], M. PANRY[2], I. RAYMOND LETRON[2], M. L. BECKER[3], J. FITREMANN[4], S. BLANQUER[5], C. CIRILLO[1], I. LOUBINOUX[1];
[1]ToNIC, Toulouse NeuroImaging Ctr., Univ. de Toulouse, Inserm, UPS, Toulouse - Cedex 3, France; [2]LabHPEC, Lab. d'HistoPathologie Experimentale et Comparée, Univ. de Toulouse, ENVT, Toulouse, France; [3]Departments of Chem., Mechanical Engin. and Materials Sci., Biomed. Engin. and Orthopaedic Surgery, Duke Univ., Durham, NC; [4]Lab. des IMRCP, CNRS UMR 5623, Univ. of Toulouse-Paul Sabatier, Toulouse, France; [5]CNRS researcher, Inst. Charles Gerhardt of Montpellier (ICGM), Montpellier, France

Abstract: In adult brain, neural cell regeneration is limited, and areas of high neural loss associated with stroke or head injury generally do not fully recover on their own. To address this limitation, innovative implantable scaffolds designed to enhance brain plasticity have attracted considerable interest. Bioprinting enables adaptation of these scaffolds to the brain's complexity, offering new prospects for tissue and functional recovery after acute brain injury. In this feasibility study, we evaluated the in vivo biocompatibility of five distinct bioeliminable or bioresorbable materials: Gelatin methacrylate (GelMA), Polyethylene glycol diacrylate (PEGDA) mixed with GelMA (PEGDA-GelMA), Poly trimethyl carbonate methacrylate (PTMC-MA), Poly Propylene Fumarate (PPF), and Polydioxanone Ethicon (PDSII) + N-nonyl-D-galactonamide hydrogel. Our in vivo evaluations were complemented by a one-month behavioral and MRI follow-up, confirming the materials safety. High-resolution T2 MRI imaging effectively captured scaffold structures and demonstrated its non-invasive utility in monitoring degradability. In this study, PDSII commonly used for human brain sutures, provided acceptable control of the cerebral inflammatory response to implantable foreign bodies. PDSII and PPF generated minimal fibro-inflammatory barriers and appeared neutral to brain tissue, neither pro-inflammatory nor pro-regenerative. No significant foreign body reactions were

observed in response to implantation of either biomaterial. They could serve well as suture or filling materials. PTMC-MA, GelMA, and PEGDA-GelMA did not induce a high fibro-inflammatory response, but provided a migration substrate for endogenous cells. These 3D implants experienced significant cell colonisation and neovascularisation. However, among this 3 biomaterials, GelMA and PTMC present poor brain integration or rise technical problems. GelMA allows cell migration to the implant periphery but not until the core, so the core is not colonized. PTMC-MA is easy to insert because more rigid, but its rigidity poses technical problems for histology. Retesting PTMC with reduced photopolymerization or mix with other materials to increase its softness could be worthwhile. The PEGDA-GelMA composite emerges as an ideal candidate for intracerebral implantation. Alongside its biophysical and bioprinting qualities, it facilitates the permissive glial barrier creation, induces neovascularization, and attracts neuronal progenitors. Collectively, these findings position PEGDA-GelMA as a convincing biomaterial option for treating severe brain lesions, paving the way for effective therapies.

Disclosures: M. Combeau: None. **J. Clauzel:** None. **N. Colitti:** None. **L. Robert:** None. **A. Brilhault:** None. **F. Desmoulin:** None. **M. Panry:** None. **I. Raymond Letron:** None. **M.L. Becker:** None. **J. Fitremann:** None. **S. Blanquer:** None. **C. Cirillo:** None. **I. Loubinoux:** None.

Poster

PSTR272: Brain Injury and Therapeutic Strategies

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR272.02/C51

Topic: C.10. Brain Injury and Trauma

Support: DGAPA

Title: The antidepressant effect of Mucuna pruriens is related to decreased nitrite and nitrate levels in a rat model of mild traumatic brain injury

Authors: *A. MATA-BERMUDEZ[1], L. NAVARRO[2];
[1]Univ. Autónoma Metropolitana, México, Mexico; [2]Fisiologia, UNAM, Cd. Mx. 04510, Mexico

Abstract: Depression is a mood disorder characterized by persistent negative thoughts and sadness. In patients with traumatic brain injury (TBI), even mild TBI (mTBI), it is one of the most frequent affectations and challenging to treat due to the multiple mechanisms involved in its pathophysiology, including increased nitric oxide (NO) levels. There are no safe and effective pharmacological strategies to treat these disorders, so in this study, we evaluated the therapeutic efficacy of Mucuna pruriens (MP), which has been shown to have neuroprotective properties that regulate damage mechanisms such as the inflammatory response and nitric oxide synthase

activity. Male Wistar rats that underwent mTBI or sham surgery were used. Immediately after mTBI, they were treated with vehicle or MP extract (50 mg/kg ip/day for five days). The preference for sucrose consumption assessed depression-like behavior. In addition, the levels of nitrites and nitrates in the cerebral cortex, striatum, midbrain, and nucleus accumbens were analyzed. Animals with mTBI showed a lower preference for sucrose consumption, whereas those treated with MP preferred sucrose, similar to sham animals. In addition, we observed that mTBI increased nitrite and nitrate levels in different brain regions; this increase was not observed in mTBI animals treated with MP. We can conclude that MP effectively reduces the behavior associated with depression and brain NO levels in rats with mTBI.

Disclosures: A. Mata-Bermudez: None. **L. Navarro:** None.

Poster

PSTR272: Brain Injury and Therapeutic Strategies

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR272.03/C52

Topic: C.10. Brain Injury and Trauma

Support: R21NS114723

Title: Intranasal delivery of KAFAK, a cell-penetrating therapeutic peptide reduces inflammation and improves neurologic function after moderate TBI

Authors: Y. YANAMADALA[1], R. ROY[1], A. A. WILLIAMS[1], N. UPPU[1], A. Y. KIM[2], M. A. DECOSTER[1], P. KIM[2], ***T. A. MURRAY**[1];
[1]Ctr. for Biomed. Engin. and Rehabil. Sci., Louisiana Tech. Univ., Ruston, LA; [2]Dept. of Biol. Sci., Grambling State Univ., Grambling, LA

Abstract: Following traumatic brain injury (TBI), secondary brain damage due to chronic inflammation is the most predominant cause of the delayed onset of mood and memory disorders. Currently no therapeutic approach is available to effectively mitigate secondary brain injury after TBI. One reason is the blood-brain barrier (BBB), which prevents passage of most therapeutic agents into the brain. Peptides have been among the leading candidates for CNS therapy due to their low immunogenicity and toxicity, bioavailability, and ease of modification. In this study, we demonstrated that non-invasive intranasal administration of KAFAK, a cell penetrating anti-inflammatory peptide, traversed the BBB in a murine model of diffuse, moderate TBI. Notably, KAFAK treatment reduced the production of pro-inflammatory cytokines that contribute to secondary injury. Furthermore, behavioral tests showed improved or restored neurological, memory, and locomotor performance in KAFAK-treated mice. This study demonstrates KAFAK's ability to cross the blood-brain barrier, to lower pro-inflammatory cytokine levels in vivo, and to restore function after a moderate TBI.

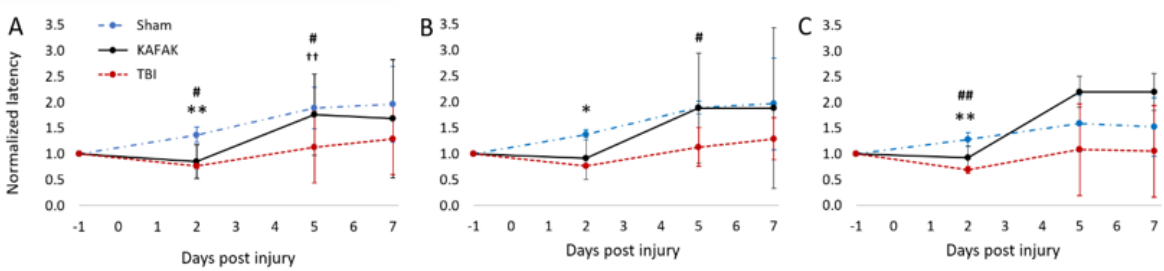

Figure 4. Mean normalized rotarod scores on Day 2, 5 and 7 for TBI-vehicle (TBI), Sham-vehicle (Sham) and TBI-KAFAK (KAFAK) groups. (A) shows the performance for all mice, while (B) and (C) represent the performance of female and male mice, respectively. (Mean ± SD, n = 8 for each group in A and n = 4 for each group in B and C; * $p < 0.05$ Sham versus KAFAK and TBI, ** $p < 0.01$ Sham versus KAFAK and TBI, # $p < 0.05$ TBI versus KAFAK, ## $p < 0.01$ TBI versus KAFAK, †† $p < 0.01$ Sham versus TBI).

Disclosures: Y. Yanamadala: None. **R. Roy:** None. **A.A. Williams:** None. **N. Uppu:** None. **A.Y. Kim:** None. **M.A. DeCoster:** None. **P. Kim:** None. **T.A. Murray:** None.

Poster

PSTR272: Brain Injury and Therapeutic Strategies

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR272.04/C53

Topic: C.10. Brain Injury and Trauma

Title: Psilocybin ameliorates chronic neurobehavioral deficits and alterations in 5-HT_{2A} receptor density in a rat model of traumatic brain injury

Authors: *S. SHULTZ;
Monash Univ., Melbourne, Australia

Abstract: Traumatic brain injury (TBI) often results in persistent neurological consequences and an increased susceptibility to psychiatric disorders. These issues are associated with alterations in neuroplasticity, neuroinflammation, and serotonergic neurotransmission. There are currently no effective pharmacotherapies available for widespread use that improve chronic TBI recovery. Psilocybin targets several serotonin receptors, including the 5-HT_{2A} receptor, and has emerged as a promising candidate due to its rapid neuroplastic, anti-inflammatory, antidepressant, anxiolytic, and pro-cognitive effects. Therefore, this study investigated whether psilocybin can mitigate enduring behavioral and neurobiological deficits induced by TBI. Rats were administered a TBI (i.e., fluid-percussion injury) or a sham injury. After a 1-year recovery period, rats received an intraperitoneal injection of either psilocybin (1 mg/kg) or saline-control. Behavioral outcomes were assessed beginning 24h after treatment. Afterwards, positron emission tomography scans

were conducted to evaluate the effects of TBI and psilocybin on 5-HT_{2A} receptor binding. The rats were then perfused for immunohistochemical analyses of brain tissue. TBI induced chronic behavioral and cognitive impairments, concomitant with reduced 5-HT_{2A} receptor binding and altered neuronal and microglial morphology. Furthermore, social deficits and anxiety correlated with 5-HT_{2A} availability in the prefrontal cortex and amygdala, respectively. The TBI-induced alterations were largely recovered by psilocybin and was associated with an enhancement of neuroplasticity and anti-inflammatory properties. These findings support the notion that psilocybin may provide a promising and clinically translatable intervention that could improve chronic TBI outcomes.

Disclosures: S. Shultz: None.

Poster

PSTR272: Brain Injury and Therapeutic Strategies

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR272.05/C54

Topic: C.10. Brain Injury and Trauma

Title: Electrophysiological profiles of the central thalamus reveal potential of recovery in disorders of consciousness

Authors: *H. ZHANG[1,2,3], Q. GE[4], X. LIU[5], Y. DANG[6], L. XU[4], Y. ZHUANG[4], S. WU[5], S. LAUREYS[7,8,9], J. HE[4], S. YU[1,2,3];
[1]Chinese Acad. of Sci. Inst. of Automation, Beijing, China; [2]Sch. of Future Technol., Univ. of Chinese Acad. of Sci., Beijing, China; [3]State Key Lab. of Brain Cognition & Brain-inspired Intelligence, CAS Ctr. for Excellence in Brain Sci. and Intelligence Technology, Chinese Acad. of Sci., Shanghai, China; [4]Dept. of Neurosurg., Beijing Tiantan Hospital, Capital Med. Univ., Beijing, China; [5]Sch. of Psychological and Cognitive Sciences, IDG/McGovern Inst. for Brain Res., Peking Univ., Beijing, China; [6]Dept. of Neurosurg., The First Med. Ctr. of Chinese PLA Gen. Hosp., Beijing, China; [7]CERVO Brain Res. Ctr., Laval Univ., Quebec City, QC, Canada; [8]Liège Univ. Hosp., Liege, Belgium; [9]Hangzhou Normal Univ., Hangzhou, China

Abstract: Disorders of consciousness (DoC), a spectral of states with prolonged altered consciousness due to damages of the brain, are usually associated with prolonged bedridden status and low spontaneous recovery rates. To reveal the neural mechanisms underlying consciousness recovery in DoC is therefore vital. The central thalamus (CT) is essential to arousal regulation, and deep brain stimulation (DBS) targeting CT has been suggested as a promising option for treating DoC. However, the specific role of CT in consciousness recovery remains elusive. Here we identify a core set of electrophysiological features of the multiunit activity (MUA) with machine learning, including the theta band stability (stab-θ), gamma band synchronization (sync-γ), theta band power (pow-θ), and high-gamma band stability (stab-hγ), in

CT in a DoC patient cohort [n=23, including those with minimally conscious state minus (MCS-) and unresponsive wakefulness syndrome/vegetative state (UWS/VS)] before receiving CT-DBS treatment. We found that this set of CT features accurately predicted individual recovery for all patients involved. Patients exhibiting recovery after CT-DBS treatment tended to have higher value for multiple features in this set before the treatment. In addition, higher percentage of traumatic patients exhibited more promising CT state compared to anoxic patients, and the same was true for younger patients compared to older patients, indicating the electrophysiological profile of CT may account for the diverse effects of clinical factors such as etiology and age on DoC recovery. Importantly, within the patients that eventually showing recovery, using the CT state we could further identify two subgroups, with characteristic high pow-θ and high stab-θ, corresponding to the patients showing favorable and less favorable behavioral scores before the treatment, respectively. The latter was sporadically observed in clinics but has not been characterized using electrophysiological features before. Last, through a biophysical model we show that these two sub-groups may indicate distinct dynamics of recovery involving CT. Taken together, our results provide strong evidence that the status of CT plays a pivotal role in DoC recovery and reveal the electrophysiological underpinnings of this process, paving the way to develop more precise CT-based therapies customized for individual DoC patients.

Disclosures: H. Zhang: None. **Q. Ge:** None. **X. Liu:** None. **Y. Dang:** None. **L. Xu:** None. **Y. zhuang:** None. **S. Wu:** None. **S. Laureys:** None. **J. He:** None. **S. Yu:** None.

Poster

PSTR272: Brain Injury and Therapeutic Strategies

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR272.06/C55

Topic: C.10. Brain Injury and Trauma

Support: NIAAA R01AA025380

Title: Transcriptome analysis of rat hippocampus following weekly binge alcohol and voluntary exercise.

Authors: T. ANDRE[1], E. MEHRHOFF[2], M. A. EHRINGER[2], *J. LEASURE[1];
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Abstract: Binge alcohol exposure, a pattern of drinking that rapidly raises the blood alcohol level to .08g/dl or above, is associated with neurodegeneration in corticolimbic regions in both humans and animal models. Using a rat model of binge exposure in which animals are given a single weekly dose of alcohol, we have found significant cell loss in the hippocampus and widespread neuroimmune activation. In contrast to the damaging effects of binge alcohol,

exercise enhances brain health and function. We have previously shown that exercised rats exposed once weekly to binge alcohol show no hippocampal cell loss and limited neuroimmune activation. The protective underlying mechanisms are unclear. We therefore conducted a transcriptome analysis on the hippocampus following exposure to binge alcohol and/or exercise. Male and female rats were gavaged once weekly for 5 weeks with 5 g/kg ethanol or isocaloric control solution. To assess exercise effects, half of the animals had access to running wheels 4 d/wk and were euthanized immediately following their final exercise session/~16 hours after the last binge exposure. The hippocampus was microdissected and total RNA extracted and submitted for sequencing. Differential expression analysis was initially performed using the main effects of sex, binge alcohol, exercise and their interactions. Very few genes were differentially expressed based on sex, so subsequent analyses collapsed across sexes. Transcription of 806 genes was altered by exercise, but only 7 by binge alcohol exposure. Only 1 gene, Zfp62, showed evidence for an interaction effect in the combined binge/exercise condition. Gene ontology (GO) analysis identified pathways associated with receptor signaling, namely protein kinase signaling, as differentially impacted by exercise. Most of the genes differentially impacted by alcohol were transcription factors. These differential expression findings were reflected in a weighted gene co-expression network analysis (WGCNA). Ongoing efforts are aimed at deconvolution of bulk transcriptomes to a single cell level, to identify gene expression changes in specific cell populations. The small number of genes impacted by binge alcohol is in stark contrast to the large number impacted by exercise and may be related to the timing of tissue harvest being closer to exercise than binge. Nonetheless, this indicates that the transcriptomic effects of exercise outweigh those of binge alcohol, however the lack of interaction effects seen in the combined binge/exercise condition suggests the transcriptomic effects identified in this study do not account for the neuroprotective effects detected in earlier studies.

Disclosures: T. Andre: None. **E. Mehrhoff:** None. **M.A. Ehringer:** None. **J. Leasure:** None.

Poster

PSTR272: Brain Injury and Therapeutic Strategies

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR272.07/C56

Topic: C.10. Brain Injury and Trauma

Support: NIH R01 NS 129573

Title: Targeting noradrenergic tone to compensate the glymphatic dysfunction in post-traumatic headache

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Abstract: Background. Mild traumatic brain injury (mTBI) is a common and complex head injury, affecting 69 million people worldwide. This condition results in many symptoms, including migraine-like post-traumatic headaches (PTH). The mechanisms underlying these headaches are still unknown. A clue to PTH may be the shared clinical associations between mTBI, migraine, and sleep-wake disruption (SWD). Indeed, sleep disruption, which is common after mTBI, is also a migraine trigger. Instead, sleep is known as migraine abortive. In this setting, sleep disruption may impair the glymphatic system, a recently characterized brain-wide network of perivascular spaces that supports the rapid exchange of cerebrospinal and interstitial fluid. Glymphatic function is most rapid during sleep, while it is inhibited during wakefulness by central noradrenergic tone and is reduced by mTBI. Therefore, our hypothesis is that the disruption of glymphatic function after mTBI may contribute to the trigger and endurance of PTH symptoms. **Methods.** Repetitive impact mTBI mouse models have been used for developing PTH phenotypes. Afterwards, the mice were treated with the α1-adrenergic antagonist prazosin. Their PTH symptoms, induced by the injection of a sub-threshold calcitonin gene-related peptide (CGRP) dose, the main migraine mediator, were assessed through light aversion and mechanical facial allodynia tests. Moreover, glymphatic system impairment was measured via glymphatic CSF tracer influx compared to sham control mice.**Results.** Our preliminary data demonstrate that treatment with the centrally active α1-adrenergic antagonist prazosin prevents and treats CGRP sensitivity in mice, with a faster effect in males. Furthermore, prazosin administration in mice increases glymphatic CSF tracer influx compared to vehicle-treated mice. **Conclusion.** These results suggest noradrenergic tone as novel target to improve PTH symptoms and glymphatic function, potentially improving sleep disruption as therapeutic strategies for mTBI patients.

Disclosures: A. Della Pietra: None. **A. Kuburas:** None. **E. Partridge:** None. **M. Sevao:** None. **J.D. Cho:** None. **A.G. Schindler:** None. **M.A. Raskind:** None. **J.J. Iliff:** None. **A.F. Russo:** None.

Poster

PSTR272: Brain Injury and Therapeutic Strategies

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR272.08/C57

Topic: C.10. Brain Injury and Trauma

Support: VA Merit Review Grant I01BX004344
VA Research Career Scientist Award IK6BX005690
Department of Neurological Surgery, University of Wisconsin-Madison

Title: An antioxidant and anti-ER stress combo therapy after TBI elevated α-Synuclein S129 phosphorylation and alleviated PD-like pathology in a sex-specific manner

Authors: *C. KOZHIKKADAN DAVIS[1], S. BATHULA[1], S. JEONG[1,2], V. K. ARRURI[1], S. SUBRAMANIAN[1], J. CHOI[1], C. OSTROM[1], R. VEMUGANTI[1,3,2];
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Abstract: Traumatic brain injury (TBI) elevates the propensity of Parkinson's disease (PD) later in life. Oxidative stress, endoplasmic reticulum (ER) stress, and neuroinflammation are shown to promote aggregation of proteins leading to neurodegeneration in preclinical studies. We presently targeted oxidative stress and ER stress following a moderate controlled cortical impact-induced TBI in mice of both sexes, using a combination of apocynin, tert-butylhydroquinone, and salubrinal, to understand if abating these pathological events curtails TBI-induced α-Synuclein (α-Syn) aggregation and dopaminergic neuron loss and improve motor and cognitive function. Compared to the vehicle treatment, the combo therapy elevated the level of phosphorylation at serine 129 (pS129) of α-Syn in the pericontusional cortex of male mice at 3 days post-TBI. Motor and cognitive deficits induced by TBI lasted at least 3 months and the combo therapy curtailed these deficits in both sexes. At 3 months post-TBI, male mice given combo therapy exhibited significantly lesser α-Syn aggregates in the substantia nigra (SN) and higher TH^+ cells in the SN pars compacta, compared to vehicle control. However, the aggregate number was not significantly different between groups of female mice. Moreover, TBI-induced loss of TH^+ cells was negligible in female mice irrespective of treatment. Thus, the present study indicates that mitigation of TBI-induced oxidative stress and ER stress at the acute stage could potentially reduce the risk of post-TBI PD-like pathology at least in male mice, plausibly by elevating pS129-α-Syn level.

Disclosures: C. Kozhikkadan Davis: None. **S. Bathula:** None. **S. Jeong:** None. **V.K. Arruri:** None. **S. Subramanian:** None. **J. Choi:** None. **C. Ostrom:** None. **R. Vemuganti:** None.

Poster

PSTR272: Brain Injury and Therapeutic Strategies

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR272.09/C58

Topic: C.10. Brain Injury and Trauma

Title: Prophylactic and therapeutic beta-hydroxybutyrate improved motor dysfunction and cortical damage in a mouse model of traumatic brain injury

Authors: E. OLIVER[1], **C. W. BIRD**[2], L. E. HOOD[2], J. SHUSTERMAN[2], N. M. MEINERZ[2], *C. M. BUTT[2];
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Abstract: Over 1.7 million traumatic brain injuries (TBIs) are documented yearly in the United States alone because of their association with hospitalization. This figure likely underestimates the true number of TBIs because many “mild” injuries go unreported. However, lower-grade TBIs can still have negative effects on brain health, including motor coordination, cognition, tissue damage, and post-concussive syndrome, and few therapies exist for reducing these effects. Beta-hydroxybutyrate (BHB) is one potential approach as it may serve as an energy source when glycolysis is disrupted during the acute phase after TBI. After a time-course study determined optimal tissue levels of BHB, a closed-head, weight-drop model (~125N at impact) induced TBI in C57BL/6J mice (N=20/arm). Animals were then assessed in the beam walk test for motor function at post-injury days 1 and 3 (N=10-12/arm). In comparison to shams, vehicle-treated TBI controls had significantly more slips in the beam walk, while animals given intranasal BHB (50 mg/kg) either 30 minutes before injury (pre-), directly after injury (post-), or both before and after injury (pre+post) had significantly fewer slips than TBI controls. Pre+post animals provided a higher dose of BHB (100 mg/kg; 2x pre+post) were equivalent to shams in the beam walk. All post-injury treatments continued daily until the cerebral cortex was harvested at 4 hours, 24 hours, and 14 days after injury to determine levels of ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) as a measure of damage (N=5/arm/timepoint). No major group differences in UCH-L1 expression were detected at 4 hours. However, at both 24 hours and 14 days post-injury, the vehicle-treated TBI controls had significantly higher UCH-L1 than all other groups, and all BHB-treated animals had UCH-L1 levels that were equivalent to those observed in uninjured shams. These findings suggest that intranasal BHB may mitigate the negative sequelae of TBI with a wide therapeutic window.

Disclosures: E. Oliver: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CEO, 2508 Biosciences. **C.W. Bird:** None. **L.E. Hood:** None. **J. Shusterman:** None. **N.M. Meinerz:** None. **C.M. Butt:** None.

Poster

PSTR272: Brain Injury and Therapeutic Strategies

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR272.10/C59

Topic: C.10. Brain Injury and Trauma

Support: 1K22NS125179

Title: Brain Injury Knowledge Ontology Knowledge base (BIKObase): Formalizing traumatic brain injury (TBI) knowledge

Authors: *M. SURLES-ZEIGLER[1], F. T. IMAM[2], T. GILLESPIE[3], C. DIXON[4], J. S. GRETHE[5], A. R. FERGUSON[6], M. E. MARTONE[7];
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Abstract: While traumatic brain injury (TBI) is a major cause of morbidity and mortality, no effective clinical therapeutics currently exist for this injury. Several therapies and procedures have been deemed successful in preclinical TBI research but have not been translated to human patients. A major hurdle in translating the knowledge gained from the preclinical studies to the clinic is the difficulty in interpreting preclinical studies. One reason for this difficulty is the methodological variance between and among published research studies. This variance can hinder the ability to draw conclusions from conflicting studies and aggregate data across different research studies. The increasing volume of papers and associated data published daily makes this hurdle even more challenging to overcome. To help address this, we are developing the Brain Injury Knowledge Ontology for TBI (BIKO), which creates a standardized machine-readable format to formalize terminology to differentiate (1) the main preclinical TBI models (Controlled Cortical Impact Model, Fluid Percussion Model, Blast Injury Model, and Weight-Drop Model) based on significant phenotypes and (2) behavioral assessment used to understand injury deficits. It uses multiple knowledge sources to increase reuse and interoperability. BIKO currently contains over 300 terms and 2,000 statements relating explicit definitions of a preclinical TBI model with preclinical model attributes, phenotypes, and the measures used to assess them. Efforts are also underway to create an accompanying knowledge base (BIKObase). BIKObase is a searchable resource backed by the BIKO semantic framework that assists in categorizing TBI information to facilitate scientific discovery. It includes experimental details from the TBI published literature, starting with metadata from the PRECISE-TBI model catalog. The PRE Clinical Interagency reSearch resource for Traumatic Brain Injury (PRECISE-TBI) model catalog is a federally led interagency (VA, NIH, DoD) effort to develop a queryable online resource for preclinical TBI model literature metadata. BIKObase contains experimental metadata from over 200 published papers, allowing for the ability to ask sophisticated and complex questions across multiple studies. The knowledge base and accompanying ontology allow researchers to ask questions such as: identify all papers (1) with preclinical TBI model of open head injury and (2) that assess motor function in adult rats. BIKObase is an initial step toward disambiguating preclinical TBI knowledge, with hopes of translating this knowledge to the clinical realm.

Disclosures: M. Surles-Zeigler: None. **F.T. Imam:** None. **T. Gillespie:** None. **C. Dixon:** None. **J.S. Grethe:** None. **A.R. Ferguson:** None. **M.E. Martone:** None.

Poster

PSTR272: Brain Injury and Therapeutic Strategies

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR272.11/C60

Topic: C.10. Brain Injury and Trauma

Support: ARC grant
Hotchkiss Brain Institute, University of Calgary
Canadian Institute of Health Research

Title: Remote ischemic conditioning treatment in mild traumatic brain injury

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Abstract: Traumatic Brain Injury (TBI) is a leading cause of death and disability globally, with tremendous health and socioeconomic consequences. Repetitive mild TBI (RmTBI; like concussion) can significantly alter brain structure and function and manifest a breadth of neurological symptoms and neurodegeneration; however, there are no effective treatments. Remote Ischemic Conditioning (RIC) is an endogenous, multifunctional protective intervention that shows promise as a safe treatment in ischemic stroke. RIC involves brief, cyclical periods of restricted blood flow that leads to release of neuroprotective factors which can reduce neuroinflammation and blood-brain barrier impairments - key drivers of RmTBI pathology. RIC has not been studied RmTBI, rendering this current study novel. This study aims to explore if RIC can improve functional recovery following RmTBI. Thirty two P56 male C57BI/6 mice were divided into four groups: sham/sham, RIC/sham, sham/RmTBI, and RIC/RmTBI. RIC treatment consisted of four cycles of 5 mins of ischemia and 5 mins of reperfusion with pressure cuffs placed around the animals' hindlimbs, repeated daily for 14 consecutive days. Sham groups did not receive cuff inflation/deflation. Closed-headed RmTBI was then delivered using the lateral impact model where acutely anesthetized animals received 1 mTBI at 24-hour intervals for 5 consecutive days at 5 m/s projectile velocity. Sham animals were anesthetized but not impacted by the projectile. 1-3 days following RmTBI, a neurobehavioral test battery was performed to evaluate anxiety, motor function and cognition. Animals were then perfused for immunohistochemical analysis of inflammatory markers. Among behavioral tests, we found that RmTBI significantly increased footslips on the beam walk assay compared sham animals ($p < 0.05$). Notably, RIC significantly reduced this motor dysfunction ($p < 0.05$). Other behavioral tests assessing anxiety (open field test) and cognition (novel object recognition) did not show any

significant differences between the groups. Neuroinflammatory analyses are ongoing. Our initial findings support the efficacy of RIC for reducing neurological dysfunction caused by RmTBI. Future efforts will focus on sex-based differences in RIC effects on RmTBI pathology. RIC's effects on neuroinflammation will also be investigated by exploring gliosis and cytokine release. There is an unmet need for effective and safe interventions to modify the course of RmTBI. Given RIC's accessibility and efficacy in motor improvement, translation into clinical practice holds considerable promise, marking a paradigmatic shift in traditional approaches in RmTBI management.

Disclosures: A. Salim: None. **T. Carr:** None. **N. Batty:** None. **C. Camara Lemarroy:** None. **A.W. Lohman:** None.

Poster

PSTR272: Brain Injury and Therapeutic Strategies

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR272.12/C61

Topic: C.10. Brain Injury and Trauma

Support: NIGMS grant P20 GM109098

Title: Sex differences in exercise tolerance following traumatic brain injury

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Abstract: Exercise intolerance, defined as an inability or decreased ability to exercise for an extended duration, is a common experience for survivors of moderate to severe traumatic brain injury (TBI). As such, the capacity to use physical exercise to promote recovery may be limited in this patient population. Moreover, using a mouse model of brain injury, we recently reported that aerobic exercise promotes sex-dependent differences in recovery following controlled cortical impact (CCI). In order to gain greater insight into factors that affect exercise capacity following brain injury, male and female Swiss Webster mice underwent a CCI, and exercise tolerance was assessed acutely (3 days post injury) and again after 2 weeks of moderate treadmill training. Energy metabolism was assessed in a separate cohort that was housed in metabolic CLAMS cages following CCI or sham injury. Compared to sedentary (non-exercised) mice, two weeks of moderate treadmill exercise significantly increased average time- and distance run to exhaustion in brain injured female, but not male, mice. Metabolic rates, measured via indirect calorimetry, revealed decreased heat production following CCI, as well as significant sex differences in respiratory exchange ratio and wheel rotations. Overall, these data indicate biological sex as an important variable mediating metabolism and exercise tolerance after brain

injury. A greater understanding of these variables will bring us closer to developing a method for optimizing exercise as a recovery strategy for TBI.

Disclosures: K. Karelina: None. **D.R. Corbin:** None. **Z.M. Weil:** None.

Poster

PSTR272: Brain Injury and Therapeutic Strategies

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Program #/Poster #: PSTR272.13/C62

Topic: C.10. Brain Injury and Trauma

Support: DARPA ElectRX: HR0011-16-C-0094
NIH R43NS105500-01A1
NIH R43AA030512-01
NIH R43AG084801-01
NIH R44NS105500-02

Title: Axial ultrasonic vibration of neural implants during insertion reduces tissue compression, improving measures of neural activity and reducing tissue damage.

Authors: *R. B. BAGWELL[1], J. K. GREASER[2], A. M. YORITA[3], K. W. GHERES[1], S.-H. LEE[4], R.-U. HAQUE[5], M. L. MULVIHILL[2];
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Abstract: Preclinical neuroscience research is reliant on surgical implantation for the delivery of transgenic vectors, optical imaging such as fiber photometry, and monitoring and stimulation of neural activity with electrodes. While significant work has been performed optimizing electrodes to minimize acute and chronic brain tissue damage, little research has focused on the development of tools to minimize tissue damage and reduce the risk of breakage or deflection of implants.The brain is a porous anisotropic solid suspended in and permeated by cerebrospinal fluid (CSF). Previous research has demonstrated how fluid-solid interactions during compression produce frequency dependent changes in brain stiffness during loading conditions such as those during surgical placement of electrodes, with higher loading rates resulting in less tissue deformation. We investigated whether micron-scale ultrasonic vibration of neural implants (electrodes, infusion cannula, optical lenses) during insertion reduces the force required to puncture brain tissue, and whether minimizing mechanical forces during surgery is sufficient to improve chronic measures of neural activity and reactive gliosis. Using a custom designed ultrasonic actuator (NeuralGlider® Neural Implant Inserter, Actuated Medical, Inc.), we demonstrate that ultrasonic vibration of neural implants reduces the required puncture force into

the pial surface of ex vivo rodent and porcine brains. Surprisingly, in vivo acute recordings from porcine cortex using silicon shank microelectrodes implanted with vibration demonstrated higher single unit yield compared to non-vibrated insertion controls, highlighting consequences of acute tissue damage caused during implantation.Continued research focuses on implantation of large diameter optical lenses implanted for in vivo microscopy experiments and ultra-small flexible electrodes. Early results demonstrated that ultrasonic vibration reduces the force required to insert lenses in ex vivo brain tissue and vibration not only reduces the force required to puncture the pial surface but also modulated implant buckling force of ultra-small flexible implants demonstrating an as-yet unexplored mechanical mechanism for shuttle free device insertion. Ongoing ex vivo experiments are investigating the role of vibrated insertion on reducing tissue shear stress along implant walls and chronic in vivo imaging experiments in rodents will provide insight as to whether vibrated insertion reduces tissue compression and damage at the optical lens-tissue interface of fiber optic cannula and GRIN lenses used for chronic optogenetic and neuroimaging modalities.

Disclosures: R.B. Bagwell: A. Employment/Salary (full or part-time):; Actuated Medical Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Actuated Medical Inc. **J.K. Greaser:** A. Employment/Salary (full or part-time):; Actuated Medical Inc.. **A.M. Yorita:** None. **K.W. Gheres:** A. Employment/Salary (full or part-time):; Actuated Medical Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Actuated Medical Inc.. **S. Lee:** None. **R. Haque:** None. **M.L. Mulvihill:** A. Employment/Salary (full or part-time):; Actuated Medical Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Actuated Medical Inc..

Poster

PSTR272: Brain Injury and Therapeutic Strategies

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR272.14/C63

Topic: C.10. Brain Injury and Trauma

Support: NIA AG000994

Title: Novel 3-monothiopomalidomide attenuates neuroinflammation as a neurodegenerative disorder candidate drug

Authors: *B. BATSAIKHAN[1], P. PARICK[2], S.-C. HSUEH[3], D. TWEEDIE[2], B. J. HOFFER[4], D. KIM[5], N. GREIG[2];
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Abstract: Quelling microglial-induced excessive neuroinflammation provides a potential treatment strategy across neurological disorders, including neurodegenerative diseases, and can potentially be achieved by Immunomodulatory imide drugs (IMiDs). IMiDs, based on the backbone of thalidomide and pomalidomide (Pom), are anti-inflammatory and clinically efficacious in multiple myeloma but are compromised by their toxicity/teratogenicity. IMiD pharmacological actions are primarily mediated via interaction with cereblon, a crucial component of the E3 ubiquitin ligase complex. IMiD-cereblon binding leads to the degradation of specific endogenous neosubstrates, such as SALL4 that is critical for embryo development. In pursuit of safer IMiDs, we synthesized 3-monothiopomalidomide (3-MT-Pom) that binds cereblon and alters its neosubstrate preference away from SALL4. We evaluated 3-MT cellular toxicity, anti-oxidative and anti-inflammatory effects vs. Pom in LPS-challenged immortal microglial (IMG) cells. IMG cells were dose-dependently pretreated with 3-MT or Pom and, thereafter, challenged with LPS. At 24 hr, cell viability (MTS assay), anti-oxidation (Griess nitrite assay) and cytokine levels (ELISA) were quantified in the media. Well tolerated 3-MT doses inhibited nitrite and pro-inflammatory cytokines. This anti-inflammation was replicated in rats following systemic administration of 3-MT and 4 hr LPS challenge. 3-MT was hence evaluated in an acute neurodegenerative animal model known to involve neuroinflammation: controlled cortical impact (CCI) traumatic brain injury (TBI) in mice. 3-MT dosing post CCI TBI mitigated markers of neuroinflammation and behavioral impairment evaluated at 14 days. Teratogenicity was not evident in preliminary evaluation of 3-MT in chicken embryos. These studies indicate that suppressing excessive inflammation provides a treatment strategy in TBI and potentially other neurodegenerative disorders involving excessive inflammation, and identify 3-MT as a new lead IMiD drug candidate for further development.

Disclosures: B. Batsaikhan: None.

Poster

PSTR272: Brain Injury and Therapeutic Strategies

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR272.15/C64

Topic: C.10. Brain Injury and Trauma

Support: R01 NS119472

Title: Transplantation of human cortical organoids after traumatic brain injury in rodents

Authors: *D. JGAMADZE[1], S. HAMIMI[1], S. SINGH[1], J. KIM[1], J. LEE[1], S. KUMAR[1], V. E. JOHNSON[1], H. SONG[2], G.-L. MING[2], H. CHEN[1];

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Abstract: Traumatic brain injury (TBI) can cause a wide range of clinical manifestations such as cognitive impairments, behavioral changes, and physical disabilities, depending on the severity of the trauma. Recovery of brain function is often only partial due to the brain's limited capacity for repair, leading to long-term neurological deficits. A promising avenue to restore lost brain function is neural transplantation into the injured area.
Recently we and others have demonstrated the feasibility of transplanting pluripotent stem cell-derived cortical organoids (CO) into the rodent cortex. In this study, we investigated the feasibility of transplanting induced pluripotent stem cell-derived cortical organoids (COs) into a rodent TBI model (controlled cortical impact). We used a 3-mm cortical impactor at a speed of 2.5 m/s to a depth of 2 mm to induce injuries in secondary visual areas of the rodent cortex. One week (acute) or one month (sub-acute) after the injury, day 50 COs were transplanted into the injury cavity. Before transplantation, two additional conditions were examined by either aspirating the glial scar before transplantation (GS-) or leaving the glial scar intact and transplanting the CO directly into the injury cavity (GS+). Two months after transplantation animals were sacrificed and brains analyzed for histological measures of organoid graft survival and the condition of the host cortex.
We found that all COs (N=4) transplanted into the GS- condition at both acute and sub-acute time points survived and integrated into the host tissue, whereas none of the GS+ organoids survived. The animals in which the glial scar was removed exhibited significantly less GFAP+ cells at the perimeter of the injury cavity. Additionally, we observed reduced injury cavity sizes in the animals with surviving organoid grafts. These results suggest a complex interplay between neural tissue grafts and the traumatically injured brain. The brain microenvironment appears to play a role in determining the survival of transplanted tissue. Conversely, organoid grafts appear to mitigate the ongoing degeneration of cortical tissue after injury.

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Poster

PSTR272: Brain Injury and Therapeutic Strategies

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Program #/Poster #: PSTR272.16/C65

Topic: C.10. Brain Injury and Trauma

Support: Astrocyte Pharmaceuticals, Inc., Sponsored Research Agreement
Brain Injury Research Center, BIRC, State of California

Title: Adenosine receptor agonist targets injury-specific astrocyte subtypes - therapeutic potential and pharmacodynamic biomarkers in a human trauma model.

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Abstract: Improving outcomes for traumatic brain injury (TBI) patients through neuroprotective therapies necessitates elucidating and tracking drug-targeted pathomechanisms. This translational study uses a novel promising therapeutic adenosine A1R/A3R receptor agonist, AST-004 that successfully improved outcomes in animal TBI and stroke models (Bozdemir, 2021; Fisher, 2022; Liston 2022). Here a human trauma culture model (n=8 donors) was used to determine drug dosage, target engagement, cytomechanisms, and pharmacodynamic biomarkers (Halford 2017). Fetal neocortical astrocytes were matured in defined medium on deformable membranes (Wanner 2012). Abrupt pressure pulses inflicted mechanical trauma followed by addition of 0-100 nM AST-004 doses. Unbiased outcome measures were cell integrity (propidium iodide uptake), mitochondrial energy capacity (JC10 dye aggregate/monomer ratiometry), survival (cell density, contact assays), cytoskeletal proteolysis and fluid biomarkers. AST-004 significantly reduced traumatized astrocyte mechanoporation and cell death at various doses (ANOVA, Tukey). A1 and A3 adenosine receptors were expressed and glycosylated in human astrocytes. Neuroprotective AST effects were attenuated by antagonist to either the A1 receptor (DCPCX) or the A3 receptor (MRS7799) suggesting both receptors contribute beneficially. Astrocyte injury-defined biomarker release was dose-dependently reduced for cell wounding markers aldolase C, brain lipid binding protein and α crystallin. The drug reduced severity-dependent cell death and attenuated cytoskeletal proteolysis shown by reduced calpain and caspase GFAP and spectrin fragments in fluids, adherent and detached cells. AST-004 elevated mitochondrial potential in acutely traumatized astrocytes (6-10 hours), with process-bearing and star-shaped astrocytes being selectively receptive to the drug, versus rounded, and senescent cells (Tukey). Drug-treated star-shaped astrocytes also had significantly restored cell integrity (effect size 2, Tukey). Trauma reduced areas with aligned astrocyte choreography and inflicted disorganized zones with increased cell wounding by severity (marginal means; injury-severity interaction). Interestingly, drug treatment retained larger areas of organized astrocytes with fewer disrupted zones (effect sizes +/-1.4, Tukey). In conclusion, AST-004 improves cell health, energy metabolism and astrocyte contact behavior with pharmacodymanic biomarkers indicating efficacy. This work offers early endpoint diagnostic tools and mechanistic insights to guide ongoing clinical trial design for improving TBI patient recovery.

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Poster

PSTR272: Brain Injury and Therapeutic Strategies

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR272.17/C66

Topic: C.10. Brain Injury and Trauma

Support: US Department of Veterans Affairs RR&D Award #K9J8XA7VF6T7
US DoD Spinal Cord Injury Research Program Investigator-Initiated Research Award #SC210266

Title: Comparative analysis of neuroinflammatory burden in rodent models of Traumatic Brain Injury using PET/FDG-18: Implications for Age and Injury Duration

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Abstract: Traumatic brain injury (TBI) represents a multifaceted global health challenge, exerting significant impacts on health outcomes, mortality rates, and socioeconomic dynamics. Its etiology spans a spectrum of injuries stemming from external mechanical forces to the cranium, manifesting in diverse cognitive, motor, and affective impairments. Notably, chronic neuroinflammation emerges as a pivotal mechanism underpinning these sequelae, characterized by sustained microglial activation and the consequent release of inflammatory mediators, culminating in synaptic dysfunction and neuronal demise. Given the prolonged duration of neuroinflammatory processes, there is a growing imperative to longitudinally monitor their progression, prompting a heightened interest in non-invasive imaging modalities, particularly positron emission tomography (PET). PET technology has evolved to enable the visualization of biomarkers indicative of activated neuroimmune cells, with [^{18}F]FDG emerging as a widely accessible tracer for this purpose. [^{18}F]FDG, being a glucose analog, selectively binds to glucose transporters, thereby reflecting cellular metabolic activity. Given the heightened metabolic demands of activated microglia, [^{18}F]FDG PET offers a promising avenue for delineating regions of neuroinflammation post-TBI. Studies employing [^{18}F]FDG PET have catalyzed a reevaluation of its utility in mapping neuroinflammation following TBI. Specifically, investigations have sought to delineate patterns of chronic neuroinflammation in brain regions pertinent to TBI-related deficits. To this end, a comparative study was undertaken, contrasting FDG uptake profiles in young adult and aged rats, both pre- and post-TBI. Baseline glucose levels were meticulously assessed following a fasting period, with subsequent FDG administration and PET imaging. Data analysis, facilitated by specialized software, encompassed quantification of whole brain uptake alongside region-specific uptake relevant to motor, sensory, cognitive, and limbic functions. Preliminary findings indicate a discernible age-related inflammatory burden, potentially mirrored in disparate FDG uptake patterns. Future endeavors will entail expanding

sample sizes to corroborate these observations and elucidate the impact of age-matched TBI on neuroinflammatory processes.

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Poster

PSTR272: Brain Injury and Therapeutic Strategies

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Topic: C.10. Brain Injury and Trauma

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Merit Review Award # 1 I01 RX003123-01A1, from the United States (U.S.) Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D)

Title: Therapeutic effects of electroacupuncture on TBI-induced chronic pain in a rodent model

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Abstract: Traumatic brain injury (TBI) can result in long-lasting sensory disabilities. It was reported that more than half of the TBI patients suffer from chronic pain, including headache and hypersensitivity (allodynia) to mild tactile and thermal stimuli. Unfortunately, there is no effective therapy available. In addition, TBI significantly increases the additional risk of addiction, as the frequent use of addictive pain medications can lead to dependency and, ultimately, substance abuse disorders. Therefore, it is urgent to find an alternative therapeutic approach to treat TBI-induced chronic pain. Here, we evaluated the therapeutic effects of electroacupuncture (EA) on TBI-induced chronic pain in a rat model of TBI. Twelve Sprague Dawley rats received mild/moderate closed-head TBI. At post-TBI week 8, they were randomly divided into TBI-Ctrl and TBI-EA groups (n=6/group). The TBI-EA group started to receive the EA treatments (30 min/session) every other day for two weeks (six sessions in total). EA was provided in three pairs of acupuncture points: ST 36 (Zusanli), LI 4 (He Gu), and LIV 3 (Tai

Chong). Operant orofacial allodynia testing (OPAD), lick-guard responses (thermal pain testing), and Magnetic Resonance Imaging (MRI) were conducted at pre-injury, pre-treatment (Pre Tx), and post-treatment (PO Tx) timepoints. The animals were then euthanized with 4% PFA and the spinal cord and brain tissues were collected for immunohistochemistry (IHC) study. Our data showed that EA treatment can significantly improve the chronic TBI-induced reduced lick/facial contact ratio at 42 °C in OPAD. In the lick-guard responses testing, both groups showed a significant reduction in hind paw lick latency compared to pre-injury; but the TBI-EA treated animals showed persistent and significant recovery compared to the TBI-Ctrl group. Blood Oxygenation level-dependent (BOLD)-based resting state fMRI (rsfMRI) showed significant alterations in connection degrees in certain regions of the brain network for pain pathway after chronic TBI. Alterations of the node degree and betweenness centrality were also observed in TBI-EA treated animals compared to the untreated TBI animals. IHC studies showed that TBI-EA treatment significantly reduced the chronic TBI-induced upregulation of the pain and pro-inflammatory molecules in the spinal cord dorsal horn and the regions of the brain related to main pain pathway. Our data showed that the EA treatment significantly reduced the TBI-induced chronic orofacial and somatic hypersensitivity via the downregulation of pain and inflammatory signaling. EA could be a promising therapeutic intervention for managing TBI-induced chronic pain.

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Poster

PSTR272: Brain Injury and Therapeutic Strategies

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Title: Locus coeruleus injury and dysregulated nociceptive system following traumatic brain injury in rats

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Abstract: Traumatic brain injury (TBI) has been affecting the health conditions of over 10 million people in the world every year. Common co-morbidities following closed-head TBI (cTBI) include balance disability, anxiety disorder, motor impairment, cognitive deficit, and allodynia, although their precise pathological mechanisms are not well understood. Our previous work showed a cTBI-induced severe loss of LC cells whose noradrenergic (NA) projections to the spinal dorsal horn are known to play a critical anti-nociceptive role. Thus, compensatory (or dysregulated) nociceptive mechanisms for the LC cell loss might be mobilized in other nociception-regulating brain regions containing NA cells following cTBI. These regions include the lateral reticular nucleus (LRN) and area postrema (AP) which have been shown to play anti- and pro-nociceptive roles, respectively. However, the precise nociceptive roles of NA cells in these brain regions in response to cTBI-induced LC injury are not well understood. To this end, the purpose of the present study was to quantitate the number of NA cells in the LRN and AP as well as examine its correlation to abnormal orofacial and somatic thermal sensitization (i.e., allodynia) following chronic mild-to-moderate cTBI in rats. It was hypothesized that the number of NA cells in the LRN and AP is altered in response to cTBI-induced LC cell loss, which may contribute to allodynia and alteration of nociceptive neural signaling. Adult female rats were randomly assigned into the normal and cTBI groups, and a weight-drop model of mild-to-moderate cTBI was induced in the latter group. Eighteen weeks after injury, the lick/face contact ratio and hindpaw lick latency were assessed. After these tests, the animals were perfused with 4% paraformaldehyde and harvested brains were cryosectioned in 40-μm thickness. The sections were incubated with primary antibodies against neuronal nuclei (NeuN) and dopamine beta-hydroxylase (DβH). On the next day, the sections were further incubated with secondary antibodies conjugated to fluorescent dyes. The samples were pictured using a confocal microscope for quantitative analyses of NA cells. Our results showed that the number of NeuN/DβH-immunoreactive NA cells was strongly elevated in the LRN and AP, whereas a significant LC cell loss and allodynia were observed following cTBI. The TBI-induced LC cell loss followed by compensatory elevation of NA-immunoreactivities in the LRN and AP, in part, may contribute to altered responses in orofacial and somatic thermal pain sensitization following cTBI.

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Poster

PSTR272: Brain Injury and Therapeutic Strategies

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR272.20/C69

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01AG068168
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Title: Rhesus monkey mesenchymalstromal cell extracellular vesicles reduce inflammation and phagocytic microglial markers in cortical injury

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Abstract: While cortical injury is a leading cause of disability, there is a lack of therapeutics available for post-injury recovery. We have demonstrated in a rhesus monkey model, the efficacy of mesenchymal stromal-cell derived extracellular vesicles (MSC-EVs) as a therapeutic for motor cortical injury. Specifically, MSC-EVs from bone marrow of young monkeys, intravenously administered 24 hours and again at 14 days in aged female monkeys facilitates recovery of fine motor hand function. Functional recovery is associated with dampened microglial inflammatory phenotypes and neuronal damage at 16 weeks following cortical injury. MSC-EVs carry proteins, lipids, and microRNA cargo that can modulate inflammation and repair, but the mechanisms and time-course of action are unclear. Building on our previous work, we used a high sensitivity Olink proximity extension assay (PEA) to assess peripheral biomarkers of inflammation in plasma samples collected 24 hours, 14 days, 28 days, and 6 weeks post injury. We found that relative to vehicle, MSC-EV treatment was associated with a sustained downregulation of pro-inflammatory markers across the recovery period, and a transient downregulation of a smaller subset of anti-inflammatory proteins at 14 days post-injury. Using the NIH Database for Annotation, Visualization, and Integrated Discovery (DAVID), Gene Ontology (GO) and KEGG pathway functional annotation analyses revealed that the proteins downregulated with MSC-EV treatment are associated with pro-inflammatory signaling, suggesting these pathways are being downregulated and the system is in a less inflammatory state. These data are consistent with our previous studies suggesting that MSC-EVs promoted a shift from more pro- to anti-inflammatory microglial phenotypes, within a chronic recovery period. To assess whether this microglial shift occurs earlier in the recovery period compared to our previous work, we analyzed microglial phenotypes in perilesional cortex, 6 weeks post-injury in a new cohort of female monkeys (MSC-EV n=4, Vehicle n=4). Immunofluorescence was performed to label microglia (Iba1), together with Galectin-3, a marker for active

phagocytosis, and C1q a complement protein that tags debris for phagocytosis. While no significant differences were found in C1q+ microglia, a greater density and proportion of presumably “non-phagocytic” ramified microglia and Gal3- microglia was found in MSC-EV monkeys compared to controls. Taken together, these data suggest that MSC-EV treatment promotes an early shift from pro-inflammatory to homeostatic states, which likely facilities repair and recovery of function after cortical injury.

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Poster

PSTR272: Brain Injury and Therapeutic Strategies

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR272.21/C70

Topic: C.10. Brain Injury and Trauma

Support: The EENT Foundation of New Orleans

Title: Single-cell multiome gene analysis and MRI imaging reveal cell and brain region-specific neuroprotective mechanisms by elovanoids in traumatic brain injury

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Abstract: Traumatic brain injury (TBI) is one of the major contributors to physical disabilities and mortality in the world. Blunt trauma to the brain causes axon shearing, which triggers widespread damage, impairing sensorimotor and cognitive functions. Currently, there are no effective treatments for TBI. Elovanoids (ELVs) are homeostatic lipid mediators that provide potent neuroprotection after TBI and experimental ischemic stroke. The current study examined whether ELVs protected the tractography of the brain using high-resolution MRI imaging and interrogated cell-cell communication patterns using single-cell multiome analysis data after ELV treatment in TBI. Male Sprague-Dawley rats were used for all experiments. Craniotomy was made overlying the right parietal cortex, and TBI was induced by fluid percussion injury (FPI) or by controlled cortical impact (CCI). Neurological deficit scores were measured by composite sensorimotor tests. ELVs or Free Fatty Acid precursors (FFA-P) were intranasally delivered (IND) at 1h and 24h (20 ug/delivery) after TBI. The cortex was harvested for single-cell multiome analysis 3 days after TBI. High-resolution MRI was taken from fixed brains 14 days post-TBI. Both IND ELVs and FFA-P treatment reduced neurological deficits after TBI compared to vehicle. Using a cell-cell communication database, we found significant differences in the number and strength of signaling between many cell types after ELV treatment.

Specifically, ELVs preferentially signaled through Wnt and Galectin in IT-ET Glutamatergic neurons. Transcriptional factor footprinting analysis revealed ELVs increased AP-1 activity in the ELV group over vehicle. Gene expression analysis found that ELV or FFA-P treatment reduced pro-apoptotic signaling and increased gene expression of antioxidant defenses in neurons and oligodendrocytes. MRI analysis revealed protective changes after ELV intervention. Finally, diffusion tensor imaging showed greater preservation of the cortex and improved corpus callosum integrity with ELV treatment. Tractography analysis of the brain found that ELVs protected the integrity of the white matter tracts. In summary, these results shed light on ELV regulation of complex patterns of cell-cell communication and revealed that ELVs protected against white matter loss after TBI. The use of IND ELVs or FFA-P may open novel therapeutic avenues to noninvasively bypass the blood-brain barrier and provide neuroprotection against TBI/concussion and other neurodegenerative diseases.

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Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

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Program #/Poster #: PSTR273.01/C71

Topic: C.10. Brain Injury and Trauma

Support: Congressionally Directed Medical Research Programs, the United States Department of Defense Traumatic Brain Injury and Physiological Health Research Program grant W81XWH-22–1–0616
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New Jersey Commission on Brain Injury Research CBIR20PIL004
New Jersey Commission on Brain Injury Research CBIR17PIL007
New Jersey Commission on Brain Injury Research CBIR19IRG025

Title: Perturbations in Risk/Reward Decision Making and Frontal Cortical Catecholamine Regulation Induced by Mild Traumatic Brain Injury

Authors: *C. KNAPP[1], E. PAPADOPOULOS[2], J. A. LOWETH[3], R. RAGHUPATHI[4], S. B. FLORESCO[5], B. D. WATERHOUSE[6], R. L. NAVARRA[7];
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Abstract: Mild traumatic brain injury (mTBI) disrupts cognitive processes that influence risk taking behavior. Little is known regarding the effects of repetitive mild injury (rmTBI) or whether these outcomes are sex specific. Risk/reward decision making is mediated by the prefrontal cortex (PFC), which is densely innervated by catecholaminergic fibers. Aberrant PFC catecholamine activity has been documented following TBI and may underlie TBI-induced risky behavior. The present study characterized the effects of rmTBI on risk/reward decision making behavior and catecholamine transmitter regulatory proteins within the PFC. Rats were exposed to sham, single (smTBI), or three closed-head controlled cortical impact (CH-CCI) injuries and assessed for injury-induced effects on risk/reward decision making using a probabilistic discounting task (PDT). In the first week post-final surgery, mTBI increased risky choice preference. By the fourth week, males exhibited increased latencies to make risky choices following rmTBI, demonstrating a delayed effect on processing speed. When levels of tyrosine hydroxylase (TH) and the norepinephrine reuptake transporter (NET) were measured within subregions of the PFC, females exhibited dramatic increases of TH levels within the orbitofrontal cortex (OFC) following smTBI. However, both males and females demonstrated reduced levels of OFC NET following rmTBI. These results indicate the OFC is susceptible to catecholamine instability after rmTBI and suggests that not all areas of the PFC contribute equally to TBI-induced imbalances. Overall, the CH-CCI model of rmTBI has revealed time-dependent and sex-specific changes in risk/reward decision making and catecholamine regulation following repetitive mild head injuries.

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Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.02/C72

Topic: C.10. Brain Injury and Trauma

Support: NIH NS110609
Research Advisory Committee, Children's Hospital of Pittsburgh
Foundation for PM&R Materson New Investigator Grant (NSR)

Title: Aberrant social behavior, impaired stress coping, and altered social preferences following parietal or frontal experimental TBI in rats

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Abstract: Cognitive integration of social and emotional behavior can be impaired by traumatic brain injury (TBI). Post-TBI socioemotional dysfunction negatively impacts quality of life and societal reintegration. Preclinical work exploring socioemotional behavior after TBI is sparse. Social Familiarity-induced Anxiolysis (SoFiA) is a behavioral phenomenon where repeated stress exposure with a familiar social partner decreases anxiety/stress via social safety learning. We hypothesized rats receiving controlled cortical impact (CCI) to the frontal or parietal lobe would exhibit impaired SoFiA acquisition. Adult male Sprague-Dawley rats (n=10-13/group) were randomized and exposed to moderate severity right parietal CCI, right frontal CCI, or sham injury (anesthesia + craniotomy, no impact) while anesthetized and ventilated. After 14 day recovery, animals acclimatized to open field and completed serial social interaction (SI) sessions with novel and familiar naïve partners under dim or anxiogenic bright conditions while SI time was recorded. Social memory (SM) was assessed (novel vs familiar SI). Shock probe defensive burying (SPDB) measured active and passive coping capacities. A separate cohort received pre-CCI SoFiA training, with post-CCI testing in the same fashion. One and two-way ANOVA (repeated measures as appropriate) assessed main effects of injury (sham, parietal CCI, frontal CCI) and time (day) with Tukey (between group) or Dunnett (within group vs. day 1) post-hocs. Age, sex, and species influences will be explored in future studies. Analysis revealed persistent dysfunction in the SoFiA paradigm, the severity of which differed between parietal and frontal CCI groups. Both TBI groups performed significantly worse than sham ($p<0.05$). Decreased SI times in each CCI group indicated persistently increased anxiety-like behavior despite the presence of a social safety signal. Sham rats demonstrated full recovery to baseline SI times, representing healthy socioemotional behavior. Decreased SPDB active coping (less time spent burying, $p<0.05$) and stressor-dependent social preference alterations in the SM task ($p<0.05$), occurred only in CCI rats. Preliminary pre-trained cohort results indicate trends toward improved post-injury SoFiA performance with familiar pre-injury partners but not new post-injury partners (study ongoing). To better understand socioemotional dysfunction after TBI and translate successful interventions, controlled interrogation of underlying neurocircuitry and neurochemistry in animal models will be required. SoFiA is a useful model to study post-TBI socioemotional dysfunction.

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Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

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Program #/Poster #: PSTR273.03/C73

Topic: C.10. Brain Injury and Trauma

Support: NS110609
NS084967
NS121037
UPMC Children's Research Advisor Committee Dissertation Fellowship
Brain Injury Association of America Dissertation Grant

Title: Examining cholinergic neurotransmission and chronic galantamine efficacy in sustained attention after pre-clinical traumatic brain injury

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Abstract: Attentional deficits are common after traumatic brain injury (TBI), posing challenges due to limited treatment options. The cholinergic network, crucial for attention, extends from the nucleus basalis of Meynert (nBM) to the medial prefrontal cortex (mPFC). In TBI, disruptions in this network, including reduced acetylcholine (ACh) levels, may contribute to attentional impairments. Therefore, galantamine (GAL), a dual-action cholinomimetic, may restore cholinergic tone and improve attention post-TBI. In Experiment 1, we hypothesize that TBI will decrease task-evoked ACh release in the mPFC via *in vivo* microdialysis, correlating with impaired attentional performance on the 3-Choice Serial Reaction Time Test (3-CSRT). Observed attentional impairments (i.e., 3-CSRT) and reduction in cholinergic tone (i.e., in vivo microdialysis) will be mitigated by GAL in a dose-dependent manner after injury (Experiment 2). Adult male rats trained in 3-CSRT underwent either right parietal controlled cortical impact or a Sham surgery (n=10-16/group). In Experiment 1, on post-injury day (PID) 14, a guide cannula was implanted in the right mPFC. Measures of sustained attention and distractibility (i.e., 3-CSRT) were assessed on days 21-27. Dialysate was collected before and during 3-CSRT onset on day 21. In Experiment 2, rats were randomly assigned to TBI or Sham groups and administered GAL (0.5, 2.0, or 5.0 mg/kg) or VEH i.p once daily from 24 hours post-surgery until sacrifice (PID 28). 3-CSRT testing occurred on PID 21-25. A subset of behaviorally-naive TBI rats underwent microdialysis procedures followed by post-drug dialysis collection at PID 21. All dialysate samples were analyzed using HPLC with electrochemical detection. At PID 28,

cortical lesion volume and probe location were examined. In Experiment 1, cholinergic neuron ultrastructure in the nBM and mPFC was quantified using brightfield microscopy coupled with IMARIS. Statistical analyses included ANOVAs with Newman-Keuls post-hoc tests. TBI rats showed attention deficits compared to Sham controls ($p<0.05$) and exhibited reduced task-evoked ACh release in the mPFC ($p<0.05$). The high GAL dose increased ACh levels ($p< 0.05$) but did not restore attention, with higher doses exacerbating deficits ($p<0.05$). GAL did not reduce lesion volume ($p>0.05$). Preliminary findings suggest that TBI reduced cholinergic neuron soma, indicative of degenerative morphology. To our knowledge, this study is the first in neurotrauma to utilize in vivo microdialysis in awake, freely moving rats during a cognitive task, offering real-time insights into behaviorally and drug-induced changes in ACh tone.

Disclosures: E. Moschonas: None. **E.H. Moschonas:** None. **A.J. Vellore:** None. **E.M. Annas:** None. **H. Capeci:** None. **H. Donald:** None. **V. Domyslawski:** None. **J.P. Cheng:** None. **A.E. Kline:** None. **C.O. Bondi:** None.

Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.04/C74

Topic: C.10. Brain Injury and Trauma

Support: NIH NS110609
Research Advisory Committee Children's Hospital of Pittsburgh (COB)
UPMC Children's Research Advisor Committee Dissertation Fellowship (EHM)
NS084967
NS121037 (AEK)

Title: Positive outcomes of α7 nicotinic acetylcholine receptor modulation and environmental enrichment on sustained attention and cholinergic neurotransmission after controlled cortical impact injury

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Abstract: Traumatic brain injury (TBI) is a leading cause of cognitive disability. Enhancing acetylcholine (ACh) transmission may ameliorate post-injury cognitive deficits, especially when

combined with noninvasive rehabilitation. We predicted that chronic NS-1738, a α7 nicotinic ACh receptor (α7-NAChR) positive allosteric modulator (PAM), will improve sustained attention post-TBI, alone and in combination with environmental enrichment (EE) housing, as well as brain markers of cholinergic transmission post-TBI in both male and female adult rats. Moreover, blocking α7-NAChRs with methylycaconitine (MLA) will attenuate the effects of NS-1738, confirming its mechanism of action. We also cross-compared effects of 4BP-TQS, a α7-NAChR allosteric agonist (ago-PAM), on attention post-injury in males. Adult male and female rats were trained in the 3-choice serial reaction time task (3-CSRT), reaching pre-injury baselines prior to right parietal controlled cortical impact (CCI) or sham injury. Rats were randomized to NS-1738 (5 mg/kg, i.p.) or vehicle, as well as daily EE (6h) or standard housing for 28d starting post-injury day (PID) 1. Male subgroups also received daily α7-NAChRs blockade via MLA (3 mg/kg) injections. 3-CSRT retrials occurred on PID 14-24, when 4BP-TQS (1 mg/kg) was administered daily in the specific male cohort. Cortical lesion volumes were performed for both sexes. Medial prefrontal cortex (mPFC) Western blots assessed cholinergic markers [acetylcholinesterase (AChE), choline acetyltransferase (ChAT), and α7-NAChR]. ChAT immunostaining was performed in the basal forebrain where ACh projections originate. Statistical analysis utilized ANOVAs with repeated measures when appropriate, and Newman-Keuls post hoc tests. TBI rats of both sexes exhibited impaired sustained attention and increased distractibility versus shams ($p<0.05$), with chronic NS-1738 improving accuracy in females and omissions in both sexes ($p<0.05$). Daily EE was beneficial on 3-CSRT measures in male rats ($p<0.05$), while combining NS-1738+EE rendered an additive effect on lowering omissions and reducing cortical cavitation ($p<0.05$). Both male and female TBI rats reflected ChAT disruptions in mPFC and basal forebrain, which were improved by chronic NS-1738+EE housing in male rats ($p<0.05$). Chronic 4BP-TQS, given during testing (14 day delay post-surgery) was promising at restoring accuracy and premature responding, albeit prompt intervention post-TBI is warranted. Our findings support benefits of α7-NAChR PAM and ago-PAM compounds and/or EE treatment after TBI on sustained attention and cholinergic neurotransmission in both males and female rats.

Disclosures: H. Donald: None. **E.H. Moschonas:** None. **E.M. Annas:** None. **T. Ranellone:** None. **J.P. Cheng:** None. **N. Race:** None. **A.E. Kline:** None. **C.O. Bondi:** None.

Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.05/C75

Topic: C.10. Brain Injury and Trauma

Support: NIH NS110609
Research Advisory Committee
Children's Hospital of Pittsburgh (COB)
UPMC Children's Research Advisor Committee Dissertation Fellowship (EHM)
NS084967
NS121037 (AEK)

Title: Effects of a non-amphetamine central nervous system stimulant on sustained attention after controlled cortical impact injury in males and females

Authors: *V. DOMYSLAWSKI[1], E. H. MOSCHONAS[1], E. M. ANNAS[2], H. DONALD[1], P. L. RENNERFELDT[3], T. RANELLONE[1], N. RACE[2], J. P. CHENG[1], A. E. KLINE[4], C. O. BONDI[5];
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Abstract: Traumatic brain injury (TBI) stands as a significant contributor to global mortality and morbidity. The ensuing disabilities often encompass long-term impairments in memory, attention, and overall cognitive function. Pharmacotherapeutic interventions involving stimulants following experimental TBI have shown promise in enhancing cortical function, thereby mitigating several cognitive deficits commonly observed. We predicted that modafinil (Provigil ®), a non-amphetamine central nervous system (CNS) stimulant thought to act in a concerted fashion on multiple catecholamines in the brain (dopamine, norepinephrine), as well as glutamate, GABA, and histamine, to enhance arousal and focus, will improve sustained attention post-TBI. Adult male and female Sprague Dawley rats were trained in the 3-choice serial reaction time task (3-CSRT) before receiving either a controlled cortical impact (CCI) of moderate severity to the right hemisphere or a sham injury. Rats were then randomly assigned to either receive modafinil (10 mg/kg) or vehicle (saline, 1 ml/kg) being administered once daily throughout behavioral tasks beginning 14 days post-injury. 3-CSRT retrials were conducted post-injury days 14-24 to assess sustained attention via percent accuracy and distractibility via percent omissions. Ongoing histological analyses include assessments of cortical lesion volumes and hippocampal cell survival. TBI induction in both male and female rats resulted in significant and lasting deficits in both sustained attention and distractibility on the 3-CSRT task. Chronic modafinil administration concurrent with post-surgery behavioral testing produced promising results by attenuating TBI-related deficits in percent accuracy, as well as percent omissions in males, however, not in females, suggesting prompt intervention post-TBI may be therefore warranted. Our findings support the benefits of modafinil on sustained attention post-TBI and can be further expanded upon in future studies with adjustments in treatment timelines and paradigms that may allow for observation of higher efficacy in reinstating complex cortical function.

Disclosures: V. Domyslawski: None. **E.H. Moschonas:** None. **E.M. Annas:** None. **H. Donald:** None. **P.L. Rennerfeldt:** None. **T. Ranellone:** None. **N. Race:** None. **J.P. Cheng:** None. **A.E. Kline:** None. **C.O. Bondi:** None.

Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.06/C76

Topic: C.10. Brain Injury and Trauma

Support: NIH NS110609 (COB)
Research Advisory Committee Children's Hospital of Pittsburgh (COB)
UPMC Children's Research Advisor Committee Dissertation Fellowship (EHM)
NS084967 (AEK)
NS121037 (AEK)

Title: Rescuing sustained attention capability in aged rats using a combined therapy of nicotinic acetylcholine receptor allosteric modulation and environmental enrichment after experimental brain trauma

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Abstract: Traumatic brain injury (TBI) is a leading cause of death and disability and poses significant challenges for elderly populations, often exacerbating existing age-related cognitive decline. Empirical evidence suggests disruptions in cholinergic neurotransmission following TBI may contribute to cognitive deficits. Therapies that enhance acetylcholine (ACh) transmission may ameliorate cognition, especially in conjunction with noninvasive rehabilitation, which is akin to the real world. We have shown that a parietal cortex TBI induces deficits of complex attention in young adult rats, males and females. We predicted that parietal injury in aged (15-16 months old) male rats will augment sustained attention deficits compared to young adults. We then hypothesized that chronic NS-1738, a novel positive allosteric modulator (PAM) of the α7 nicotinic ACh receptor (α7-NAChR) will improve sustained attention post-TBI in aged rats, alone and in combination with environmental enrichment (EE), a pre-clinical neurorehabilitation model. Aged male rats were trained in the 3-choice serial reaction time task (3-CSRT) prior to a right parietal controlled cortical impact (2.8 mm cortical deformation depth) or sham injury. They required more sessions to reach criterion than young adults, especially as cue durations shorten. Following a controlled cortical impact (CCI) of moderate severity to the right parietal

lobe or sham injury, rats were randomized to daily NS-1738 (5 mg/kg) or vehicle, as well as daily EE (24h) or standard housing for a month starting post-injury day (PID) 1. 3-CSRT retrials occurred on PID 17-27. Statistical analysis utilized repeated measures ANOVAs with Newman-Keuls post hoc tests. Anxiety-like behavior was assessed via the well-validated open field test (OFT) on PID 28. Cortical lesion volumes were assessed post-sacrifice. TBI-induced cognitive deficits were pronounced in aged rats ($p<0.05$) and were rescued by chronic NS-1738 ($p<0.05$). Moreover, NS-1738+EE rendered an additive effect on restoring accuracy and lowering omissions ($p<0.05$). TBI reduced OFT center exploration without reductions in ambulation ($p<0.05$). NS-1738 and EE housing individually restored center exploration, suggestive of ameliorating anxiety-like behavior ($p<0.05$). While both NS-1738 and EE rendered trends on reducing the extent of cavitation, the combined therapy was ineffective at promoting tissue preservation in preliminary findings. Our findings reflect the vulnerability of the elderly following TBI and support benefits of α7-NAChR PAM and/or EE treatment after experimental brain trauma on sustained attention through cholinergic neurotransmission.

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Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.07/C77

Topic: C.10. Brain Injury and Trauma

Support: NS084967
NS121037

Title: Enriching rats prior to traumatic brain injury does not protect against subsequent neurobehavioral deficits

Authors: *H. CAPECI[1], J. STEBER[1], A. J. VELLORE[1], P. GOYAL[1], R. VANGALA[1], T. MINDEL[1], E. H. MOSCHONAS[1], C. O. BONDI[2], A. E. KLINE[3];
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Abstract: Environmental enrichment (EE) reliably produces behavioral and histological benefits when initiated after experimental traumatic brain injury (TBI). However, no benefit or

prophylactic effect was revealed in a recent study where EE was provided for 2-weeks before a single controlled cortical impact (CCI) impact to the right hemisphere. The lack of protection with Pre-TBI EE may have been due to limited exposure and thus to verify the puzzling finding, the current study utilized a 4-week Pre-TBI EE paradigm to test the hypothesis that pre-TBI EE can exert a prophylactic effect. A group receiving EE before and after TBI was included to determine whether Pre-TBI EE affects the robust effectiveness of Post-TBI EE. After 4 weeks of EE or standard (STD) housing, anesthetized adult male rats were subjected to a right hemisphere CCI injury (2.8 mm deformation at 4 m/s) or sham surgery and then randomly assigned to post-operative EE or STD conditions. Beam-walk agility and acquisition of spatial learning were assessed on post-operative days 1-5 and 14-19, respectively. The Post-TBI EE groups performed better than the Post-TBI STD groups ($p<0.05$) but did not differ from each other ($p>0.05$). However, despite 4 weeks of EE prior to TBI, no prophylactic effect was observed as there were no differences between the STD-housed TBI groups regardless of whether they received EE or STD housing before surgery ($p>0.05$). These data reproduce previous findings showing that EE post-TBI is effective and replicate a recent report that providing EE prior to TBI does not confer protection.

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Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.08/C78

Topic: C.10. Brain Injury and Trauma

Support: NSTC113-2636-B-038-001

Title: Transcranial interfering electric field stimulation (TIS) mediates EAAT1 activity and microglia polarization to achieve neuroprotective effects in traumatic brain injury

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Abstract: Transcranial interfering electric field stimulation (TIS) mediates EAAT1 activity and microglia polarization to achieve neuroprotective effects in traumatic brain injury Authors*N.D. HIEU[1], C.W. PENG[2], J.Y. CHUANG[1,3,41]Ph.D. Program in Medical Neuroscience;[2]The School of Biomedical Engineering;[3]International Master Program in Medical

Neuroscience;[4]TMU Research Center of Neuroscience, Taipei Medical University, Taipei, Taiwan, R.O.C.DisclosuresN.D. Hieu: None. C.W. Peng: None. J.Y. Chuang: None.AbstractTraumatic brain injury, especially mild traumatic brain injury (mTBI/concussion) mostly happens in daily life results in long-term memory loss and cognitive impairment which are consequences of hippocampal dysfunction. Recently, the majority of deep-brain stimulation has been used to treat the hippocampus, however, they are invasive and side effects remain significant challenges. To address this issue, we have used TIS, which is non-invasive and also can deeply impact the hippocampal or thalamic region for treatment. By performing several tests in animals such as (1) rotarod test and (2) beam walking test, we initially found TIS could improve the balance and motor function of rats after a concussion, that brought us a big question mark about which mechanism could promote the efficacy of TIS after TBI. Moreover, we understand that during TBI, the heterogeneity of cells undergoes striking changes, but much is still unknown due to complex cell states and functional differences. We used single-cell droplet technology following 10x genomic protocol and analyzed how TIS improves recovery after TBI at the cellular level. R studio and Seurat were the main tools that we used to perform bioinformatic analysis. After performing quality control, we eventually discovered 13 cell types and observed significant changes in immune cell responses following TIS treatment. We advanced to analyze differential gene expression among groups and found that TBI causes neurotoxicity by excessively increasing extracellular glutamate as well as neuroinflammation, and TIS prevents these consequences from further damage by mobilizing microglia to reuptake glutamate release through EAAT1 signaling pathway, meanwhile polarizing microglia into M2 phenotypes to ameliorate inflammatory response. Using BV2 cell line combined with the LPS condition for in-vitro validation, we successfully found TIS could reduce the expression of several common inflammatory markers while improving EAAT1 expression , which partly suggests the protective activity of microglia through EAAT1.

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Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.09/C79

Topic: C.10. Brain Injury and Trauma

Support: DOD - TBIPHRP - HT9425-23-1-0484

Title: Amnion cell secretome mediated therapy for traumatic brain injury

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Abstract: Background: Traumatic brain injury (TBI) poses a significant health risk for both combat Veterans, Service members, and civilians alike. Among military personnel returning from recent conflicts in the Middle East, repetitive mild TBI (r-mTBI) stands out as a leading cause of chronic illness. The proposed project aims to investigate the potential advantages of administering ST266 via the intranasal route for addressing defects associated with TBI. ST266 represents a proprietary secretome derived from culturing a unique cell population known as Amnion-derived Multipotent Progenitor cells under exclusive, pharmaceutical-grade GMP conditions. **Method:** For this study, there will be 2 exposure groups (Sham or TBI), 2 treatments (ST266 treatment or Vehicle starting at 1 month post last injury), 2 sexes and 1 daily treatment duration until euthanasia. With 12 mice per group this totals 96 mice. At 1 month post last r-mTBI/sham mice will receive daily treatment with 10µL/dose ST266 or vehicle control (saline) via intranasal delivery (ID) for a period of 4 months (until euthanasia). Learning, memory and visual deficits were assessed with the Barnes Maze and optomotor behavioral tests starting at 4 months post injury. The dose that gives the optimal recovery was assessed by combining the scores of the following end points: 1) Optomotor, a rodent visual test, 2) Barnes Maze, a learning and spatial memory test, 3) Elevated plus maze an anxiety test, 4) Rotarod a locomotor test and 5) neuropathology of the brains, eyes, and optic nerves. **Results:** All mice were healthy throughout the course of the study and tolerated both the low and high dose. Following euthanasia, no difference in morphology was observed in any of the major organs dissected in the first group of male mice. Our initial investigation involving male subjects revealed decreases in TBI-induced astroglial (GFAP) and microglial (IBA1) markers, along with a potential improvement in learning and cognitive abilities among injured animals treated with the intervention. These early results suggest the potential effectiveness of administering ST266 at a later stage in mitigating short-term behavioral and pathological consequences following r-mTBI. **Conclusion:** By assessing nuanced aspects of neurobehavioral and pathological deficits, we will provide a framework from which informed decisions can then be made about the cellular and molecular mechanisms that are most important to improve the understanding, prevention, and treatment of psychological health conditions and TBI-related pathology.

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Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.10/C80

Topic: C.10. Brain Injury and Trauma

Support: Canadian Institutes of Health Research
Capes Print UNIFESP

Title: Deep brain stimulation mitigates memory deficits and increased hippocampal cell counts in a rodent model of traumatic brain injury.

Authors: T. RABELO[1], A. CAMPOS[1], F. MAHMUD[1], **T. H. ALMEIDA SOUZA**[1], M. R. POPOVIC[2], L. COVOLAN[3], V. CARDOSO BETTA[4], L. DA COSTA[5], N. LIPSMAN[1], M. DIWAN[6], *C. HAMANI[1];
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Abstract: Introduction: Traumatic brain injury (TBI) is a major life-threatening event. In addition to neurological deficits, it can lead to long-term impairments in attention and memory. Deep brain stimulation (DBS) is an established therapy for movement disorders, such as Parkinson's disease and tremor. To date, DBS has mainly been used as a symptomatic treatment. Whether it induces neuroprotective effects is a matter of debate. In rodent models, DBS delivered to the anterior nucleus of the thalamus (ANT) induced anti-inflammatory effects following strong excitotoxic insults and improved memory performance in various behavioural tests. We tested whether DBS administered following TBI improved memory performance and induced neuroprotective effects in the lateral fluid percussion (LFP) model. Methods: Male rats were implanted with ANT DBS electrodes one week prior to being exposed to LFP. Thereafter, animals received active or sham stimulation for 6 hours. Four days later, they were tested in a novel object/ novel location recognition and a Barnes maze paradigm. After the experiments, hippocampal cells were counted. Separate groups of animals were sacrificed 24h or 72h after TBI for the measurement of cytokines and brain derived neurotrophic factor (BDNF). In a second set of experiments, TBI-exposed animals receiving active or sham stimulation were injected with the tropomyosin receptor kinase B (TrkB) antagonist ANA-12 (5 mg/Kg) alongside DBS. Four days later, animals underwent memory testing. Results: Rats receiving DBS had an improved performance in the Barnes Maze, a higher expression of hippocampal BDNF and an increase in the number of hippocampal cells. No changes in proinflammatory cytokines were noticed. The administration of ANA-12 did not change the behavioural effects f DBS. Conclusion: DBS delivered immediately after TBI improved memory performance, increased the expression of BDNF and the number of hippocampal cells in rats. Mechanisms for these effects were not related to an anti-inflammatory effect of mediated via TrkB receptors.

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Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.11/C81

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01NS116384

Title: Electrical stimulation of the deep cerebellar nuclei promotes consolidation of suppressed innate anxiety in a rodent medial prefrontal cortex traumatic brain injury model.

Authors: *M. KIM[1], H. H. CHAN[1], A. G. MACHADO[1,2], K. B. BAKER[1,2];
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Abstract: Prefrontal cortex traumatic brain injury (PFC-TBI) increases the risk of neuropsychiatric disorders, particularly anxiety and fear. The association between the PFC and learned fear and innate anxiety regulation circuits has been well-documented as have efforts to find treatments for those with associated disorders, including chronic traumatic encephalopathy, panic disorders, and post-traumatic stress disorder. However, research on the reduction of innate anxiety response disorders after TBI is lacking, and therapeutic interventions to prevent or treat post-traumatic anxiety are limited. We have shown previously that deep brain stimulation (DBS) of the lateral cerebellar nucleus (LCN) enhances cognitive recovery after medial PFC (mPFC)-TBI using the controlled cortical impact (CCI) model in rats. Here, we used the same model to evaluate whether the benefits of LCN DBS would extend to innate anxiety as measured using the open field (OF) and elevated plus maze (EPM). All animals underwent mPFC-CCI (week 0), unilateral LCN DBS electrode implantation (week 2), and DBS activation (or sham [week 5]). OF and EPM performance were measured pre-CCI, four weeks post-CCI (pre-DBS baseline), and after four weeks of treatment. Post-mortem, treatment-related effects on post-trauma neurodegenerative processes, as well as the expression of immediate early genes (IEGs) across the amygdala-PFC pathway, were characterized using immunohistochemistry and arc and c-fos expression, respectively. We found that post-CCI animals showed an inhibited innate anxiety response compared to pre-CCI and naïve animals, while LCN DBS treatment was associated with a significantly greater rate of consolidation of suppressed innate anxiety as compared to untreated animals. These behavioral findings were associated further with a suppressed reactive microgliosis and gliosis in the perilesional cortex, as well as increased IEGs expression in mPFC and ventral hippocampus and decreased IEGs expression in the basolateral amygdala. Overall, these results support a potential role for LCN DBS in promoting the consolidation of suppressed innate anxiety, possibly mediated by inhibiting the post-trauma neurodegenerative processes and the amygdala anxiety circuitry. Therefore, LCN DBS may represent a promising, novel therapeutic approach to improve the rehabilitation of innate anxiety responses in patients with post-traumatic anxiety who do not respond to traditional rehabilitation efforts.

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Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

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Topic: C.10. Brain Injury and Trauma

Support: I01BX005586 from the United States (U.S.) Department of Veterans Affairs (VA) Biomedical Laboratory Research and Development Program.

Title: Contribution of brain pericytes to neuroinflammation following repetitive head trauma

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Abstract: Neuroinflammation is a prominent pathological hallmark of traumatic brain injury (TBI), but little is known about the specific contribution of brain pericytes to the inflammatory response in TBI. In our preliminary data, we observed diminished PDGF-BB levels in brain vasculature following r-mTBI. As pericyte health is highly dependent on stimulation of the PDGF pathway, the diminished availability of PDGF-BB in the brain following TBI might be driving pericyte degeneration post-injury. In this study, we examined the role of brain pericytes in the neuroinflammatory process following r-mTBI, and the response of these cells to PDGF-BB stimulation. Firstly, to evaluate the response of pericytes to external inflammatory stimuli, we exposed mouse brain vascular pericytes (MBVP) to a cocktail of inflammatory stimuli for 2 and 24 h. We tested the physiological response of pericytes to inflammatory stimuli by examining PDGFRβ expression levels and observed a 3-fold and 8-fold increase in receptor expression at 2 h and 24 h cytokine insult, respectively. To determine whether PDGF-BB might prevent or revert pericyte inflammatory status, cultured MBVP were treated with PDGF-BB (10 ng/mL) prior to, simultaneous, and following the inflammatory insult. While PDGF-BB stimulation did lead to a subtle reduction in PDGFRβ levels, administration of PDGF-BB following cytokine treatment significantly mitigated the effect of the cytokine insult, lowering PDGFRβ levels by nearly 2-fold compared to cytokine insult alone. In addition, we investigated the release of inflammatory mediators by pericytes, using both MBVP and *ex-vivo* pericytes isolated from r-mTBI animals (male and female WT C57BL/6 at 3 months age received 2 impacts per week for 3 months) at 6 months post-injury using magnetic cell sorting. We treated them with PDGF-BB directly or *via* phenytoin administration, which has been shown to induce PDGF-BB secretion from brain endothelia. Using an MSD assay, we found that r-mTBI caused a

robust inflammatory response in isolated pericytes, which was broadly attenuated upon PDGF-BB stimulation. Furthermore, we found phenytoin modulated several cytokines in the r-mTBI pericytes, including IL-1beta and TNF-alpha, which are prominently observed in human TBI brains. Overall, our results showed pericytes contribute to the propagation of neuroinflammation following r-mTBI and we found that PGDF-BB and/or Phenytoin treatment can mitigate or dampen inflamed pericytes following brain injury. Targeting vascular brain pericytes may provide novel opportunities to mitigate cerebrovascular-mediated inflammation in the aftermath of TBI.

Disclosures: A. Cembran: Other; Departement of Veterans Affairs (VA) Biomedical Lab Research and Development Program. **C. bachmeier:** None. **D. Paris:** None. **M.J. Mullan:** None.

Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.13/C83

Topic: C.10. Brain Injury and Trauma

Support: NS111378
NS117148
NS116383

Title: Protective effects of thyroid hormone on blood brain barrier integrity in traumatic brain injury

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Abstract: The blood brain barrier (BBB) shields the brain from chemicals transported in blood and plays an important role in the clearance of toxins to maintain brain homeostasis. Because of traumatic brain injury (TBI), blood vessels are damaged, tight junctions are disrupted resulting in increased paracellular permeability, neuronal loss, cognitive dysfunction. Moreover, post-traumatic injury results in brain edema due to extensive damage to BBB that results in increased intracranial pressure and ischemia. Cognitive functions such as impairment in memory and motor functions, are also affected because of injured BBB. We wanted to determine means to reduce BBB dysfunction after TBI. Thyroid hormone is crucial for brain development and function. Hypothyroidism is a threating complication found in individuals suffering from TBI causing neuronal impairment and intellectual deficit. Membrane transporters facilitates the transport of thyroid hormone T4 and prohormone T3 across the blood brain barrier. In the current

study, mice were subjected to fluid percussion injury (FPI) followed by acute T4 treatment (1 hour and 6 hours post-TBI) to investigate several questions: 1) Does T4 enhance PDGF-B binding to PDGFRβ and reduce toxin permeability across tight junctions? 2) Does T4 prevent BBB edema and hyperpermeability? 3) What are the roles of transporters and enzymes in thyroid hormone transport across the BBB? 4) How do astrocytes contribute to neurotoxicity or neuroprotection in T4-treated mice? 5) How does BBB dysfunction affect cognitive behavior? Our results showed that T4 treatment post-TBI, reinstated the levels of pericyte and endothelial cells associated with BBB dysfunction. Additionally, T4 treatment differentially regulates hyperpermeability and edema, mainly in the frontal cortex and hippocampus, through the suppression of AQP4 and MMP-9. Thus, T4 shows promise in alleviating vascular leakage and reducing inflammation following TBI.

Disclosures: M. Khandelwal: None. **F. Gomez-Pinilla:** None.

Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.14/C84

Topic: C.10. Brain Injury and Trauma

Support: 1R01NS123051-01A1S1

Title: Feasibility of hydrogel anti-inflammatory drug delivery to the brain after traumatic brain injury

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Abstract: Motivation: Nearly 5.5 million people suffer a traumatic brain injury (TBI) each year. A standard treatment for severe injury is to perform a decompressive craniectomy (DC). This project aims to develop an anti-inflammatory drug delivery system to augment the standard DC treatment. Intracranial pressure can result in decreased cerebral blood flow and herniation leading to death. Although DC can immediately decrease intracranial pressure, the brain is unprotected for weeks to months and is associated with neurological symptoms. However, local and controlled drug delivery can be implemented during the DC procedure. Numerous candidate drugs can be encapsulated and loaded into a pentenoate functionalized hyaluronic acid hydrogel. Using a rat model of TBI, this study is assessing the efficacy of this delivery system for motor outcomes. Methods: To assess the feasibility of this approach, Long Evans rats (male, 10-12

weeks old, 350-415g) were trained on a reach task requiring rats to reach and grasp small food pellets placed outside of a Plexiglas behavioral box with the preferred forelimb. Food pellets were singularly presented for 60 trials per session. Once a rat reached >60% success rate, a pre-injury baseline was established. Rats were assigned randomly to one of three experimental (hydrogel) conditions. Experimental (hydrogel) conditions: 1) dexamethasone (DEX); 2) indomethacin (INDO); 3) metformin (MET). Reach performance was assessed once per week for 8 weeks after the CCI injury. A controlled cortical impact (CCI) procedure was then used to deliver a TBI to the rat forelimb motor cortex contralateral to the trained forelimb. A 5mm diameter craniectomy was made to access the cortex in all groups. Immediately following the CCI injury an 8 X 5 mm craniectomy was performed, mimiking DC. Results: Our initial data showed a significant motor deficit for each TBI group during the first week post-TBI. Over the course of 8 weeks, INDO and DEX rats showed an increase in motor performance. MET showed an initial robust effect but declined over time. Historical data show that under the same CCI conditions, rats with DC only or DC plus hydrogel without drugs did not recover to baseline levels of performance. This adds to the information on effects of controlled and local drug delivery to the cortex on motor recovery post-TBI. Conclusion: Based on our preliminary feasibility study, a more extensive study is in progress to assess the efficacy of various drug eluting hydrogels after TBI.

Disclosures: J. Deng: None. **S. Barbay:** None. **J. Townsend:** None. **R.J. Nudo:** None. **M. Detamore:** None.

Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.15/C85

Topic: C.10. Brain Injury and Trauma

Support: NIH grant UO1 CA266981

Title: The neuroprotective effects of a novel hybrid compound targeting oxidative stress for traumatic brain injury

Authors: *J. GREEN[1], J. THOMPSON[2], S. ZHANG[3], Q. CHEN[2], D. SUN[4];
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Abstract: Traumatic Brain Injury (TBI) is a debilitating condition associated with pathological changes in the brain caused by an external force. Following TBI, there are many identifiable events that propagate the developing pathology including the production of reactive oxygen and

nitrogen species (ROS and RNS respectively) as a result of mitochondrial damage and dysfunction. Thus far, mitochondria dysfunction related secondary brain injury following TBI is well studied from mechanistic and pharmacological perspectives. In TBI therapeutic development, published studies have found that two natural products, curcumin (a dietary product) and melatonin (the major pineal hormone), have beneficial effects for TBI. Recently, we have developed a novel hybrid compound derived from curcumin and melatonin, ZCM-I-1, with specifics on mitochondrial dysfunction induced oxidative stress. In a mouse transgenic model of Alzheimer's disease, this novel compound showed significant beneficial effects in reduction of oxidative stress and microglial activation, and enhancement of synaptic plasticity via its interaction with mitochondrial complex I. In this study, we have explored the therapeutic effect of ZCM-I-1 for TBI. Adult male Sprague-Dawley rats were subjected to a moderate cortical impact injury. Following injury, animals received 4 doses of ZCM-I-1 (50mg/kg, i.p.) at 30 minutes, 6, 24 and 30 hours after CCI. Animals were survived for 2 or 28 days post-injury. The groups of animals which survived for 2 days were subjected to assessment of injury-induced degenerative neurons and mitochondrial function using using a Clark electrode for oxidative phosphorylation studies. Those survived for 28 days, sensorimotor and cognitive functions were assessed, and brain tissues were processed for histological examination to measure cortical lesion volume. We found that following TBI, ZCM-I-1 administration significantly reduce the number of degenerative neurons in the injured cortex and hippocampus. Injured animals received ZCM-I-1 treatment also had significant improvement in both motor and cognitive functions. We have also found an improved mitochondria complex I mediated respiration in the injured cortex in animals treated with ZCM-I-1. Our data suggest that our novel compound has neuroprotective effects for TBI. Further study examining the effect of ZCM-I-1 treatment on post-TBI neuroplasticity is ongoing. Sponsored by NIH grant UO1 CA266981.

Disclosures: J. Green: None. **S. Zhang:** None. **D. Sun:** None.

Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.16/C86

Topic: C.10. Brain Injury and Trauma

Support: NIH RF1AG059321-01A1

Title: Treatment with isolated functional mitochondria rescues tau pathology after traumatic brain injury

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Abstract: Traumatic brain injury (TBI) disrupts brain structure and function, triggering metabolic, inflammatory, and degenerative changes, including accumulation of hyperphosphorylated tau aggregates that can spread throughout the brain in a prion-like manner. An important abnormality associated to both TBI and Tau deposition is mitochondrial dysfunction which may play an important role in neurodegeneration. We propose systemic administration of purified mitochondria as a treatment to halt Tau aggregation triggered by TBI. Five-month-old male and female PS19 Tau-P301S transgenic mice were distributed into three groups (n=11-18 per group): 1-TBI; 2-TBI+Mitochondria; 3-Untreated. Each mouse underwent a single TBI event using controlled cortical impact (CCI) and treated with isolated functional mitochondria. Treatment with fresh mitochondria, extracted from mouse neural precursor cells, began immediately post-TBI and continued weekly for up to four months post-TBI. We used the rotarod apparatus to test all mice at 7, 14, 21, 28 days post-TBI, with a final test at the endpoint. After euthanizing all animals, we studied the impacted hemisphere using ELISA to evaluate pS199-Tau levels. We used the contralateral hemisphere for histology studies (IHC) performing semiquantitative evaluations of cortical thickness, NeuN+ cell densities, brain inflammation and Tau deposition. Results showed significant improvement in the latency to fall at 14 DPI and 21 DPI, reaching a recovery plateau at 28 DPI in both groups. At nine months, TBI-exposed mice receiving treatment outperformed the TBI alone group, with no significant differences compared to the untreated group. ELISA results showed TBI significantly increased pS199-Tau levels compared to untreated animals. Importantly, mitochondria treatment significantly reduced this increase, keeping pS199 at levels comparable to those of animals not exposed to TBI. Histological studies revealed conserved cortical thickness and increased NeuN+ cell density in treated animals. Our study suggests that systemic administration of mitochondria could be a potential treatment strategy to halt Tau pathology triggered by TBI and associated neurodegeneration. Further studies are needed to validate these findings and explore the potential therapeutic implications for patients affected by TBI.

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Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.17/C87

Topic: C.10. Brain Injury and Trauma

Support: NIH 1R21NS135301-01A1 to DAP and RAM

Title: Selective sequential viral vector delivery of transgenes to glial progenitor cells with temporal specification as a reprogramming strategy to facilitate neuronal subtype lineages

Authors: E. REISENBIGLER[1], *R. A. MARR[2], D. A. PETERSON[1];
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Abstract: Viral delivery of transgenes to the CNS has been widely used for experimental, and increasingly, therapeutic objectives. If the study objectives require expression of multiple genes, the current strategy is coinfection with separate vectors. In addition to the lack of efficiency in ensuring each cell gets one copy of the desired transgenes, all transgenes are expressed simultaneously. However, cell lineage reprogramming studies need to introduce a second gene construct to specify the cell lineage once reprogramming is initiated, thereby mimicking the sequential instructions presented for cell subtype specification during development. The EnvA pseudotyping of rabies virus offers one suggestion for the selective, subsequent infection of a specific cell, in this case, one ectopically expressing the avian TVA receptor. This approach has been used successfully for transsynaptic labeling following a second gene delivery of the EnvA-pseudotyped rabies virus. Inspired by this strategy, we generated a retroviral construct expressing TVA and dsRed and used this to infect adult rat cortical glial progenitor cells (oligodendrocyte progenitor cells- OPCs) in vitro. Infection with the RV-TVA-dsRed resulted in dsRed detection in proliferating OPCs. To achieve a subsequent specific targeting of these TVA-dsRed-expressing OPCs, we pseudotyped lentiviral constructs expressing eGFP using either 1) the commonly used VSVG envelope protein , or 2) an EnvA envelope protein altered to contain the transmembrane region from VSVG. While VSVG-pseudotyping allows entry into a wide variety of cells, EnvA-pseudotyped lentiviral particles will only enter cells expressing the avian TVA receptor. We confirmed this selectivity by the absence of any cells expressing eGFP only in naïve rat OPCs treated with EnvA-pseudotyped lentivirus in vitro. However, EnvA-LV-eGFP infected TVA-dsRed-expressing rat OPCs, leading to detection of the eGFP reporter, but only in TVA-dsRed-expressing cells (resulting in yellow cells). In contrast, VSVG- LV-eGFP infected both TVA- and non-TVA-expressing cells resulting in detection of both yellow and green-only cells, respectively. These results demonstrate the ability to express a reprogramming factor in the first infection where ectopic expression of TVA will allow for selective “follow-up” infection with additional genes for lineage specification at defined temporal intervals. This strategy facilitates neuronal reprogramming and could have much wider implications for any experimental study or therapeutic objective using gene delivery or gene editing.

Disclosures: E. Reisenbigler: None. **R.A. Marr:** None. **D.A. Peterson:** None.

Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

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Topic: C.10. Brain Injury and Trauma

Support: Merit Review Award # B3986-R/1 I01 RX003986-01A1, from the United States Department of Veterans Affairs Rehabilitation Research and Development Service (RR&D)
Spinal Cord Injury Research Program (SCIRP) Investigator-Initiated Research Award # SC210266 from the United States Department of Defense (DoD)

Title: Longitudinal progression of basal ganglia intracerebral hemorrhage in a rat model: A multimodal MRI study

Authors: *N. M. WESTON[1], J. HOU[1,2], R. A. CARRASCOSA[1], D. PLANT[1], F. J. THOMPSON[1], P. K. BOSE[1,2,3];
[1]BRRC, North Florida/South Georgia Veterans Hlth. Syst., Gainesville, FL; [2]Anesthesiol., [3]Neurol., Univ. of Florida Col. of Med., Gainesville, FL

Abstract: Intracerebral hemorrhage (ICH) is a subtype of stroke that is characterized by a bleed into the brain parenchyma. ICH is an emergency medical event that results in high mortality and can result in significant brain atrophy in those who survive. Patients undergo an initial CT or MRI image screening upon arrival at the hospital, which serves to guide the urgency of treatment. Often, after the initial bleed, neurologic symptoms are the primary guide for prognosis and treatment, and do not commonly involve further imaging. Understanding the progression of pathology in relation to recovery would be beneficial to the field, and multimodal MRI may serve this goal. Basal ganglia hemorrhages are the most common ICH, with symptoms that include motor impairments, headache, nausea, emotional dysregulation, and cognitive impairments. In this study, we developed a rat model of basal ganglia ICH and tracked the longitudinal progression of the hematoma pathology along with parameters of neurological function. Five female young-adult Sprague Dawley rats received 0.3µl of 1mg/mL Collagenase IV intracerebrally at a rate of 0.2µl/min with the stereotaxic coordinates of 3.5mm left of Bregma and 4.5mm beneath the skull. MRI data was collected on a 7T/17cm scanner and included T2, Susceptibility Weighted Images (SWI), and Diffusion Tensor Imaging (DTI) at pre-ICH and post-ICH days 0, 1, 2, 5, 7, 14, 21, 28, and 56. The T2 images allowed for careful tracking of the path from the point of injection to the targeted area, in addition to confirming the accuracy of hematoma location between animals. The largest volume of hematoma was observed post-ICH day 2 consistently among animals with a total volume average of 23mm3. Additionally, at post-ICH day 2, we observed a dramatic shift in hematoma composition consistently across all animals, specifically an increase in edema. The SWI data serves to generate quantitative

susceptibility mapping (QSM) to measure direct concentration changes of iron within the hematoma. This allows us to measure the direct proportion of iron to the overall hematoma/edema volume. Midline shift is commonly observed in association with ICH, and in our model, midline shift is presented within post-ICH day 1 and resolved by post-ICH day 7. We examined the performance of quantitative anisotropy (QA) in facilitating deterministic fiber tracking in the ipsilateral hemisphere of the hematoma compared to corresponding areas of the contralateral unaffected hemisphere. The establishment of this chronic basal ganglia ICH model will serve as a foundation for interventional pharmaceutical studies to target the hematoma progression that is associated with these symptoms.

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Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.19/C89

Topic: C.10. Brain Injury and Trauma

Support: NIH grant 1R01NS133233-01A1
NIH grant 1R21AA030625-01

Title: Transmigration of leukocytes and the formation of neutrophil extracellular traps in TBI: a novel peptide therapeutic strategy

Authors: B. SAIKIA, Y. POOVANTHODI, ***P. ABDUL-MUNEER**;
Hackensack Meridian Hlth. JFK Univ. Med. Ctr., Edison, NJ

Abstract: Traumatic brain injury (TBI) causes the blood-brain barrier (BBB) dysfunction and transmigration of inflammatory immune cells into the brain, an important mechanism underlying neurovascular damage and neuroinflammation. Adhesion of leukocytes to endothelial cells is a critical step in the migration of leukocytes into injured tissues. Although multiple factors are involved in the causation of the transmigration of leukocytes to the brain, recently, we have demonstrated that Intercellular Adhesion Molecule 1 (ICAM-1) is one of the key regulators of the transmigration of leukocytes to the brain after TBI. Previously, it has been demonstrated that the activation of leukocytes, especially neutrophils causes the release of nuclear and granular contents to form an extensive web-like structure of DNA called neutrophil extracellular traps (NET). Although the mechanism of the formation of NET and its role in exacerbating neurological deficits in stroke is evident, the role of NET in TBI is not yet fully elucidated. Moreover, it is not clear whether blocking of formation of NET provides better outcomes after

TBI. Therefore, an approach to suppress the formation of NET would be a valuable therapeutic strategy and to analyze the efficacy of the therapy in the functional recovery level after TBI. We hypothesize that inhibition of peptidyl arginine deiminase type 4 (PAD4), an enzyme required for NET formation, using PAD4 antagonistic peptide (PAP) will attenuate the formation of NET and promote neovascularization after TBI. Here, we tested whether PAP reduces PAD4 expression, inhibits NET formation, and promotes neovascularization. We validated the role of PAD4 in the formation of NET by CRISPR/Cas9 mediated PAD4 gene deletion in human brain microvascular endothelial cells (hBMVECs) and human neutrophil co-culture *in vitro* and PAD4 knockout (KO) mice (*PAD4−/−*) *in vivo*. Therefore, in this project, we target a subset of events towards unraveling a larger picture of neurovascular remodeling and functional recovery after TBI by attenuating PAD4 activity using a novel small peptide developed in the PI's lab. This work was supported by the NIH grants 1R01NS133233-01A1 and 1R21AA030625-01.

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Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.20/C90

Topic: C.10. Brain Injury and Trauma

Support: DoD Grant AZ180035

Title: Chronic glial activation and behavioral alterations induced by acute/subacute pioglitazone treatment in a mouse model of traumatic brain injury

Authors: L. ESTRELLA, J. MANGANARO, A. SHELDON, B. LAMBERTY, ***K. STAUCH**; Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Traumatic brain injury (TBI) is a disabling neurotraumatic condition and the leading cause of injury-related deaths and disability in the United States. Attenuation of neuroinflammation early after TBI is considered an important treatment target; however, while these inflammatory responses can induce secondary brain injury, they are also involved in the repair of the nervous system. Pioglitazone, which activates peroxisome proliferator-activated receptor gamma (PPAR-γ), has been shown to decrease inflammation acutely after TBI, but the long-term consequences of its use remain unknown. For this reason, the impacts of treatment with pioglitazone during the acute/subacute phase (30 min after injury and each subsequent 24 hours for 5 days) after TBI were interrogated during the chronic phase (274 days after injury) in mice using the controlled cortical impact model of experimental TBI. Acute/subacute pioglitazone treatment after TBI results in long-term deleterious consequences, including

disruption of tau homeostasis, chronic glial cell activation, and worsened injury severity, with male mice being more susceptible than female mice. Further, male pioglitazone-treated TBI mice exhibited increased dominant and offensive-like and decreased non-social exploring behaviors. This work reveals that timing and long-term consequences of treatment with glitazones must be considered and further studied prior to their use in the clinic for TBI therapy.

Disclosures: L. Estrella: None. **J. Manganaro:** None. **A. Sheldon:** None. **B. Lamberty:** None. **K. Stauch:** None.

Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.21/C91

Topic: C.10. Brain Injury and Trauma

Support: David's Fund of the Community Foundation of New Jersey

Title: The impact of music-based interventions on the psychiatric and psychological outcomes of traumatic brain injury: a systematic review

Authors: *R. TABUCHI, A. MARINO, E. KANG, J. SANDRY;
Montclair State Univ., Montclair, NJ

Abstract: Traumatic brain injury (TBI) is caused by an external force inflicted on the head, which disrupts normal brain functioning. Moderate to severe TBI is particularly associated with psychiatric and neurobehavioral issues, engendering an emergent need for rehabilitative care. In accordance with this need, different interventions have been explored including, in a limited capacity, music. Recent literature has discussed the effectiveness of music for a range of psychopathologies, such as anxiety, apathy, and mood disorders, noting music's ability to induce feelings of pleasure and its relationship to brain reward circuitry. However, whether music can effectively mitigate the social, emotional, and behavioral difficulties of TBI is currently unclear. Addressing these concerns, this systematic review investigates the impact of music-based interventions on psychiatric and psychological outcomes of TBI. We searched five databases (MEDLINE via PubMed, Embase, APA PsycINFO, Cochrane Central Register of Controlled Trials, and CINAHL Complete), and the population of our interest was restricted to adults (age 18+) with TBI. Two reviewers independently completed the study screening, data extraction, and quality assessment using the Cochrane risk of bias 2 tool for randomized controlled trials (RCTs) and risk of bias in non-randomized studies - of interventions tool for observational studies. Data on study and intervention characteristics, measures, and psychiatric/psychological outcomes (stress, mood, anxiety, depression, social interaction, agitation, inertia, and sleep quality), were

extracted. PROSPERO ID: CRD42024528029. We identified 2,467 articles, and 8 studies were included (n = 85 TBI patients). Five studies (62.5%) were observational studies while three studies (37.5%) used RCTs. The mean time since injury ranged from 40 days to 8 years, and the severity covered mild (n = 8), moderate (n = 22), and severe (n = 55). The methods of music engagement varied, including music listening, song singing, instrumental playing, musical improvisation, relaxation with music, and music lessons. The included studies ranged from some concerns to critical in quality. Our review suggests that a broad scope of music-based interventions, both receptive and active, may improve the psychiatric and psychological outcomes in adult TBI patients. This result proposes the use of music as an accessible and cost-effective nonpharmacological intervention to address psychiatric and neurobehavioral problems of TBI. Future studies should address limitations on the heterogeneity of interventions and poor study quality.

Disclosures: R. Tabuchi: None. **A. Marino:** None. **E. Kang:** None. **J. sandry:** None.

Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.22/C92

Topic: C.10. Brain Injury and Trauma

Support: CDMRP/DoD Gulf War Illness Research Program Innovative Treatment Evaluation Award # 11488016
VA Office of Academic Affiliations

Title: Changes In Brainstem Structure Following Treatment For Chronic Pain In Gulf War Illness

Authors: P. J. BAYLEY[1,2], Y. ZHANG[1], D. C. MATHERSUL[3], J. W. ASHFORD, Jr.[1,2], *A. J. FURST[1,2];
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Abstract: Nonpharmacological therapies have shown efficacy for treating chronic pain in veterans with Gulf War Illness (GWI). We have previously shown that brainstem structures play an important role in pain sensation and modulation in GWI. The current study aimed to use brainstem imaging to assess the effects of pain treatments in a subset of veterans who were participating in a randomized controlled trial comparing yoga therapy to cognitive behavioral therapy (CBT) for chronic pain and other symptoms of GWI.
At baseline 13 veterans with GWI (aged 45-64 years, 10 males) were assessed using a range of

standard clinical measures and a structural MRI. Participants then received 10 weeks of either yoga therapy (n=6) or CBT (n=4) consisting of 10 weekly group sessions with home practice. At the end of treatment, a total 10 participants were given clinical assessments and a second MRI. Pre- and post-treatment MRI data analysis measured gray matter volumes in brainstem regions-of-interests, and fractional anisotropy (FA) of brainstem white matter tracts.At baseline, after controlling for age, sex and education, lower FA in the dorsal longitudinal fasciculus (DLF) was associated with higher pain severity ($r = -.916$, $p = 0.001$) on pain intensity ratings of the Brief Pain Inventory (BPI), and the McGill Pain Questionnaire-Short Form (MPQ-SF) visual analogue pain scale ($r = -.809$, $p = 0.015$). At end of treatment, improvements were found in the degree to which pain interferes with daily function (BPI pain interference, $t = -3.03$, $p = .01$) and the affective dimension of pain (MPQ, $t = -3.25$, $p = .01$). Improvements in mood were also found, as measured by the Tension scale of the Profile of Mood States-Short Form ($t = -4.39$, $p = .002$), and the Emotional/Wellbeing scale of the SF-36 Health Questionnaire (SF-36) ($t = -3.17$, $p = .01$). Brainstem imaging showed limited changes following treatment. Correlational tests showed that decreased BPI pain interference ($r = -.831$, $p = .006$) and BPI pain intensity ($r = -.640$, $p = .46$) following treatment were associated with increased volume in the periaqueductal gray (PAG). Increased PAG volume after treatment was also associated with improved energy ($r = .653$, $p = .041$) and emotion ($r = .708$, $p = .022$) as measured by the (SF-36).
The findings suggest treatment of chronic pain in GWI is associated with an increase in the PAG volume, possibly due to a treatment-related enhancement of endogenous pain modulation. Furthermore the integrity of the DLF may be related to chronic pain perception, rather than a direct response to pain treatment.

Disclosures: P.J. Bayley: None. **Y. Zhang:** None. **D.C. Mathersul:** None. **J.W. Ashford:** None. **A.J. Furst:** None.

Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.23/C93

Topic: C.10. Brain Injury and Trauma

Title: Direct Motor Thalamus Stimulation Facilitates Motor Output: Immediate and Long-Term Effects

Authors: *L. W. TANG[1], E. M. GRIGSBY[2], J. C. HO[1], A. DAMIANI[2], D. J. CRAMMOND[3], T. CONSTANTINE[3], M. CAPOGROSSO[3], J. A. GONZÁLEZ-MARTÍNEZ[3], E. PIRONDINI[4]; [1]Sch. of Med., [2]Physical Med. & Rehabil., [3]Dept. of Neurolog. Surgery, [4]Rehabil. and Neural Engin. Labs., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Neuromodulation using deep brain stimulation (DBS) has been established as a safe and effective treatment to relieve symptoms for movement disorder patients. In recent work, we demonstrated in anesthetized non-human primates (NHPs) that low-frequency (50-80Hz) stimulation of the motor thalamus recruits afferent fibers to the motor cortex to enhance the excitability of the corticospinal tract (CST) and corticobulbar tract (CBT), potentiating upper-limb and facial muscle motor evoked potentials, even after lesions of the white matter tracts. We then tested whether these effects translated to human subjects in one patient who suffered severe traumatic brain injury with bilateral lesions of the CST and CBT and underwent DBS implants in the motor thalamus to treat tremors. The patient presented with hyperreflexia, bilateral arm and facial paresis, and profound dysarthria. We assessed the immediate effect of motor thalamus stimulation on voluntary movements of the left arm and the face. The participant performed grip strength, reach tests, and voluntary facial motor tasks with and without stimulation. Bilateral motor thalamus stimulation at 55 Hz led to an immediate statistically significant increase in shoulder abduction, elbow extension, and arm elevation. Similarly, stimulation of the motor thalamus enhanced voluntary facial movements as compared to periods without stimulation. Importantly, both EMG and kinematic recordings showed the largest and fastest movements with acute stimulation at 55Hz as compared to standard clinical stimulation at 130Hz further confirming the optimal stimulation parameters as determined in NHPs. With the clinical stimulation parameters set to 55Hz, we then evaluated the long-term effects of thalamic DBS and continued longitudinal follow-up for 2 years. In addition to completing voluntary facial motor tasks across 3 sessions, the participant's caregivers documented their daily activity and motor recovery exercises for over 5 months. Results were plotted across time for visualization of longitudinal trends. Repetition of the voluntary facial tasks showed a consistent increase in amplitude and mean velocity of facial movement over time. Documented daily activities tolerance, specifically steps and standing time, increased by 40%. These results demonstrate the immediate and long-term restorative effects of thalamic stimulation on enhancing facial and limb muscle activation, to ultimately improve motor function. Further experiments in a larger patient population will be necessary to confirm our results.

Disclosures: L.W. Tang: None. **E.M. Grigsby:** None. **J.C. Ho:** None. **A. Damiani:** None. **D.J. Crammond:** None. **T. Constantine:** None. **M. Capogrosso:** None. **J.A. González-Martínez:** None. **E. Pirondini:** None.

Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.24/C94

Topic: C.10. Brain Injury and Trauma

Support: Private donation

Title: Changes in brain structure and age in Veterans with TBIs following treatment with magnesium-ibogaine

Authors: A. GEOLY[1], ***J. COETZEE**[1], W. STRUCKMANN[1], D. BUCHANAN[1], A. AZEEZ[1], B. KIM[1], K. CHERIAN[1], M. M. ADAMSON[2], N. WILLIAMS[1];
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Abstract: Introduction: TBI is common among Veterans of recent conflicts, and may lead to a range of symptoms, as well as accelerated brain aging. Ibogaine, a psychoactive alkaloid, has neuroplasticity-promoting properties. It may help remodel neural circuitry and improve functioning in Veterans with TBI. **Methods:** We conducted an observational study with 30 Veterans with multiple blast TBI (mbTBI) and complex clinical problems who received ibogaine treatment, preceded and followed by preparation and integration. At baseline, immediate post, and 1-month, we performed clinical assessments and structural MRI scans. We derived cortical thickness (CT) measures with the ANTs longitudinal CT pipeline and evaluated CT and volume in cortical and subcortical gray matter, and in cerebellar ROIs. To evaluate longitudinal changes in CT across ROIs, we used linear mixed effects (LME) models. We used the algorithm brainageR to measure brain age. **Results:** A Wald $X2$ test of regional LME models revealed a significant (pFDR less than 0.05) effect of study visit on CT in 13 ROIs. Pairwise t-tests demonstrated significant (pholm<0.05) increases in CT following ibogaine relative to baseline visit in 11 regions. For subcortical volume, Wald $X2$ test of the subcortical LME models revealed a significant (pFDR less than 0.05) main effect on the log-jacobian determinant in the Right Ventral Diencephalon. Wald $X2$ test of the LMEs revealed a significant change in brain age across time points [$X2(2)=10.64$, $p=0.0049$]. Post-hoc t-tests gave a significant (pholm less than 0.05) reduction of 1.60 years in predicted brain age relative to baseline one month after treatment ($t=3.18$, $p=0.0082$, $d=1.035$).
Conclusions: This provides the first evidence of measurable brain morphometric changes in humans following ibogaine therapy. More research is needed to understand the mechanisms by which ibogaine works and to determine long-term impact on cortical structure.

Disclosures: A. Geoly: None. **J. Coetzee:** None. **W. Struckmann:** None. **D. Buchanan:** None. **A. Azeez:** None. **B. Kim:** None. **K. Cherian:** None. **M.M. Adamson:** None. **N. Williams:** None.

Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.25/C95

Topic: C.10. Brain Injury and Trauma

Support: private donor

Title: Exploring Neural Correlates Of Ibogaine In Special Forces Combat Veterans Through Multimodal Imaging

Authors: A. AZEEZ[1], M. SRIDHAR[1], A. GEOLY[1], A. FAERMAN[1], K. CHERIAN[1], J. P. COETZEE[1,2], S. HUNEGNAW[1], D. BUCHANAN[1], J. N. KEYNAN[3,4], C. ROLLE[1], ***M. M. ADAMSON**[1,2], M. SAGGAR[1], N. WILLIAMS[1];
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Abstract: Objective: This analysis sought to identify the neural mechanisms underlying the strong therapeutic results from a recent study that evaluated the safety and clinical impact of ibogaine in treating military veterans with traumatic brain injury (TBI). TBI is a leading cause of disability with sequelae of psychiatric symptoms such as post-traumatic stress disorder (PTSD), major depressive disorder (MDD), and generalized anxiety disorder (GAD).
Methods: We collected arterial spin labeling (ASL) and blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) data at three-time points pre and post-treatment on 30 Special Operations Veterans (SOV) who had voluntarily enrolled in tabernanthe iboga exposure at a clinic in Mexico. We used a multimodal whole-brain resting-state exploratory approach of examining changes to regional Cerebral Blood Flow, Functional Connectivity, and Network communication to characterize neural features that were altered post-ibogaine treatment.
Results: Significant changes were identified in blood flow (p less than 0.001, PFDR less than 0.05), functional connectivity (p less than 0.005), and networks of the limbic and sensory-motor system, regions associated with TBI and PTSD. We found associations between neuroimaging findings in the left hemisphere insula, anterior cingulate cortex, and hippocampus-dorsal attention network with clinical measures of disability index and PTSD symptomology.
Conclusions and Relevance: Our novel multimodal neuroimaging approach revealed potential mechanisms underlying the therapeutic benefits of ibogaine for SOV suffering from TBI with comorbid disability and psychiatric symptoms. Further research with larger and diverse populations would be beneficial to establish clinical and neuroimaging alterations from ibogaine on subjects without lifetime TBIs or combat-induced PTSD.

Disclosures: A. Azeez: None. **M. Sridhar:** None. **A. Geoly:** None. **A. Faerman:** None. **K. Cherian:** None. **J.P. Coetzee:** None. **S. Hunegnaw:** None. **D. Buchanan:** None. **C. Rolle:** None. **M.M. Adamson:** None. **M. Saggar:** None. **N. Williams:** None.

Poster

PSTR273: Therapeutic Strategies for Brain Injury With a Human and Non-Primate Mammal Focus

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR273.26/C96

Topic: C.10. Brain Injury and Trauma

Support: NJCBIR20PIL004
Department of Defense W81XWH-22-1-0616
Osteopathic Heritage Foundation

Title: Sex-dependent restoration of risky decision making and prefrontal catecholamine regulatory proteins using low-dose methylphenidate following repeated mild traumatic brain injury

Authors: *E. PAPADOPOULOS[1], A. ABRIMIAN[1], C. KNAPP[2], B. D. WATERHOUSE[2], R. L. NAVARRA[2];
[1]Rowan-Virtua SOM, Stratford, NJ; [2]Cell Biol. and Neurosci., Rowan-Virtua STBES, Stratford, NJ

Abstract: Head trauma often impairs decision making processes mediated by the prefrontal cortex (PFC) leading to increased risk-taking behavior. Although many individuals including athletes and military personnel experience repeated mild traumatic brain injuries (rmTBIs), the consequences of sustaining multiple traumatic events and whether such effects are sex-dependent remain elusive. The catecholamine neurotransmitters, dopamine (DA) and norepinephrine (NE), modulate the PFC's actions and require precise regulation for optimal PFC operations. Imbalances in catecholamine function are theorized to underlie aberrant decision making following TBI. The psychostimulant, methylphenidate (MPH), elevates catecholamine levels by blocking DA and NE reuptake transporters (NET). Due to MPH's efficacy in reducing risky behavior in patients with ADHD, it has been considered as treatment for alleviating similar neurocognitive symptoms following TBI. Here we used a closed head-controlled cortical impact model to induce up to 3 mTBIs, the probabilistic discounting task of risky decision making, and western blotting to determine the effects of chronic low-dose MPH (0.5 and 2 mg/kg, i.p.) on risky behavior and catecholamine regulatory protein levels within specific PFC subregions following rmTBI in male and female rats. rmTBI alone increased risky choice preference in saline-treated females, but not males. MPH *prevented* rmTBI-induced risky choice in females but *promoted* risky choice preference in rmTBI males. Within the medial PFC, expression levels of packaging protein vesicular monoamine transporter (VMAT) were decreased in both male and female saline-treated rmTBI groups. MPH treatment normalized VMAT levels in injured females but not males. Within the orbitofrontal cortex, VMAT and NET were decreased only in males exposed to both rmTBI and MPH. Our results suggest that females are more susceptible to rmTBI-induced behavioral disruption and rmTBI reduces transporter levels within subregions of

the PFC. In addition, MPH treatment produces *restorative* benefits in females, but *exaggerated* pathological outcomes in males. This study is the first to reveal a potential sex-specific psychostimulant therapeutic strategy for rmTBI-induced risky behavior and neuropathological outcomes.

Disclosures: E. Papadopoulos: None. **A. Abrimian:** None. **C. Knapp:** None. **B.D. Waterhouse:** None. **R.L. Navarra:** None.

Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR274.01/C97

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH T32
HHMI

Title: Sars-cov-2 Main protease activates sensory neurons

Authors: *Z. GONG[1], S. S. MALI[2], C. COOK[2], D. BAUTISTA[3,4];
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Abstract: SARS-CoV-2 infection elicits a range of symptoms including sneezing, coughing, respiratory distress, and pain. These symptoms are mediated by sensory neurons that innervate the skin and viscera, but little is known about how these neurons are activated by viral infection. Proteases derived from bacteria, allergens and plants directly act on sensory neurons to trigger itch, pain and airway dysfunction. We thus asked if the SARS-CoV-2 Main protease (Mpro), that is required for viral replication, also activates sensory neurons.Using in vivo calcium imaging in Pirt-cre; GCaMP6s mice (n=8 animals), we found that 35.45% of trigeminal neurons displayed an increase in intracellular calcium following intranasal protease administration. 51.28% of Mpro-activated neurons also respond to capsaicin, suggesting that Mpro activates a subset of airway innervating nociceptors. To assess whether Mpro acts directly on neurons, we used in vitro calcium imaging dorsal root ganglia neurons (n=3 animals). We see 3.07% neurons show an increase in calcium after Mpro treatment, 88.73% of which are also activated by both AITC and capsaicin. These findings reveal a novel role for SARS-CoV-2 Mpro in the activation of nociceptors that may promote pain and inflammation.

Disclosures: Z. Gong: None. **S.S. Mali:** None. **C. Cook:** None. **D. Bautista:** None.

Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR274.02/C98

Topic: D.01. Somatosensation – Pain and Itch

Support: Howard Hughes Medical Institute (HHMI)
NSF Grant DGE 1752814
NSF Grant DGE 2146752

Title: Characterizing pain-like behaviors and inflammation in a mouse model of Atopic Dermatitis

Authors: *L. N. MURPHY[1], C. COOK[1], Y. SCOTT[1,3], E. A. LUMPKIN[1,2], D. M. BAUTISTA[1,2,3];
[1]Mol. and Cell Biol., [2]Helen Wills Neurosci. Inst., UC Berkeley, Berkeley, CA; [3]Howard Hugh Medicial Inst., Berkeley, CA

Abstract: Atopic dermatitis (AD), also known as eczema, is a chronic inflammatory skin disease that causes redness, skin lesions, and debilitating itch. AD-induced itch is mediated by aberrant interactions between keratinocytes, immune cells, and sensory neurons. Clinical studies have shown that approximately 60% of individuals with AD also experience pain with some describing their itch using terms commonly associated with neuropathic pain, such as burning, prickling, and tingling.[1,2] However, little is known about the mechanisms by which this skin disease leads to persistent pain. We developed an unbiased approach to characterize a range of behaviors that occur in the MC903 mouse model of AD. Specifically, we applied MC903 to the shaved left cheek of 8-14 week old wild-type male and female mice for 7 consecutive days. Mice were acclimated to recording chambers on the last day of MC903 application. On day 8, mouse behavior was recorded using cameras positioned at multiple angles for subsequent behavior scoring. The treated cheek skin tissue was collected at the end of the experiment to conduct flow cytometry to assess immune cell infiltration. As previously described, MC903-treated mice exhibited increased total scratching time, number of scratching bouts, and number of neutrophils, basophils, and $CD4^+$ T cells in the skin, compared to vehicle-treated mice. We also observed and quantified a range of behaviors including single arm wiping as a metric of pain-like behavior and grooming. We found that the MC903-treated mice displayed more single forearm wipes than vehicle-treated mice and spent less time grooming. Furthermore, we observed unreported behaviors and disruptions to the highly stereotyped pattern of grooming behavior. Our preliminary data shows that mice treated with MC903 wiped their face with both forearms outside of grooming sequences more compared to vehicle-treated mice. This work establishes a platform to study the mechanisms that drive different pain- and itch-like behaviors in a mouse model of Atopic Dermatitis.
REFERENCES: [1]Silverberg, Jonathan I., et al. "Pain is a common and burdensome symptom of

atopic dermatitis in United States adults." The Journal of Allergy and Clinical Immunology: In Practice 7.8 (2019): 2699-2706. [2]Vakharia, Paras P., et al. "Burden of skin pain in atopic dermatitis." Annals of Allergy, Asthma & Immunology 119.6 (2017): 548-552.

Disclosures: L.N. Murphy: None. **C. Cook:** None. **Y. Scott:** None. **E.A. Lumpkin:** None. **D.M. Bautista:** None.

Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR274.03/C99

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH grant F31NS129269
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NIH grant R61NS127271
The Eugene McDermott Foundation
American Physiological Society SURF

Title: Loss of Sigma-2/TMEM97 is associated with increased mechanical hypersensitivity during the inflammatory pain resolution in both germline and nociceptor specific knockout mice

Authors: *V. M. HONG[1,2], A. RADE[1], Z. SYED[1], B. A. JAISWAL[1], C. FOFIE KUETE[1,2], M. YOUSUF[1,2], D. J. LIEBL[3], S. MARTIN[4], T. J. PRICE[1,2], B. J. KOLBER[1,2];
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Abstract: The Sigma-2 receptor/TMEM97 (σ_2R/TMEM97) is a transmembrane protein located in the plasma and endoplasmic reticulum (ER) membrane with promising analgesic properties, as indicated by recent studies. A ligand targeting σ_2R/TMEM97, FEM-1689 (20 mg/kg), has shown efficacy in reducing neuropathic pain-induced mechanical hypersensitivity. However, the involvement of *Tmem97* in inflammatory pain-like responses has been less studied compared to neuropathic pain. Our study aims to elucidate the role of σ_2R/TMEM97 in inflammatory pain-like responses that resemble pathological pathways implicated in diseases affecting a larger population, thereby providing significant translational impact. Based on previous studies, we hypothesized that inhibiting σ_2R/TMEM97 function would increase the inflammatory pain-like response. To test this hypothesis, we used global *Tmem97* knockout (KO) and nociceptor-specific conditional *Tmem97* knockout mice (cKO) models (both male and female at 2-3 months) for target validation to examine the cellular role of σ_2R/TMEM97, as compared to its pharmacological response. First, we assessed mechanical and thermal stimulated pain-like

behaviors using a Complete Freud's Adjuvant (CFA)-induced inflammatory pain model. Studies were completed blinded to mouse genotype. We then measured *Tmem97* RNA expression changes in the dorsal root ganglion (DRG) before and after CFA injection using RNAscope fluorescent in situ hybridization (FISH) followed by immunohistochemistry (IHC). Our results showed that *Tmem97* KO mice exhibited prolonged hypersensitivity to mechanical stimulation using von Frey testing, but not to thermal stimulation using the thermal plantar assay and acetone test compared to wildtype (WT) mice. The *Tmem97* cKO mice showed a similar behavioral effect, albeit to a lesser degree. We also observed a decrease in *Tmem97* RNA expression in the paw-innervating DRG, reaching a maximum decrease three days post-CFA injection, which returned to baseline as inflammatory pain-like responses resolved. We found that the loss of *Tmem97* exacerbated the resolution of inflammatory pain in a sensory modality-dependent manner. Our study provides insight into the peripheral role of σ_2R/TMEM97 in inflammatory pain modulation, suggesting it as a promising therapeutic target for inflammation-associated pain. Future research aims to characterize the functional electrical activity of isolated mouse DRG from WT, *Tmem97* KO, and nociceptor-specific *Tmem97* cKO cells to delineate potential analgesic effects at the cellular level and explore possible mechanisms of action.

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Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR274.04/C100

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH T32
USASP MAYDAY Clinical/Translational Research Award

Title: Inflammatory and hormonal mediators in patients with endometriosis.

Authors: *A. DOURSON[1], M. FLUEGEL[2], A. MCMICHAEL[1], J. BROWN[3], R. W. GEREAU IV[4], H. NAHMAN-AVERBUCH[5];
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Abstract: Endometriosis is a chronic inflammatory disease affecting an estimated 10% of females. Adherent, benign lesions arise in this disease forming on multiple visceral organs including the uterus, bladder, and peritoneal wall. There is no agreed upon cause of

endometriosis although reverse menstruation has been proposed as a possible mechanism. However, most women who experience reverse menstruation will not form the disease. Endometriosis causes extreme abdominal pain as well as infertility both of which significantly decrease patient quality of life. In addition, the time to diagnose endometriosis is, on average, 10 years from the onset of symptoms. The disease is classified into four stages defined by the location, number, and severity of lesions. As the determined stage increases, so does the reported pain and infertility of the patient. However, little is known about lesion inflammatory and hormonal microenvironment between lesions and stages of disease. Unfortunately, there are few therapeutic options available for patients.
Removal of lesions by minimally invasive laparoscopic surgery results in a rescue of abdominal pain and infertility in some women. However, of those that had successful pain remediation, about 50% will have a return of pain and lesions, necessitating multiple surgeries. Hormone therapy is another method to treat the pain caused by endometriosis, but the side effects are often unacceptable to patients. While endometriosis is known to be an inflammatory disease and that estrogen levels regulate symptoms, the underlying microenvironment of these factors within and between lesions is unknown. We hypothesized that lesions contain increased levels of inflammatory and hormonal regulators which correlate to disease stage and lesion severity.
We evaluate women undergoing surgery to remove lesions. Women are first identified by obstetric and gynecological providers with specialties in gynecological surgery. Following consent and during surgery, we collect biopsies including both healthy and abnormal tissues. Disease severity score is provided by surgeons. Collected samples are snap frozen for downstream analyses including real-time quantitative PCR (qPCR) and ELISA. Preliminary results indicate increases in inflammatory and hormonal mediators within lesions compared to healthy peritoneal tissue. Specifically, interleukin-1 beta (IL-1β) and IL-6, as well as estrogen receptors one and two (ESR1 and ESR2) are elevated within lesions. In addition, pro-inflammatory mediators positively correlate with one another and lesion severity. These data suggest a possible role of these factors underlying endometriosis.

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Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR274.05/C101

Topic: D.01. Somatosensation – Pain and Itch

Title: The mechanism of CB_1R mediated analgesia in post-operative pain via regulating ASIC3

Authors: *L. JI[1], K. XU[2], Z. WANG[3];
[1]Southern Univ. of Sci. and Technol., Shenzhen, Guangdong, China; [2]Southern Univ. of Sci. and Technol., Shenzhen, China; [3]Sch. of Med., Southern Univ. of Sci. and Technol., Shenzhen, China

Abstract: Post-operative pain is the most common and urgent pain in the clinic. Previous studies have shown that cannabinoid type 1 receptor (CB_1R) can exert analgesic effects in pain, but due to its widespread expression in the central nervous system, drugs targeting CB_1R may have adverse effects such as psychosis and addiction due to its widespread activation in the brain, limiting its therapeutic potential. In the peripheral nervous system, CB_1R is mainly expressed in dorsal root ganglion (DRG) neurons. The inhibitory effect of peripheral restricted CB_1R agonists on neurons may involve various ion channels and receptors, including acid sensitive ion channels, among which acid sensitive ion channel 3 (ASIC3) is a subtype mainly expressed in the peripheral nervous system. In this study, we aim to determine whether CB_1R in DRG neurons can alleviate post-operative pain caused by incision injury by regulating the function of ASIC3. We establish mouse toe incision post-operative pain model to test the role of ASIC3 and CB_1R with the non-selective agonist of cannabinoid receptors (WIN55, 212-2, WIN) and the selective antagonist of ASIC3 (APETx2), and Von Frey test results show that both WIN and APETx2 can significantly alleviate post-operative pain sensitivity, but CB_2R selective agonist AM1241 can't alleviate mechanical hyperalgesia. Afterwards, planter injection of GMQ (0.1-3 mM) can induce spontaneous nociceptive behavior in WT mice, and planter injection of WIN 15 min before GMQ injection can reduce the spontaneous nociceptive behavior and raise the mechanical pain threshold in both WT and $CB_2R^{-/-}$ mice, while the selective antagonist of CB_1R (AM251) can significantly reverse the analgesic effect. Meanwhile, we have demonstrated the expression characteristics of CB_1R and ASIC3 in DRG neurons using immunofluorescence and in situ hybridization techniques, nearly 30% co-expression of Cnr1 and ASIC3 in $ASIC3^+$ DRG neurons, 70% co-expression of *Cnr1* and ASIC3 in *Cnr1*$^+$ DRG neurons, 50% co-expression of *Cnr1* and CGRP in $CGRP^+$ DRG neurons. Besides, ASIC3 was mainly expressed in $CGRP^+$ and $IB4^+$ DRG neuronal endings in the spinal dorsal horn. Transgenic mice ($Advillin^{CreER/+}$;*Cnr1*$^{flox/flox}$, $Trpv1^{CreER/+}$;*Cnr1*$^{flox/flox}$, $Trpv1^{Cre/+}$;DTR) have been used to demonstrate that activation of CB_1R in DRG large diameter neurons can inhibit GMQ-induce spontaneous pain. Using calcium imaging assay, we find that attenuation of WIN-induce intracellular calcium mobilization in primary DRG neurons treated by GMQ. Together, activation of CB_1R in DRG neurons can regulate the function of ASIC3, which may be a potential mechanism for CB_1R to inhibit post-operative pain sensitivity.

Disclosures: L. Ji: None. **K. Xu:** None. **Z. Wang:** None.

Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR274.06/C102

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant NS132398

Title: G9a in primary sensory neurons promotes inflammatory pain and Trpa1 and Trpv1 transcription via bivalent histone modifications

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Abstract: Transient receptor potential ankyrin 1 (TRPA1) and vanilloid 1 (TRPV1) play crucial roles in detecting and transmitting nociceptive stimuli. Inflammatory pain is associated with increased TRPA1 and TRPV1 expression in primary sensory neurons. However, the epigenetic mechanisms underlying their upregulation remain largely unknown. G9a (encoded by *Ehmt2*) catalyzes H3K9me2 and is typically involved epigenetic repression of gene transcription. In this study, we found that intrathecal administration of UNC0638, a specific G9a inhibitor, or G9a-specific siRNA substantially reduced pain hypersensitivity induced by complete Freund's adjuvant (CFA) in rats. Correspondingly, CFA treatment failed to induce persistent pain hypersensitivity in mice with conditional *Ehmt2* knockout in dorsal root ganglion (DRG) neurons. Quantitative PCR and RNA sequencing analyses showed that CFA treatment caused a sustained increase in *Trpa1* and *Trpv1* mRNA levels in the DRG. Conditional *Ehmt2* knockout in DRG neurons potentiated baseline *Trpa1* and *Trpv1* mRNA levels but remarkably reversed CFA-induced persistent increases in *Trpa1* and *Trpv1* expression. Furthermore, chromatin immunoprecipitation revealed that CFA treatment diminished the abundance of G9a and H3K9me2 but concurrently enhanced the enrichment of H3K9ac and H3K4me3—two activating histone marks—at *Trpa1* and *Trpv1* promoters in the DRG. Strikingly, conditional *Ehmt2* knockout in DRG neurons not only diminished H3K9me2 at *Trpa1* and *Trpv1* promoters but also reversed CFA-induced increases in H3K9ac and H3K4me at these promoters. These findings suggest that G9a in primary sensory neurons has a dual function: constitutively repressing *Trpa1* and *Trpv1* transcription whereas paradoxically promoting their transcription during tissue inflammation. This latter activity primarily mediates inflammatory pain.

Disclosures: K. Ghosh: None. **Y. Huang:** None. **D. Jin:** None. **S. Chen:** None. **H. Pan:** None.

Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR274.07/C103

Topic: D.01. Somatosensation – Pain and Itch

Support: DoD CDMRP award W81XWH-20-1-0854
Charles Henry Leach II Fund

Title: Rnaseq of dorsal root ganglia uncovers sexual dimorphism in response to neuroimmune modulation with cox-2 inhibiting nanoemulsions

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Abstract: Pain is present in disease and health. Understanding pain begins in the peripheral nervous system where different injuries elicit different pain sensations through distinct biological mechanisms. Sex is a biological variable for pain. Using a rat sciatic nerve chronic constriction injury, hypersensitivity assessed by the von Frey technique shows that males and females develop pain-like behavior on the same time course and to the same degree[1]. Treated with an intravenous injection of a nanoemulsion containing the COX-2 inhibitor celecoxib[2], the nanodroplets are phagocytosed by circulating monocytes. As macrophages, they naturally accumulate at the site of injury, delivering the drug there. A single dose that relieves pain for 6 days and thousands-of-fold less drug as compared to oral dosing[1,3]. The significant reduction in hypersensitivity is associated with a reduction of macrophages at the injury[1,3]. While males achieve complete, multi-day relief, females only realize partial relief[1] revealing that differences in the neuro-inflammatory responses are at play in males as versus females. Increasing the dose of celecoxib per nanodroplet provides equivalent relief from hypersensitivity for both sexes[4]. RNA sequencing identified differential gene expression in the affected dorsal root ganglia (DRG) between sexes during pain and also during pain relief[4]. Significant sex differences in expression are evident as a result of nerve injury and during pain relief resulting from the COX-2 inhibition. While the nanotherapeutic achieved equivalent behavioral pain relief between the sexes, the sex differences in RNA expression reveals that comparable behavior does not necessitate the same gene expression.
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2. Herneisey M *et al.* (2018) AAPS PharmSciTech 20, 65 (2019). https://doi.org/10.1208/s12249-018-1287-6
3. Janjic J M et al (2018) J Neuroimmunol 318: 72-79. https://doi.org/10.1016/j.jneuroim.2018.02.010.
4. Deal B et al. (2023) International Journal of Molecular Sciences 24, no. 11 (2023): 9163. https://www.mdpi.com/1422-0067/24/11/9163.

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Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

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Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant U19NS130608
NIH Grant R01NS065926
NIH Grant R01NS111929
NIH Grant K00NS120636

Title: Behavioral and electrophysiological characterization of dorsal root ganglion neurons from Complete Freund's Adjuvant-treated rats

Authors: *K. E. MCDONOUGH[1], P. M. DOUGHERTY[2];
[1]Univ. of Texas MD Anderson Cancer Ctr., Houston, TX; [2]Pain Med., Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract: Spontaneous activity of neurons within the dorsal root ganglion (DRG) is associated with various pain models. Lipid rafts are highly dynamic cholesterol-rich domains on the cell membrane which can organize excitatory ion channels and receptors. Thus, the disruption of these lipid rafts impacts neuronal excitability. Apolipoprotein A-1 binding protein (AIBP) binds to toll-like receptor 4 (TLR4), augmenting efflux of cholesterol from cell membranes, therefore disrupting lipid rafts in TLR4-expressing neurons, which has recently been shown to attenuate the generation of neuropathic and inflammatory pain. In this study we assess whether CFA injection produces spontaneous activity in DRG neurons, and if so, whether this could be attenuated by *in vitro* treatment with AIBP. To do so, we administered a single intraplantar injection of 100 µg of CFA into the left hind paw of adult male rats. Paw width was measured with calipers before injection and at 48h and 8d post-injection. Mechanical hypersensitivity was measured using the vonFrey behavioral assay. A secondary aim of this study was to assess whether proprioceptive changes could also be assessed in our rodent pain models, therefore gait was measured using the CatWalk system. 72h or 8d after CFA injection, the lumbar DRG were dissociated. Electrophysiology experiments were conducted on primary cultured DRG neurons. Spontaneously active cells were treated with bath-perfusion of AIBP (0.5 µg/mL). Preliminary data shows that intraplantar CFA injection produces spontaneous activity in DRG neurons. This spontaneous activity is inhibited by AIBP. Further experiments are underway to assess whether this also occurs in females.

Disclosures: K.E. McDonough: None. **P.M. Dougherty:** None.

Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR274.09/C105

Topic: D.01. Somatosensation – Pain and Itch

Support: Stanford-Coulter Translational Research Grant

Title: Focused ultrasound-induced changes to animal pain behaviors and peripheral nerve structure

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Abstract: Introduction Moderate-to-severe acute pain is prevalent in many healthcare settings and associatedwith adverse outcomes. Opioids primarily block C fibers, not A-delta fibers and areassociated with many adverse outcomes. Peripheral nerve blockade improves painoutcomes for some patient populations but has shortcomings limiting use. Focusedultrasound (FUS) is capable of inhibiting the peripheral nervous system and haspotential as a pain management tool. In an in vivo acute pain model, we investigatedfocused ultrasound's effects on behavior and peripheral nerve structure.**Methods**FUS was applied directly to the sciatic nerve of rats just prior to a hindpaw incision;three control groups (FUS sham only, hindpaw incision only, FUS sham+hindpawincision) were also included. For all four groups (intervention and controls), behavioraltesting (thermal and mechanical hyperalgesia, hindpaw extension and flexion) tookplace for 24 weeks. Structural changes were assessed using transmission electronmicroscopy for controls and for 28 weeks after FUS application.**Results**Compared with controls, after FUS application, animals had: 1) increased mechanicalnociceptive thresholds for 2 weeks; 2) increased thermal nociceptive thresholds for 9weeks; 3) decreased hindpaw motor response for 1 week; and 4) decreased hindpawplantar sensation for 3 weeks. Histologically, FUS altered nerve structure significantly,but phasically, with nerve anatomy returning to normal by 20 weeks after FUSapplication.**Conclusion**FUS, using a distinct parameter set, reversibly inhibits C and A-delta peripheral nervenociceptive, motor, and non-nociceptive sensory fiber-mediated behaviors, and altersnerve structure. FUS may have potential as a peripheral nerve blockade technique foracute pain management. However, further investigation is required to determine thesignificance of nerve structural changes.

Disclosures: T.A. Anderson: None. **C. Pacharinsak:** None. **D.C. Yeomans:** None.

Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR274.10/C106

Topic: D.01. Somatosensation – Pain and Itch

Title: Exploration of peripheral graded electrical stimulation on local field potential in the rat brain

Authors: *D. IBARRA[1], J. TREJO[2], Y. B. PENG[3];
[1]Univ. of Texas at Arlington, Grand Prairie, TX; [2]Psychology, Univ. of Texas, Arlington, Arlington, TX; [3]Dept Psychol, Life Sci. Bldg., Univ. of Texas at Arlington, Arlington, TX

Abstract: Electrical stimulation (ES) is a valuable technique used for the intervention of pain, both in the peripheral or central nervous system. For instance, the use of transcutaneous electrical nerve stimulation (TENS) has been shown to alleviate acute and chronic pain. However, more exploration is needed to understand the underlying mechanisms of electrical stimulation. We hypothesize that with graded peripheral stimulation in the periphery it will elicit an increased power in the brain. To investigate the efficacy of ES for acute pain, we utilize ES in the left ankle and record local field potentials (LFP) simultaneously from four different brain areas to observe alterations in power across frequency bands of the male adult Sprague-Dawley rat brain from intracranially implanted electrodes in four distinct rat brain regions: the anterior cingulate cortex (ACC), bilateral amygdala (AMG), and ventral tegmental area (VTA). The LFP can be subdivided into five frequency bands: delta, theta, alpha, beta, and gamma. Under isoflurane anesthesia, LFP was recorded in 2 separate conditions for each animal with 3 different parameters: 1ms pulse width, 10s duration at 5v, 10v, 50v, and 100v, at 1, 50, and 100 Hz, with interstimulus interval of 3 minutes. The combination of ES parameters has been done in the following groups: pre-formalin ES; 2. post-formalin plus ES; 3. formalin only plus ES. Nociceptive input was induced by injection of 50ul of 3% formalin in the left paw. The results reveal that (1) without formalin, high intensity ES, induced a brief inhibition followed by a rebound phase in all frequency bands, but not observed in low ES. (2) No obvious LFP power changes to formalin injection as well as no changes induced by ES. (3) In formalin plus ES group, we observed an increase of formalin response and an inhibition by ES. These results are consistent with what has been found in human TENS study, where lower frequency of stimulation reduces acute pain perception. Due to the big variation among individual rats, more research needs to be performed to validate our findings.

Disclosures: D. Ibarra: None. **J. Trejo:** None. **Y.B. Peng:** None.

Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR274.11/C107

Topic: D.01. Somatosensation – Pain and Itch

Support: DE032394

Title: Resolvin E1 regulates neutrophil signaling and inhibits inflammatory temporomandibular joint pain

Authors: *S. BANG[1], R.-R. JI[2];
[1]Duke Univ., durham, NC; [2]Pain Res. Div., Anesthesiol., Duke Univ. Med. Ctr., Durham, NC

Abstract: Pain associated with temporomandibular joint (TMJ) is a common problem with various causes like injury, inflammation, and teeth grinding. Although the inflammatory processes involved in temporomandibular disorder (TMD) are well-understood, the pathways to recovery and resolution process remain unclear. Recent research highlights s the role of omega-3 fatty acid derivatives known as Specialized Pro-Resolving Mediators (SPMs) in resolving inflammation. Specifically, Resolvin E1 (RvE1), an EPA-derived SPM, has gained attention for its potent anti-inflammatory and analgesic actions. This study aims to explore the role of RvE1 in TMJ pain and its pro-resolving mechanisms using a mouse model of TMD, induced by CFA injection into TMD. We developed a new technique to assess TMJ pain in mice. Our method uses sensors to detect bite force and grinding frequency, enabling real-time assessment of voluntary biting patterns. We found that mice with TMJ inflammation showed reduced biting and grinding behaviors than healthy control mice (n=10 of both sexes). However, pretreatment of mice with TMJ administration of RvE1 increased occlusal force and grinding frequency. We also used electronic Von-Frey filament to measure mechanical pain sensitivity in the TMJ region and found that RvE1 decreased CFA-induced mechanical pain hypersensitivity. Furthermore, CFA increased neutrophil netosis, a form of programmed cell death that specifically aims at immobilizing and destroying pathogens extracellularly, and Lipocalin-2 (LCN2) levels in the TMJ and trigeminal ganglia , but both were blocked by the RvE1 treatment . These findings indicate that RvE1 can effectively relieve TMJ pain by netosis clearing in TMJ and trigeminal ganglia. These findings highlight the potential therapeutic value of RvE1 in treating inflammatory conditions related to TMJ. We are currently investigating the involvement of the RvE1 receptor ChemR23/CMKLR1 in TMD. This project was supported by an NIH R03 grant (DE032394) to S.B.

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Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

Location: MCP Hall A

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Topic: D.01. Somatosensation – Pain and Itch

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STI 2030-Major Projects (2021ZD0203200-5)
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Title: Ssao inhibitor attenuates inflammatory pain by regulating trpv1 in primary sensory neurons

Authors: *Y. CHANG, Z. ZHOU, Y. LANXING;
Fudan Univ., Shanghai, China

Abstract: Objective It is the high efficacy with few side effects analgesic drugs that are important for inflammatory pain. Semicarbazide-sensitive amine oxidase (SSAO) catalyzes the oxidative deamination of primary amines, and then produces aldehydes, hydrogen peroxide and ammonia. Hydrogen peroxide modulates neuronal excitability and activates transient receptor potential subtype V1 (TRPV1), which is known as a nociceptive sensor. However, whether and how SSAO regulates inflammatory pain in the primary sensory neurons remains unclear. **Methods** ECC0509 is a small molecule drug developed as a specific inhibitor of SSAO. The inflammatory was elicited by complete Freund's adjuvant (CFA) injected into the knee cavity in mice. Mechanical allodynia and thermal hyperalgesia were measured. Electrophysiological recording and molecular experiments were employed to investigate the role of ECC0509 in inflammatory pain. **Results** (1) CFA-induced inflammatory pain significantly upregulated TRPV1 and SSAO expression, increased TRPV1 currents, enhanced neuronal excitability in ipsilateral dorsal root ganglion (DRG), and induced pain behaviors. (2) ECC0509 reduced the expression of SSAO and TRPV1, depressed DRG neuronal excitability and TRPV1 currents, and dose-dependent relieved inflammatory pain behaviors elicited by CFA. (3) Knockout of TRPV1 prevented ECC-induced analgesic effects. **Conclusion** SSAO inhibitor, ECC0509, attenuates inflammatory pain via TRPV1 in primary sensory neurons.

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Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

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Topic: D.01. Somatosensation – Pain and Itch

Support: NRF-2020R1C1C1010245
NRF-2022M3E5E8017395
NRF-2022R1C1C2003317
RS-2023-00254795
2E31502
2V09106
RS-2024-00351160

Title: Uncaria Rhynchophylla and hirsutein alleviate inflammatory pain via TRPV1 desensitization

Authors: *T. HA[1,2], G. HONG[3], U. OH[4];
[1]Korea Inst. of Sci. and Technol., Seoul, 02792 Republic of Korea, Korea, Republic of; [2]Korea University, Seongbuk-Gu, Korea, Republic of; [3]Neurosci., Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of; [4]Brain Sci. Inst., KIST, Seoul, Korea, Republic of

Abstract: *Uncaria rhynchophylla* (UR), an herb traditionally used in Eastern medicine, is recognized for its therapeutic applications in treating hypertension and inflammation. However, the specific molecular mechanisms how UR and its bioactive constituents modulate inflammatory pathways remain unknown. Based on the analgesic effects of UR extract in inflamed mice, we discovered and defined the underlying molecular mechanism of UR. UR extract selectively activated TRPV1 in sensory neurons as well as in a heterogeneous expression system. Notably, consecutive application of UR extract on TRPV1 exhibited inhibitory action through channel desensitization. Further analysis led to the isolation of hirsuteine (HST), a compound within UR extract that robustly activates and subsequently desensitizes TRPV1 in HEK293T cells. The residues S501, F507, Y511, L515, and E570 in human TRPV1 were associated with the binding of HST. Notably, HST administration significantly attenuated the initial pain responses in a formalin-induced model of acute inflammatory pain. Our findings collectively suggest that UR and HST possess distinct agonistic and desensitizing effects on TRPV1, highlighting their therapeutic potential as targeted agents for managing inflammatory pain.

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Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

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Program #/Poster #: PSTR274.14/C110

Topic: D.01. Somatosensation – Pain and Itch

Title: Macrocystis pyrifera ferment modulates neurocutaneous inflammatory signals by regulating endocannabinoid signaling

Authors: *J. EMMETSBERGER[1,2], A. MESSINA[1], T. MAMMONE[1];
[1]The Estee Lauder Companies, Melville, NY; [2]The Max Huber Research Labs, Melville, NY

Abstract: As the largest barrier organ of the body, skin is continuously exposed to a variety of insults which can lead to a dysregulated inflammatory state. Peripheral sensory neurons (SNs) in the skin perform a role, not only in transducing environmental stimuli to the CNS, but also in promoting local inflammatory and cutaneous immune responses via antidromic release of neuropeptides and cytokines. The crosstalk between non-neuronal skin cells and SNs can generate feedback loops that can exacerbate inflammation. We have generated a ferment of *Macrocystis pyrifera*, a kelp known for its anti-inflammatory and antioxidant properties, to produce unique secondary metabolites and facilitate cutaneous absorption. We investigated whether *M. pyrifera* ferment (MPF) could modulate inflammatory signaling in SNs and skin cells through the ubiquitously expressed endocannabinoid (eCB) system. To address this, we performed RNA-Seq on human *ex vivo* skin explants (n=3) isolated from panniculectomies, to comprehensively evaluate the effect of topical application of MPF on gene expression of inflammatory mediators. Since *ex vivo* skin explants lack functional sensory nerve endings, capsaicin-stimulated cocultures of human keratinocytes and human induced pluripotent stem cell-derived sensory neurons (hiPSC-SNs; n=6) were utilized to immunochemically assess the effect of MPF on neuropeptide and cytokine release, in the presence or absence of AM281 and AM630, inverse agonists to cannabinoid CB1 and CB2 receptors, respectively. Monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH) direct enzyme inhibition assays (n=3) were performed to determine if MPF could influence the activity of eCB hydrolyzing enzymes. RNA-Seq analysis of skin explants revealed that after 24h MPF significantly down-regulated gene expression of various inflammatory mediators including *IL1B*, *IL6*, *CXCL8*, *IL33*, and several matrix metalloproteinases. MPF treatment of capsaicin-stimulated hiPSC-SN and keratinocyte cocultures significantly reduced the release of the neuropeptide CGRP-α ($p<0.0001$) and the cytokines TNF-α ($p<0.001$), IL-1β ($p<0.05$), IL-6 ($p<0.001$), and MCP-1 ($p<0.0001$). These effects were abrogated by the presence of AM281 and AM630, suggesting that MPF is modulating eCB signaling. MPF inhibited MAGL activity with an IC50 of 500 µg/mL; however, no appreciable effect was observed on FAAH activity. Together, these data suggest that MPF modulates eCB signaling, in part, by inhibiting MAGL, potentially increasing the eCB 2-arachidonylglycerol, and in turn reducing downstream expression of inflammatory mediators generated by SNs and non-neuronal cells in skin.

Disclosures: J. Emmetsberger: A. Employment/Salary (full or part-time):; The Estee Lauder Companies. **A. Messina:** A. Employment/Salary (full or part-time):; The Estée Lauder Companies. **T. Mammone:** A. Employment/Salary (full or part-time):; The Estée Lauder Companies.

Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

Location: MCP Hall A

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Topic: D.01. Somatosensation – Pain and Itch

Support: JSPS KAKENHI Grant Numbers JP20K10116
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Title: Meloxicam prevents carrageenan-induced chronic hyperalgesia by suppressing microglial activation

Authors: *M. YOSHIDA[1], T. YAMAMOTO[2], E. IMADO[1], Y. MULPURI[3], K. OUE[1], S. KURATA[2], M. DOI[4], Y. SHIMIZU[4], Y. TANAKA[2], N. KISHIMOTO[2], H. HANAMOTO[4], K. SEO[2];
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Abstract: Objectives: The aim of this study is to investigate the mechanisms underlying development of chronic muscle pain in orofacial region and the potential preventive effect of meloxicam. **Methods:** All experimental procedures were approved by the Animal Research Committee of Hiroshima University (approval number: A15-46, A22-120) and Niigata University (approval number: SA01111). Male Sprague-Dawley rats (6 weeks old, 200-250g) were utilized. 3% carrageenan (100μL, diluted with saline) was injected into the masseter muscle in the carrageenan group (CA group: n=6). In the control group, the carrageenan solution was replaced with an equal volume of saline (n=6). To determine whether suppression of microglial activation is involved in the increased mechanical hypersensitivity by carrageenan, minocycline hydrochloride (40mg/kg i.p.) which is a microglial inhibitor was administered daily from the day of the carrageenan injection for up to 1 week (MI group: n=6). To investigate the potential preventive effect of non-steroidal anti-inflammatory drugs for the increased mechanical hypersensitivity by carrageenan, meloxicam (2mg/kg, s.c.) which is a non-steroidal anti-inflammatory drug was injected at the same time as carrageenan injection (ME group: n=6). Then, we evaluated the mechanical sensitivity of the masseter muscle before injection (baseline), 1, 3, 8, 24h post-injection and the day from 1 to 6 weeks by Electronic von Frey device in all groups. To determine whether suppression of microglial activation is involved in the increased mechanical hypersensitivity by carrageenan, we also assessed microglial activation in the trigeminal spinal subnucleus caudalis by immunohistology on the 3rd day after treatment in CA and ME group. Two-way analysis of variance, followed by Dunnett's multiple comparison test was used to compare differences between baseline and values at each time point or between experimental groups. The results were considered statistically significant when $p<0.05$. **Results:** Rats which were injected with 3% carrageenan into the masseter muscle exhibited acute and chronic hyperalgesia to mechanical stimuli for 6 weeks and significantly activated microglial cells at the acute phase. In this model, administration of minocycline hydrochloride at the acute

phase prevented chronic hyperalgesia. Administration of meloxicam not only prevented chronic hyperalgesia but also significantly suppressed the microglial activation at the acute phase. **Conclusion:** Microglial activation may be involved in the development of chronic muscle pain and meloxicam may suppress the development of chronic muscle pain by suppressing microglial activation.

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Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR274.16/C112

Topic: D.01. Somatosensation – Pain and Itch

Support: NSC Taiwan 10-2314-B-006-078-MY2

Title: The role of the gut microbiome in regulating histone acetylation of the SLC12A5 (KCC2) transporter expression after complete Freund's adjuvant-induced inflammation

Authors: *C.-R. LIN;
Natl. Cheng-Kung Univ. Hosp., Tainan, Taiwan

Abstract: Background:In this study, we aimed to explore the relationship among inflammatory pain, KCC2 regulation, gut microbiota, and epigenetic modifications using a CFA-induced pain model. Inflammatory pain is a complex condition characterized by heightened sensitivity and pain perception, often associated with dysregulation in pain processing pathways. KCC2, a key chloride transporter, plays a crucial role in pain modulation by maintaining neuronal excitability. The gut microbiota has emerged as a significant player in modulating various physiological processes, including pain perception, through its interactions with the central nervous system. Methods:CFA injection was used to induce inflammatory pain in animal models. Pain behaviors, including mechanical and thermal hyperalgesia, were evaluated. Acetylation of histone H3 at the KCC2 gene locus was assessed to investigate KCC2 regulation. Changes in gut microbiota composition were analyzed to understand its role in inflammatory pain. Gut decontamination strategies were employed to modulate the gut microbiome and assess its impact on pain severity. Results: Increased pain behaviors were observed following CFA injection, indicating successful induction of inflammatory pain. Decreased acetylation of histone H3 at the KCC2 gene locus was detected, leading to KCC2 downregulation in the spinal cord.Alterations in gut microbiota composition were correlated with the severity of CFA-induced pain. Gut decontamination interventions reduced the intensity of inflammatory pain, suggesting a role for the gut

microbiome in pain modulation.
Conclusion:Our study demonstrates a complex interplay among inflammatory pain, KCC2 regulation, gut microbiota, and epigenetic modifications. The findings highlight the importance of considering the gut-brain axis in pain modulation and provide insights into potential therapeutic targets for managing chronic pain conditions. Understanding the intricate relationships among these factors may pave the way for novel treatment strategies aimed at alleviating inflammatory pain and improving patient outcomes.

Disclosures: C. Lin: None.

Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

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Program #/Poster #: PSTR274.17/C113

Topic: D.04. Interoception

Support: NIH Grant F31AR083277
NIH Grant P30AR079206
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Title: Targeting Piezo2 for osteoarthritis pain in mice

Authors: *N. ADAMCZYK[1], S. ISHIHARA[1], D. REN[2], R. J. MILLER[2], A.-M. MALFAIT[1], R. E. MILLER[1];
[1]Rush Univ. Med. Ctr., Chicago, IL; [2]Northwestern Univ., Chicago, IL

Abstract: The mechanosensitive ion channel Piezo2 is responsible for the conversion of mechanical stimuli such as touch and pressure into electrical signals within the peripheral nervous system. We have previously shown in a surgical model for osteoarthritis (OA) that conditional knockout of Piezo2 from $Na_V1.8$+ sensory neurons protects mice from the development of pain behaviors[1]. It has been shown that a styryl dye, FM1-43, and similar-sized derivatives like FM4-64 can transiently inhibit responses to mechanical stimuli, likely through selective uptake of these dyes through Piezo2[2]. The goal of this study was to assess the effect of FM dyes on pain behavior and neuronal firing in response to mechanical stimulation. Experiment 1. Ten male wt mice were injected intra-articularly (i.a.) with 5 μL of complete Freund's adjuvant (CFA). On day three post injection, a dose-response study was conducted utilizing FM1-43 (0 nM, 0.05 nM, 0.5 nM, 5 nM, and 50 nM). Mice were assessed for knee hyperalgesia before and 30, 90, 180, 360 min and 24 hr after i.a. injection of 2.5 μL. Results demonstrated that the effective dose is 5 nM at 90 min post injection. Experiment 2. The CFA model was induced in both male and female $Na_V1.8$-GCaMP6s mice as in Expt 1. On day 3, under isoflurane anesthesia, the L4 DRG was exposed for imaging in response to mechanical force applied to the

ipsilateral knee (100 g) before or 90 min post i.a. injection of saline or FM4-64 (5 nM). CFA mice injected with saline exhibited no difference in calcium responses post injection (n=3, p=0.9). Mice injected with FM dyes, however, demonstrated a significant decrease in responsiveness post injection (n=3, p=0.07). Experiment 3. Male and female wt mice underwent sham or partial medial meniscectomy (PMX) surgery to induce OA. Four weeks after PMX surgery, both male and female mice developed knee hyperalgesia compared to sham controls (n=9-10 p<0.0001 female PMX vs sham, p=0.0002 male PMX vs sham). At 7 weeks, PMX mice were injected i.a. with FM dye (5 nM in 2.5 μL) or vehicle control, and tested 90 min later for knee hyperalgesia. Wt PMX mice injected with FM dye had less knee hyperalgesia compared to pre injection (n=5, p=0.0002 male, p<0.0001 female), whereas saline had no effect (n=5, p=0.96 male, p=0.56 female). Our work suggests that FM derivatives are transiently silencing Piezo2 positive sensory cells by inhibiting calcium mobilization, thus preventing neuronal firing and pain in response to mechanical stimuli. Implications of this work suggest Piezo2 is likely a fruitful target for pharmacologic intervention in chronic pain states.References1.Obeidat et al. 2023 PMID 371204272.Villarano et al. 2023 PMID 37120427

Disclosures: N. Adamczyk: None. **S. Ishihara:** None. **D. Ren:** None. **R.J. Miller:** None. **A. Malfait:** F. Consulting Fees (e.g., advisory boards); Orion, Averitas. **R.E. Miller:** None.

Poster

PSTR274: Peripheral Mechanisms of Inflammatory Pain

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR274.18/C114

Topic: D.01. Somatosensation – Pain and Itch

Support: R01NS105715
R01NS113965
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R01AI124355
U19AI070235
P30DK078392
the Campaign Urging Research for Eosinophilic Diseases (CURED) Foundation

Title: Allergic Inflammation in Eosinophilic Esophagitis Alters Esophageal Neuronal Innervation

Authors: *K. KELLERMAN[1,2,3], M. NATALE[4,5], Y. ROCHMAN[6,7], M. ROCHMAN[6,7], M. ROTHENBERG[6,7], M. P. JANKOWSKI[8,7,9];
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University of Cincinnati College of Medicine, Cincinnati, OH; [4]Dept. of Pediatrics, Univ. of Cincinnati Col. of Med., Cincinnati, OH; [5]Allergy and Immunology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [6]Allergy and Immunol., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [7]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH; [8]Dept Anesthesia, Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [9]Pediatric Pain Research Center, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Abstract: Eosinophilic esophagitis (EoE) is a chronic allergic disease in which patients can experience refractory pain that reduces quality of life. Yet, the role of the nervous system in disease development has not received much attention. We aimed to determine changes in esophageal nerve density and sensitivity in humans with eosinophilic esophagitis (EoE) as well as mice with experimental EoE, compared with respective controls. Immunofluorescence (IF) for ß-tubulin was performed on esophageal biopsies from patients with EoE and healthy controls. The sensory neuron/putative nociceptor reporter NaV1.8-Cre/tdTomato mouse line and ß-tubulin, was examined in mice, under saline control and experimental EoE conditions induced by repeat *Alternaria* allergen exposure. *In vitro* calcium imaging was then performed on esophageal innervating vagal ganglion (VG) and dorsal root ganglia (DRG) cells from mice with GCaMP6 calcium reporter expressed in sensory and vagal neurons (Pirt-GCAMP6). Neuronal sensitivity and responsiveness to capsaicin, mustard oil, and ATP were examined under control and experimental EoE conditions. In humans, we found increased myelinated nerve density in active EoE patients compared with healthy controls. Interestingly, ß-tubulin was only elevated in active EoE patients who had received topical glucocorticoid treatment. In mice, allergen exposure increased nociceptor nerve density in the lamina propria, but had no impact on myelinated nerves. Additionally, allergen exposure demonstrated an increase in sensitivity of esophageal DRG cells in response to ATP and capsaicin. Taken together, these data indicate that allergic inflammation is associated with changes in nerve density and sensitivity in the esophagus. This provides early insight into neuroimmune mechanisms that may be operational in EoE.

Disclosures: K. Kellerman: None. **M. Natale:** None. **Y. Rochman:** None. **M. Rochman:** None. **M. Rothenberg:** None. **M.P. Jankowski:** None.

Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.01/C115

Topic: D.01. Somatosensation – Pain and Itch

Support: Marie Skłodowska-Curie Actions grant agreement No 956477

Title: Investigation of GABAergic communication within the axons of primary sensory neurons in a microfluidic culture system

Authors: *S. FIGOLI[1], N. GAMPER[1,2];
[1]Fac. of Biol. Sci., Univ. of Leeds, Leeds, United Kingdom; [2]Dept. of Pharmacol., Hebei Med. Univ., Shijiazhuang, China

Abstract: The GABAergic cross-talk between the cell bodies of primary sensory neurons within the Dorsal Root Ganglia (DRGs), has been shown to modulate the transmission of painful stimuli (Du et al., 2017; Hao et al., 2023). However, the possibility of crosstalk between the nerve fibers within the peripheral nerve, is yet to be explored. To address this aspect, we recapitulated the sensory neuron axonal morphology in a novel compartmentalized microfluidic system (Vysokov et al., 2019). This setup included co-culturing DRG and dorsal horn (DH) neurons, which allowed not only to better mimic the physiology and morphology of the peripheral neuron *in vitro*, but also to carry out investigation of signaling processes at distinct segments of somatosensory axon. Our observations reveal a subset of DRG neurons exhibiting typical pseudo-unipolar morphology with T-junction and polarized axonal branches, traversing both, 'peripheral' and DH compartments. Immunocytochemical investigations revealed the expression of $GABA_A$ receptor subunits, including γ_2, α_1 and α_2, along cultured DRG neurons axons and selective axonal stimulation with GABA (100 μM), evoked calcium events associated with action potentials (APs) at the cell bodies. Interestingly, Iodide imaging of HEK_{GABAA}- EYFP H148Q/1152L reporter cells co-cultured with DRG neurons axons, showed that Capsaicin and KCl^- application at the peripheral compartment triggered GABA release both from axons themselves and from cell bodies, likely via vesicular exocytosis. These experiments reveal new level of axonal crosstalk within the peripheral nerves. The microfluidic culture system constitutes a valuable tool for exploring axonal dynamics contributing to the modulation of the transmission of sensory information.

Disclosures: S. Figoli: None. **N. Gamper:** None.

Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.02/C116

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant R01 NS050674
NIH Grant R01 CA205255
NIH Grant T32 AG000222

Title: Central and peripheral axons of nociceptive dorsal root ganglia neurons contain distinct local translatomes

Authors: *E. SILAGI[1], E. NDUKA[2], M. F. PAZYRA-MURPHY[3], R. A. SEGAL[4]; [1]Harvard Med. School/ DFCI, Boston, MA; [2]Dana-Farber Cancer Inst., Brighton, MA; [3]Dana Farber Cancer Inst., Boston, MA; [4]Harvard Med. Sch. Grad. Program In Neurosci., Brookline, MA

Abstract: Dorsal root ganglia (DRG) sensory neurons have a unique pseudounipolar morphology whereby a short axon bifurcates to form a peripheral axon, innervating viscera and limbs, and central axon, innervating the spinal cord. We hypothesize that the dynamic and spatiotemporally regulated functions of DRG axons are developed and maintained by local axonal translatomes. We generated rigorous data on the axonally translated mRNAs and RNA-binding protein (RBP) transport partners to provide new insight into pain modulation by DRG nociceptors. We performed spatial translatomics in mice containing GFP-tagged ribosomal subunits in DRG nociceptors ($NaV1.8^{Cre}$;L10a-GFP, P3-5). Using Translating Ribosomal Affinity Purification (TRAP), translating mRNAs were isolated from tissue lysates containing lumbar DRGs (soma), dorsal roots/spinal cord (central axons), and sciatic nerves (peripheral axons) (n=4 sets). We sequenced all mRNAs associated with GFP-tagged ribosomes to elucidate subcellular translatomes in nociceptors (n=4). Nonspecific mRNAs were isolated from mice without a GFP-tag for comparison. Expression and local translation of axon-specific mRNA targets were visualized *in vivo* by RNAScope, puromycin-proximity ligation assays, and expansion microscopy. Bioinformatic binding motif analyses elucidated RNA-binding proteins (RBPs) that may be responsible for sorting specific mRNAs to central and peripheral axons. These studies identified 224 translating mRNAs in central axons and 302 in peripheral axons. Axonally translated mRNAs often encoded membrane-bound receptors and channels, i.e. serotonin receptors and voltage-gated sodium channels. Surprisingly, functional enrichment analyses demonstrated central axons are implicated in nociceptor function/pain processing while peripheral axon translatomes relate to synaptic organization, ribosomal assembly, and primary cilia. We confirmed that 5-HT3A receptors are locally translated and expressed in nociceptor dorsal roots and axons innervating spinal cord lamina I/II. Considering 5-HT3RA is the only excitatory, ionotropic 5-HT receptor and global knock-out mice have reduced chronic pain, 5-HT3RA may be uniquely involved in descending serotonergic pain modulation. We identified RBPs that may be required for axon-specific mRNA transport, including EWSR1 (central) and SFPQ (peripheral), suggesting RNA regulons may synchronize translation across time and space. Overall, central and peripheral nociceptor axons maintain distinct local translatomes orchestrating their unique subcellular roles and enabling dynamic bidirectional pain processing.

Disclosures: E. Silagi: None. **E. Nduka:** None. **M.F. Pazyra-Murphy:** None. **R.A. Segal:** None.

Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.03/C117

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant R00NS116123
Genetics and Genomics Academy - NCSU

Title: Cell-specific alternative splicing controls functional coupling of calcium channels and vesicle exocytosis machinery in nociceptors

Authors: E. R. MUSTAFA[1], M. DAESCHNER[2], M. AIESI[2], A. MORGAN[2], Q. MOSLEY[2], ***E. LOPEZ SOTO**[2];
[1]Multidisciplinary Inst. of Cell Biol., La Plata, Argentina; [2]Mol. Biomed. Sci., North Carolina State University, Col. of Vet. Med., Raleigh, NC

Abstract: Voltage-gated $Ca_V2.2$ calcium channels are critical for excitation-secretion coupling in nociceptors. Interactions of $Ca_V2.2$ channels with vesicle exocytosis machinery ensure timely release of neuropeptides and neurotransmitters that contribute to neuronal sensitization during inflammation. In this study, we demonstrate that cell-specific alternative pre-mRNA splicing controls functional coupling between $Ca_V2.2$ channels and the Calcium Dependent Secretion Activator (CAPS1), vesicle priming factor essential for neuropeptide release. CAPS1 has been shown to regulate large dense core vesicle exocytosis and it has been associated with calcium-dependent release of neuropeptides in nociceptors. By using western blot and transcriptomic analyses, we showed that CAPS1 is expressed in all dorsal root ganglia sensory neurons and that *Cadps*, the CAPS1 gene, mRNA transcripts undergo extensive cell-specific alternative pre-mRNA splicing. Interestingly, *Cadps* alternative exon 16a is enriched in peptidergic nociceptors (50.75 ± 2.37%), non-peptidergic nociceptors (24.05 ± 0.64%) and C-low-threshold mechanoreceptors (39.77 ± 1.84%) in comparison with all other sensory neuron subtypes ($p<0.0001$). Then, we directly assessed the interaction between $Ca_V2.2$ channels and CAPS1 using whole-cell patch clamp in voltage-clamp mode. We recorded Ca_V currents from tsA201 cells overexpressing $Ca_V\alpha1$ subunits with required auxiliary subunits β3, α2δ1, with or without CAPS1. We found that CAPS1 isoform containing exon 16a increases $Ca_V2.2$ currents by ~70% ($p=0.001$), but not related $Ca_V1.2$ or $Ca_V3.2$ currents ($p=0.98$ and $p=0.45$), in comparison with control conditions. CAPS1 isoforms lacking exon 16a fail to induce $Ca_V2.2$ current increase ($p=0.18$). We also found that CAPS1 isoform containing exon 16a hyperpolarizes ~5 mV $Ca_V2.2$ voltage-dependence activation and increases relative channel open probability. Our data reveal that CAPS1 functionally couples to $Ca_V2.2$, when *Cadps* alternative exon 16a is included, upregulating $Ca_V2.2$ channel activity. Cell-specific alternative splicing may optimize CAPS1 interactions with $Ca_V2.2$ channels in nociceptors and C-low-threshold mechanoreceptors, likely impacting vesicle exocytosis.

Disclosures: E.R. Mustafa: None. **M. Daeschner:** None. **M. Aiesi:** None. **A. Morgan:** None. **Q. Mosley:** None. **E. Lopez Soto:** None.

Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.04/C118

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH grant R00NS116123
Genetics and Genomics Academy - NCSU

Title: Extensive alternative splicing in dorsal root ganglia sensory neurons is cell specific and selective to the regulation of neuronal ion channels and synaptic properties

Authors: *Q. T. MOSLEY[1], N. ROBIL[2], J. MAJUAL[1], P. DE LA GRANGE[2], E. LOPEZ SOTO[1];
[1]Mol. Biomed. Sci., North Carolina State Univ., Col. of Vet. Med., Raleigh, NC; [2]Genosplice, Paris, France

Abstract: Gene expression programs are essential instructions for neurons to develop, connect, and mature. As neurons differentiate, alternative pre-mRNA splicing further enriches their genetic programs by generating multiple isoforms from single genes. This molecular mechanism is most prominent across neuronal types in the central nervous system, but we know little about alternative splicing contributions to neurons in the peripheral nervous system. Here, we study genome-wide expression of alternative exons in different subsets of sensory neurons from the dorsal root ganglia (DRG) in mice. We use publicly available deep RNA-sequencing datasets and splicing-sensitive analysis to detect and quantify alternative exons in genetically identified DRG sensory neurons. We include datasets of peptidergic and nonpeptidergic nociceptors, proprioceptors, Aδ-low threshold mechanoreceptors (LTMRs), Aβ-SA (slowly adaptive)-LTMRs, Aβ-RA (rapidly adaptive)-LTMRs, Aβ-field-LTMRs. Using percent-splice-in based on junction reads, we quantify alternative exon inclusion, and we uncover hundreds of alternative spliced exons among sensory neurons (fold change >2, $p<0.05$). We find that expression of alternative exons clusters neuronal samples based on cell-type and with similar trajectories than global gene expression. Splicing of alternative first and or terminal exons is enriched in all sensory neurons (>30%, $p<0.05$), followed by cassette exons in all cases, in comparison with their expected representation in the FAST DB reference database. Differentially spliced alternative exons between neuronal subtypes are enriched in genes involved in regulation of ion channel activity, morphine addiction, presynaptic membrane, and endocannabinoid signaling pathways (fold enrichment >10, $p<0.001$). Using weighted gene co-expression network analysis, from alternative exons rather than genes, we reveal a co-expression module containing 145 exons associated with early lineage differentiation of sensory neurons. Our results demonstrate that extensive alternative splicing occurs in major subsets of sensory neurons affecting intrinsic neuronal and synaptic properties. The splicing of alternative exons in DRG neurons may arise

from mechanistically different forms of transcript diversification likely initiated early during development.

Disclosures: Q.T. Mosley: None. **N. Robil:** None. **J. Majual:** None. **P. de la Grange:** None. **E. Lopez Soto:** None.

Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.05/C119

Topic: D.01. Somatosensation – Pain and Itch

Title: Early life stress modulates neonatal somatosensation and the transcriptional profile of immature sensory neurons

Authors: *F. EID[1], K. HARBOUR[2], E. K. SERAFIN[2], M. HAYES[3], M. L. BACCEI[2];
[1]Univ. of Cincinnati, Cincinnati, OH; [2]Anesthesiol., Univ. of Cincinnati, Cincinnati, OH; [3]Univ. of Illinois, Peoria, IL

Abstract: Traumatic childhood experiences, including sexual, physical, or emotional abuse and parental neglect, evoke severe early life stress (ELS). Infants in the neonatal intensive care unit (NICU) also commonly experience stress from tissue-damaging procedures as well as non-painful stressors such as maternal separation, excessive light and noise, and stress induced by routine nursing procedures. ELS is known to sensitize nociceptive circuits within the mature nervous system, leading to exacerbated pain responses in adulthood. However, the acute effects of ELS on pain processing in the developing nervous system remain poorly understood. We sought to address this gap by investigating the effects of ELS on pain sensitivity in neonatal mice, as well as the genetic signatures of immature sensory neurons residing in the dorsal root ganglion (DRG). Neonatal limited bedding between postnatal days (P)2-P9 was used as a model of early life chronic stress, with litters housed under normal bedding conditions serving as a control. Experiments were then performed between P9-P12 to assess the short-term effects of ELS. Our behavioral results show increased sensitivity to noxious cold, mechanical pressure, and tactile stimuli in ELS pups compared to the control group. Bulk RNA-seq analysis identified 132 differentially expressed genes within the neonatal L3-L5 DRG after ELS, including a >16-fold reduction in mRNA expression of the genes encoding the neuropeptides somatostatin (*Sst*) and natriuretic polypeptide b (*Nppb*) which are known to play key roles in pain and itch. *In situ* hybridization confirmed that neurons in the neonatal DRG expressed significantly lower mRNA levels of both *Sst* and *Nppb* in pups exposed to ELS. Given that peripheral Sst has been shown to tonically inhibit nociceptive transmission, we systemically administered the stable Sst analog octreotide and observed a reversal of the cold hypersensitivity induced by ELS, thereby raising the possibility that ELS evokes pain by disrupting endogenous Sst transmission in the periphery.

Collectively, these results demonstrate that ELS elicits short-term changes in nociceptive processing within developing mice. These findings represent a critical first step towards unraveling endogenous molecular mechanisms governing the interaction between chronic stress and pediatric pain, with the long-term goal of ameliorating negative health outcomes in pain patients.

Disclosures: F. Eid: None. **K. Harbour:** None. **E.K. Serafin:** None. **M. Hayes:** None. **M.L. Baccei:** None.

Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.06/C120

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH HEAL Initiative U19NS130607
NIH HEAL Initiative R34NS126036
Dr. Seymour and Rose T. Brown Professorship
Washington University Neuroprep Program
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Title: Expression landscape of cannabinoid type 1 receptors in mouse and human sensory ganglia: implications for peripherally restricted analgesics

Authors: *A. TOLIVER[1], J. M. MWIRIGI[2], M. FLUEGEL[2], J. YI[2], R. W. GEREAU IV[2]; [1]Washington Univ., St. Louis, MO; [2]Anesthesiol., Washington Univ. Sch. of Med., St. Louis, MO

Abstract: 11% of the US population is affected by chronic pain, and current available therapies have limited efficacy, and many have undesirable properties including abuse liability and the development of tolerance. Cannabinoid type 1 (CB1) receptors, encoded by *CNR1*, are key players in the endocannabinoid system and are promising therapeutic targets for chronic pain. These receptors are broadly expressed in both the central (CNS) and peripheral (PNS) nervous system, however, CNS engagement of CB1 receptors produces untoward effects such as tolerance, dependence, and psychoactivity at analgesic doses. Our previously published work demonstrates that a peripherally acting CB1 agonist attenuates inflammatory pain in mice. Whether similar effects will be observed with peripherally restricted CB1 agonists in humans is unknown. Mapping the expression landscape in human and mouse sensory neurons is an important initial step toward understanding the prospects for peripherally restricted CB1 agonists as analgesics in humans. To do this, we used RNAscope *in situ* hybridization to characterize CB1 receptors in different neuronal populations in human (hDRG) and mouse (mDRG) dorsal

root ganglia. In the hDRG study, donors were age- and sex- matched for chronic cannabis use versus non-cannabis use. *CNR1* was found in approximately 30% of nociceptors and proprioceptors in hDRGs from non-cannabis donors with no known history of chronic pain. Although the distribution profile of *CNR1* in key neuronal subpopulations was similar for both human and mouse DRGs, cross-species comparisons revealed a higher expression of *CNR1* in the proprioceptor population compared to nociceptor populations in mDRG. Taken together, the abundance of CB1 receptors in human DRG offers promise for developing novel peripherally restricted CB1 targeting therapeutics for chronic pain.

Disclosures: A. Toliver: None. **J.M. Mwirigi:** None. **M. Fluegel:** None. **J. Yi:** None. **R.W. Gereau:** F. Consulting Fees (e.g., advisory boards); Advisory board for Doloromics.

Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.07/C121

Topic: D.01. Somatosensation – Pain and Itch

Support: R01DE032227
Duke DREAM Innovation Grant

Title: Sexually dimorphic MRAS expression in mouse and human dorsal root ganglia

Authors: *G. BASSETT[1], A. MCGINNIS[2], M. FONSECA[2], A. MOLLOY[2], S. BANG[2], R.-R. JI[2], C. DONNELLY[2], S. B. SMITH[2];
[1]Duke Univ., Durham, NC; [2]Dept. of Anesthesiol., Duke Univ., Durham, NC

Abstract: Muscle RAS Oncogene Homolog, or M-Ras, is a widely expressed GTPase recently implicated in male-specific chronic pain. A polymorphism which decreases *MRAS* expression has been tied to an increased incidence of painful temporomandibular disorders in men, and male *Mras* knockout mice are especially sensitive to inflammatory pain. How M-Ras exerts this influence and whether this protein is important in the pathogenesis of other pain conditions remains mysterious. To explore these questions, we analyzed *MRAS* expression in DRGs procured from healthy human donors. Fluorescent in situ hybridization demonstrated that *MRAS* is broadly expressed in multiple cell types in the DRG, including small-diameter sensory neurons. Realtime PCR in cDNA libraries from eight male and seven female human DRG lysates identified *MRAS* as being more highly expressed in male DRGs. We then leveraged HiPlex RNAscope, a multi-channel fluorescent in situ hybridization (FISH) technique, to analyze the expression patterns of murine *Mras*. We discovered murine *Mras* to be highly and predominantly expressed by the so-called "*Nppb*-positive" class of small-diameter c-fibers known best for their transduction of itch, significant production of the ich-inducing neuropeptide somatostatin, and

rich expression of inflammation-related peptides such as Il31ra, Cysltr2, S1pr1, and Npy2r. Our findings also revealed a higher expression of *Mras* in male mice compared to females within this class. To examine these effects in the context of neuropathic pain conditions, we analyzed DRGs from naïve mice or mice treated with paclitaxel to induce chemotherapy-induced peripheral neuropathy (CIPN)-associated neuropathic pain. Expression was primarily observed in DRG neurons, with particularly strong expression in small diameter neurons which largely consist of pain-sensing nociceptors. This expression pattern was significantly stronger in DRGs from naïve males than from CIPN males. CIPN males had similar signal intensities to naïve and CIPN females. While studies are ongoing to explore the functional significance of neuronal Mras, we posit that loss of this heightened expression may be at the root of *MRAS*'s unique role in male chronic pain.

Disclosures: G. Bassett: None. **A. McGinnis:** None. **M. Fonseca:** None. **A. Molloy:** None. **S. Bang:** None. **R. Ji:** None. **C. Donnelly:** None. **S.B. Smith:** None.

Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.08/C122

Topic: D.01. Somatosensation – Pain and Itch

Title: Generation and Comparison between Humanized MrgX1/C57BL and Humanized MrgX1/CD1 Mouse Models

Authors: *J. WANG[1], E. O'NEIL[2], H. XIAO[2], B. L. ADAMS[2], B. LI[2];
[1]Eli-Lilly and Co., indianapolis, IN; [2]Pain and Neuronal Hlth., Eli Lilly and Co., Indianapolis, IN

Abstract: Chronic pain is a major health and economic problem. MrgX1 presents a promising target for the development of novel pain blockers due to its exclusive expression in primary nociceptive neurons. It was demonstrated that MrgX1 modulators were species specific. *Li Z, et al* published a hMrgX1 mouse model that contains an 845kb deletion and removes 12 endogenous Mrgpr genes, including MrgprC11. We were concerned about the potential functions of those deleted genes, therefore we generated a C57 and CD1 based, humanized MrgX1 (hMrgX1) mouse model. Here we utilized a CRISPR/CAS9-mediated gene knock-in protocol in mouse ES cells and/or fertilized mouse eggs to swap the mouse MrgprC11 coding sequence (CDS) with human MrgX1 CDS. Two lines of hMrgX1 mice in C57 or CD1 backgrounds were generated to fit the requirements for different pain behavior or toxicological studies. It was confirmed that human MrgX1 mRNA is expressed in the dorsal root ganglia (DRG) of hMrgX1 homozygous (HOM) C57 and CD1 mice, but not in the DRG of wild-type (WT) C57 or WT CD1 mice. Human MrgX1 mRNA expression levels in the DRG of CD1 HOM hMrgX1 mice are 2.58-fold higher than levels in the DRG of C57 HOM hMrgX1 mice. Commercially available

mMrgC11 TaqMan probes detected low levels of mouse MrgC11 in the DRG of both C57 and CD1 hMrgX1 HOM mice most likely due to non-specific signal. MrgC11 expression was not detected in the DRG from HOM hMrgX1/C57 and HOM hMrgX1/CD1 mice when two custom designed TaqMan probes against mouse MrgC11 was used. We then performed RNAscope study showing that hMrgX1 expression is present in the DRG neurons of hMrgX1/C57 and hMrgX1/CD1 mice. Furthermore, co-expression of hMrgX1 and mOprm1 was detected in some mOPrm1 positive DRG neurons. With the confirmation of full length hMrgX1-V5 transcript in the hMrgX1 mice, we conclude that both hMrgX1/C57 and hMrgX1/CD1 mice are fully humanized MrgX1 mouse models. This study was supported 100% by Lilly. None of authors has a conflict of interest.

Disclosures: J. Wang: A. Employment/Salary (full or part-time):; Eli Lilly and company. **E. O'Neil:** A. Employment/Salary (full or part-time):; Eli Lilly and company. **H. Xiao:** A. Employment/Salary (full or part-time):; Eli Lilly and company. **B.L. Adams:** A. Employment/Salary (full or part-time):; Eli Lilly and company. **B. Li:** A. Employment/Salary (full or part-time):; Eli Lilly and company.

Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.09/C123

Topic: D.01. Somatosensation – Pain and Itch

Support: Swedish Research Council Starting Grant no. 2020-01107
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Intramural Research Program of the NIH (NCCIH)

Title: Piezo2-dependent rapid pain system in humans.

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Abstract: PIEZO2-dependent Rapid Pain System in Humans.
Background and Aims: The PIEZO2 ion channel is critical for transducing light touch into neural signals but is not considered necessary for transducing acute pain in humans (Szczot et al., 2018; Nagi et al., 2019). Here, we discovered an exception - a form of mechanical pain evoked by hair pulling.
Methods: To address this, first, we conducted psychophysical experiments by testing a range of pulling forces on single hairs in control participants, individuals with PIEZO2 deficiency syndrome, and individuals with Aβ deafferentation. We then performed a preferential ischemic nerve block to investigate the contributions of primary afferents to hair-pulling pain. Next, we used in vivo electrophysiological recordings (microneurography) from single mechanoreceptive cutaneous neurons to investigate the neural coding of single hair pulls. Finally, using functional imaging combined with pharmacological mapping approach, we tested hair pull pain in mice with a conditional knockout of Piezo2. **Results:** We found hair-pulling elicits a distinct, low-threshold pain sensation associated with a specific urge-to-move behavior. Interestingly, individuals with PIEZO2 deficiency syndrome had a deficit in pain perception to hair-pulling stimuli. Hair-pulling pain was abolished in the condition of blocked Aβ fibers - a finding confirmed in selective Aβ deafferented individuals who did not perceive hair-pulling stimuli as painful. Single-unit axonal recordings revealed that a class of cooling-responsive myelinated nociceptors in human skin is selectively tuned to painful hair-pull stimuli. Furthermore, using a pharmacological mapping approach, we have confirmed that these hair-pull coding fibers belonged exclusively to a transcriptomically defined class of nociceptors and that Piezo2 is critical for hair-pulling response of these neurons.
Conclusions: These findings demonstrate that a specialized class of rapidly conducting PIEZO2-dependent nociceptors associated with hair follicles serves as evolutionarily conserved mediators of hair-pull pain.
References: Szczot, M., Liljencrantz, J., Ghitani, N., Barik, A., Lam, R., Thompson, J.H., Bharucha-Goebel, D., Saade, D., Necaise, A., Donkervoort, S., et al. (2018). PIEZO2 mediates injury-induced tactile pain in mice and humans. Sci Transl Med 10. 10.1126/scitranslmed.aat9892.Nagi, Saad S., et al. "An ultrafast system for signaling mechanical pain in human skin." Science advances 5.7 (2019): eaaw1297.

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Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.10/C124

Topic: D.01. Somatosensation – Pain and Itch

Support: U19NS130608
R01NS065926

Title: Type I Interferons act directly on human dorsal root ganglion nociceptors to induce signaling and enhance excitability.

Authors: *Ú. FRANCO-ENZÁSTIGA, K. NATARAJAN, F. ESPINOSA, H. MYDUGOLAM, T. J. PRICE;
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Abstract: The type I interferon (IFN) family comprises IFN-α and IFN-β, important cytokines for antiviral defense. Our previous work showed that, when applied to the periphery, type I IFNs induce pain by activating IFN receptor (IFNR) on mouse DRG nociceptors. However, the effects of type I IFNs on human nociceptors have not been assessed. Here, we build on the study of type I IFN effects by doing patch clamp electrophysiology and signaling assays on hDRG neurons from organ donors. Our first approach was to characterize the distribution of type I IFN receptor (IFNR) subunits, IFNAR1 and IFNAR2, in hDRG neurons. Using *in situ* hybridization experiments, we found that both subunits are expressed in both *SCN10A*-positive and negative neurons, with *IFNAR1* showing a higher amount of mRNAs compared to *IFNAR2* across all the neurons. We confirmed the presence of both subunits on the membrane of cultured hDRG neurons using immunocytochemistry (ICC). Subsequently, we exposed hDRG cultures to type I IFNs and assessed p-STAT1, a downstream effector of the canonical JAK-STAT pathway induced by IFNs. We observed a concentration-dependent increase in p-STAT1 levels in peripherin positive hDRG neurons induced by IFNs, indicating the successful activation of downstream signaling pathways of IFNs in hDRG nociceptors. Previously, we demonstrated that type I IFNs stimulate MNK-eIF4E signaling in mouse DRG neurons, a pivotal pathway contributing to nociceptor hyperexcitability and chronic pain. Therefore, we incubated hDRG cultures with IFNs and, using ICC, we analyzed the levels of p-eIF4E, a specific target of MNK. We found that IFNs increase p-eIF4E in peripherin positive neurons in hDRG. To better understand IFN actions on hDRG, we performed calcium imaging recordings on hDRG cultures incubated with type I IFN. We found that IFN increases the magnitude of capsaicin-evoked calcium response, suggesting nociceptor sensitization. Moreover, using electrophysiological

recordings on nociceptors, we observed that IFN-α increases the neuronal membrane resistance and decreases the step and ramp rheobase showing that IFN-α increases excitability of hDRG nociceptors. We conclude that type I IFNs activate downstream signaling pathways on human nociceptors inducing sensitization and hyperexcitability, which might explain pain associated with increased IFN expression in viral infections and other pathologies.

Disclosures: Ú. Franco-Enzástiga: None. **K. Natarajan:** None. **F. Espinosa:** None. **H. Mydugolam:** None. **T.J. Price:** None.

Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

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Program #/Poster #: PSTR275.11/C125

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH CA200263 (PMD)
NIH NS102432 (YIM, TLY)
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NIH NS111929 (PMD)
NIH NS065926 (PMD)
NIH NS130608 (PMD)
NIH NS120636 (KEM)
NIH NS132622 (PMD)

Title: Lipid rafts contribute to TRPV1 function in human dorsal root ganglion neurons

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Abstract: Current analgesic pharmaceutical categories offer pain relief but are hindered by adverse reactions, necessitating the need for novel analgesic therapies with improved safety profiles and reduced addiction potential. In this arena, ion channel modulation has emerged as a promising strategy for pain management. Transient receptor potential vanilloid subtype 1 (TRPV1) is an ion channel involved in pain perception that is localized in nociceptors, reacting to stimuli such as capsaicin and heat. Capsaicin can desensitize TRPV1, paradoxically reducing pain sensation. While TRPV1 channels increase during heightened pain sensitivity, understanding how these changes correspond to functionality is crucial. Cholesterol in the plasma membrane has also been found to influence TRPV1 expression, lipid rafts, and ion channel activity. Other factors, such as developmental differences, have not been fully explored

in human sensory neurons. The current study examines variations in TRPV1 activation profiles upon repeated capsaicin exposure, impacting pain sensations across ages and conditions. Here we present differential TRPV1 function and expression in human sensory neurons from juvenile and adult organ donors and adult cancer patients. In Case 1 (54M, cancer patient), capsaicin responses included depolarization and bursts of action potentials, with desensitization during the second capsaicin application. In contrast, Case 2 (11F, donor) showed sensitization and multiple action potentials, even at a higher capsaicin concentration (1 μM). Case 3 (59M, cancer patient) presented substantial inward currents that were not observed in Case 2. Comparison of immunohistochemistry images of DRGs from Case 2 (11F, donor) and Case 4 (23M, donor), revealed smaller neuron diameters and more TRPV1-positive neurons in the juvenile donor, indicating that the stronger responses seen in adult sensory neurons did not correspond to higher TRPV1 expression. Donors in Case 2 and Case 4 exhibited similar CTxB (a lipid raft marker) intensity, while oncology patients with (Case 6) and without (Case 5) radiating pain symptoms showed elevated CTxB intensity. Patient DRGs affected by nerve root compression due to spinal metastases exhibited upregulated TRPV1 and lipid raft content, indicating that these changes could potentially be linked to pain symptoms. Differences in TRPV1 expression profiles and function across age groups and how these are impacted by lipid raft expression in neuropathic pain states suggest a potential role for TRPV1 and lipid rafts in novel treatments targeted at reducing aberrant nociceptor activity in chronic pain.

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Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.12/C126

Topic: D.01. Somatosensation – Pain and Itch

Support: DoD Grant W81XWH-19-2-0037

Title: Identification of molecular and cellular predictors of pain resolution after traumatic amputation from human sciatic nerve tissue

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Abstract: Pain is a natural byproduct of injury, almost universally affecting all people at some point in their lives. After injuries heal, pain usually subsides and allows the person to go about their normal lives. However, a subset of those sustaining injuries suffer from chronic pain that can last indefinitely and severely affect quality of life. The underlying causes of chronic pain remain elusive. In this study, we sought to identify molecular and cellular predictors of long-term clinical pain outcomes. Sciatic nerves were harvested from patients who required surgical amputation due to traumatic leg injury. The nerves were processed for quantitative proteomic analysis using LC-MS/MS, along with measurement of cytokines, chemokines, and vascular injury markers using the Meso Scale Discovery platform. Subsequent immunohistochemical assays were used to determine the localization of proteins which were differentially expressed between pain resolvers and non-resolvers. Surprisingly, we found nerves from resolvers exhibited more robust inflammatory signatures relative to nerves from non-resolvers. Several chemokines and vascular injury markers were enriched in nerve tissues of resolvers, and the majority of these factors were most abundant in pro-inflammatory (M1-like) perivascular macrophages and nearby vasculature within the sciatic nerve sheath. Our data appear to support an evolving conceptual framework in which acute inflammation is critical for long-term pain resolution.

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Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.13/C127

Topic: D.01. Somatosensation – Pain and Itch

Title: Utilizing Long-Read Sequencing to Facilitate Target Discovery in the Human Dorsal Root Ganglion

Authors: *D. GEORGE[1], F. VAEZ LIVARY[1], R. MCDONALD[2], B. MCDONALD[1], C. M. FLORES[3], J. BROUGHER[4];
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Abstract: Chronic pain poses a significant public health challenge, exacerbated by the notable disparity between preclinical research and the development of effective analgesics. The dorsal root ganglion (DRG) is pivotal in pain signal transduction and transmission. Disruptions within the DRG during chronic pain conditions often lead to sustained and heightened transmission of pain signals, highlighting the necessity for a nuanced understanding of the molecular changes underlying the disease. We conducted long-read sequencing (LRS) of DRG tissue obtained from male and female human donors using the PacBio Iso-seq platform to gain deeper insights into the molecular landscape. RNA isolation and cDNA generation of full-length transcripts were performed, followed by circular consensus sequencing (CCS). Subsequent preprocessing using the Iso-seq pipeline identified over three million reads, totaling approximately 8 billion base pairs, leading to the detection of 152,264 isoforms. This approach facilitates the exploration of isoform diversity within neuronal and glial cells, allowing for the delineation of alternative splicing events and specific isoform expression profiles. To investigate the complexity of gene architecture within the DRG, we integrated our LRS data with single-cell RNA sequencing profiles derived from human DRG samples. This combinatorial approach enables discernment of isoform specificity at a single-cell level and supports a highly specific cartography of networks within the DRG microenvironment. The identified targets, arising from specific cellular subsets within the DRG, offer promising avenues for therapeutic intervention. Our ongoing work focuses on the delivery of genetic payloads to modulate specific isoforms within the neuronal subpopulations to validate the function of the identified isoforms in modulating and driving hyperexcitability. By elucidating the molecular profile of chronic pain at a granular level, our study lays the foundation for the advancement of precision pain therapeutics.

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Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.14/C128

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Molecular and Physiological Characterization of Pain Targets in Human Dorsal Root Ganglion

Authors: *R. MCDONALD[1], B. MCDONALD[1], F. VAEZ LIVARY[1], D. GEORGE[2], C. M. FLORES[3], J. BROUGHER[4];
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Abstract: The prevalence of chronic pain remains greater than diabetes, heart disease, and cancer. However, progress in the development of therapeutics to alleviate chronic pain remains minimal. This is, in part, due to the difficulty in translating pain targets characterized in animal models to human medicine. To overcome this, our lab leverages the use of human dorsal root ganglion (hDRG) tissue to identify and assess targets that contribute to the development and maintenance of chronic pain. The DRG is a collection of neuronal and nonneuronal cells that play a crucial role in the detection and amplification of painful stimuli from the periphery. Molecular and physiological alterations in the DRG contribute to the etiology of chronic pain, however, these alterations can be distinct between mammalian species, rendering the translation of promising molecular targets and physiological modifications between rodent and human disease ineffective. Therefore, to identify effective chronic pain targets, a therapeutic advantage lies in the continued development of biological assays that assess and model chronic pain in donor hDRG tissue. While the use of donor hDRG tissue becomes more prevalent in research labs, much piloting work remains to efficiently leverage this limited tissue as a model for disease. Our lab has input donor hDRG tissue through a variety of laboratory protocols, enabling us to efficiently and effectively model chronic pain and validate clinical targets. To accomplish this, we optimized hDRG dissociation to maximize neuronal yield to greater than 150,000 neurons per donor (~13 thoracic/lumbar DRGs pairs on average). Downstream of neuronal isolation, we perform a battery of assays including single cell (sc)RNA sequencing, quantitative (q)PCR analysis, western blotting, immunofluorescence, microelectrode array (MEA), and calcium imaging. By optimizing the use of hDRG tissue in multiple downstream assays, our team maximizes biological information ascertained from each donor sample, allowing for a multi-omic and comprehensive physiological view of the donor hDRG tissue. As a direct result of our work, we have improved nociceptor cell models by tuning the expression of key-nociceptive genes in iPSC-derived sensory neurons. Furthermore, we have identified multiple human pain-specific targets not found in other mammalian species, supporting the notion that using human-derived tissue will improve the development of human chronic pain therapies. Together, our model allows us to define chronic pain targets both ex vivo and in vitro in a highly novel and translatable format that will promote the advancement of chronic pain research as well as therapeutics.

Disclosures: R. McDonald: A. Employment/Salary (full or part-time):; Doloromics. **B. McDonald:** A. Employment/Salary (full or part-time):; Doloromics. **F. vaez livary:** A. Employment/Salary (full or part-time):; Doloromics. **D. George:** A. Employment/Salary (full or part-time):; Doloromics. **C.M. Flores:** A. Employment/Salary (full or part-time):; Doloromics. **J. Brougher:** A. Employment/Salary (full or part-time):; Doloromics.

Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.15/C129

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Advanced Models of Nociception

Authors: *F. VAEZ LIVARY[1], B. MCDONALD[1], R. MCDONALD[2], D. GEORGE[3], C. M. FLORES[4], J. BROUGHER[5];
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Abstract: Nociception is a complex process in which the nervous system detects and responds to harmful stimuli. This process is important for understanding pain mechanisms and developing effective treatments. Animal models, especially rodent models, have been crucial in nociception research due to their physiological similarity to humans. However, these models fail to capture the full complexity of human pain conditions, making it difficult to translate research findings into treatments. The dorsal root ganglion (DRG) is a heterogeneous tissue housing various subpopulations of sensory neurons that form tight associations with non-neuronal cells, playing a central role in nociceptive signaling. Accurately recapitulating the intricate architecture and microenvironment of the DRG *in vitro* holds immense promise for identifying pain targets with greater physiological relevance. To address this need, we have developed a novel human DRG organoid model to provide a more accurate *in vitro* platform for studying neuropathic pain. DRG from human donors are dissociated and allowed to reassemble into 3D organoids, preserving their viability and structural integrity over weeks of culture. This 3D spheroid model closely mimics the natural cellular environment, enabling precise studies of nociceptive pathways. Furthermore, our access to DRG tissues from pain and non-pain patients enables a genomic and proteomic approach to identifying novel pain targets. We then validate these targets in our *in vitro* systems, using different molecular techniques. We conduct downstream functional assays, including microelectrode array-based detection of spontaneous and evoked responses, as well as calcium imaging assays to allow real-time visualization of neuronal responses to different stimuli. Additionally, our organoid model enables innovative applications such as culturing spheroids on 2D surfaces to facilitate axonal outgrowth enabling us to model for example, a chemotherapy-induced peripheral neuropathy model wherein we can monitor axonal degeneration and uncover novel therapeutics that can prevent this degeneration. We leverage microfluidic devices to model interactions with peripheral blood mononuclear cells, offering a versatile platform for studying complex cellular interactions. In summary, our organoid model of the human DRG can be a physiologically relevant platform for investigating the complex mechanisms in pain sensation and for accelerating the development of novel therapeutic interventions.

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Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.16/C130

Topic: D.01. Somatosensation – Pain and Itch

Title: High content imaging platform for accessing potential therapeutic drug compounds

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Abstract: Differentiation of human induced pluripotent stem cells (hiPSC) to neuron and glial cells represents an efficient tool for *in vitro* modeling of human neurological disorders. This, combined with highly efficient gene editing tools, enables comparative analysis of genome-modified hiPSC lines to their parental lines to study disease mechanisms in phenotypic and functional screening assays. Here, we describe our approach to building an *in vitro* model for studying peripheral pain. Following the establishment of hiPSC clones, we employed a subtype-specific differentiation protocol using transient expression of Neurogenin2 (NGN2), that consistently allowed us to differentiate sensory neurons at scale. Using the ImageXpress Micro HT, culture conditions were optimized to allow for miniaturization, in addition to automated high content imaging (HCI). Hereby, ensuring assay scalability onward. Applying in-house software packages, raw images could be subjected to ai-enabled segmentation and data quantification in an automated process. Thus, allowing for efficient phenotypic profiling, through the analysis of intricate neuron processes and subcellular markers. Results demonstrate that our NGN2-based approach propose a valuable tool, that allows the identification of new HCI biomarkers and screening of potential therapeutic drug compounds in a relevant human neuronal model.

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Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

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Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant R61 AT011938 (BJK, GD, JP)
NIH Grant U19 NS130608 (TJP)

Title: A multifaceted high-content screening platform for analgesic compounds using human induced pluripotent stem cell-derived nociceptors

Authors: *C. FOFIE KUETE[1], R. GRANJA-VAZQUEZ[2], S. BISWAS[3], V. TRUONG[4], T. J. PRICE[1], G. O. DUSSOR[1], J. J. PANCRAZIO[5], B. J. KOLBER[1];
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Abstract: Existing treatments for chronic pain often fall short or have side effects, highlighting the need for better preclinical models that can quickly identify analgesic candidates while ensuring translational relevance. High-throughput screening (HTS) is one solution, but traditional methods using non-human sensory neurons or synthetic alternatives face issues due to species differences and lack of physiological relevance. Human induced pluripotent stem cells (hiPSCs) are promising for developing nociceptor-like cells for both high content screening (HCS) and HTS. We used a multi-electrode array (MEA) platform on a HCS basis to examine both spontaneous and evoked activities of hiPSC-derived nociceptors in 48- and 96-well plates. By optimizing culture conditions, such as seeding techniques and densities, we recorded extracellular action potentials from multiple sensors per well, allowing us to measure key neurophysiological parameters like firing rates, spike counts, and cell impedance. We probed diverse pharmacological targets implicated in pain modulation under both normal and inflammatory states, demonstrating consistent sensitivity to established analgesics via distinct molecular mechanisms. The performance of our model was assessed by calculating classical and robust Z' factors, where a Z' of 0.5-1 indicates a high-quality assay. We achieved nearly complete (~100%) sensor functionality by the second week and maintained stable firing rates and spike counts for over two weeks at various cell densities. With the use of lidocaine and a cell density of 35,000 cells per well in a 48-well plate format, a high and consistent robust Z' factor above 0.6 was achieved, suggesting reliable screening performance. Similarly, when utilizing TTX and seeding 15,000 cells per well in a 96-well setup, robust Z' factor values reaching 0.6 were obtained, further indicating dependable screening abilities. Exposure to proinflammatory cytokines elicited heightened responsiveness reminiscent of native human dorsal root ganglia behavior. Pharmacologically, we discerned differential impacts on neural excitability attributable to discrete protein classes, namely sodium channels (Nav1.7, Nav1.8), potassium channels (Kv), calcium channels (Cav), transient receptor potential vanilloid type 1 (TRPV1), gamma-aminobutyric acid (GABA) receptors, glutamate receptors, and tyrosine kinases. Collectively, these findings substantiate the feasibility and efficacy of integrating hiPSC-derived nociceptors with an HCS-based MEA device for streamlined drug discovery initiatives.

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Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.18/C132

Topic: D.01. Somatosensation – Pain and Itch

Support: NINDS R01NS119476

Title: Targeted gene delivery to nociceptors

Authors: P. BHATIA[1], J. WANG[1], ***L. S. HE**[1], J. LI[1], E. SEMIZOGLOU[1], L. YANG[2], M. XU[3], S. HRVATIN[4], W. RENTHAL[1];
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Abstract: Chronic pain affects over 25 million adults in the United States and is a major cause of disability. Currently, available pain treatments such as opioids are often ineffective and linked to unacceptable side effects like respiratory depression and high abuse potential. Viral-based gene therapy offers several attractive advantages in treating refractory pain, as viruses can be engineered to deliver a wide range of molecular cargo capable of regulating the activity of neurons involved in pain perception (nociceptors). To develop novel nociceptor-specific viral tools, we performed single-nucleus multi-ome sequencing (RNA and assay for transposase accessible chromatin) on the mouse dorsal root ganglion (DRG) to identify nociceptor-specific gene expression and epigenomic features. We identified >10,000 nociceptor-specific regions of chromatin accessibility. Gene regulatory network analyses of these data identified transcription factors that are significantly enriched in DRG nociceptor subtypes, including *EBF1, POU4F3, JUN, MEF2C, NFIA,* and *ISL2*. After prioritization and screening of putative nociceptor-specific enhancers, candidate enhancers were cloned into adeno-associated viral vectors and delivered to DRGs in mice for validation. Enhancers driving expression preferentially in nociceptors were used to express inhibitory ion channels to silence the activity of nociceptors *in vitro* and *in vivo*. These nociceptor-selective vectors are likely to offer significant safety advantages over currently available viral vectors for the treatment of refractory chronic pain.

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Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.19/Web Only

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant RO1MH112205
NIH Grant U24DK116195
National Research Foundation of Korea 2022R1C1C1008786

Title: Advancing Chemogenetic Technologies: Structural and Functional Insights into a New Peripheral DREADD

Authors: *H. KANG[1], A. TASSOU[2], M. GERON[3], G. SCHERRER[4], B. L. ROTH[5];
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Abstract: Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) are the primary chemogenetic tools for remotely controlling cellular signaling, behavior, and physiology. In this study, we introduce a novel peripherally restricted DREADD based on the structure of a GPCR, which has minimal expression in the brain, and a new chemical actuator that does not penetrate the blood-brain barrier. This was achieved through a combination of mutagenesis, analoging using an extensive make-on-demand library, structural analysis of the new DREADD receptor using cryo-electron microscopy, and in vivo validation. The development of a chemogenetic system activated by peripheral-only ligands broadens the research scope into many peripheral systems without significantly affecting the central nervous system. Additionally, our structure-guided methodology will likely speed up the creation of new chemogenetic tools for both fundamental and applied sciences.

Disclosures: H. Kang: None. **A. Tassou:** None. **M. Geron:** None. **G. Scherrer:** None. **B.L. Roth:** None.

Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.20/C133

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH/NINDS R61NS122298
NIH/NCI R43CA206796-01A1

Title: A Novel Biomarker of Painful Peripheral Neuropathy

Authors: *M. I. NEMENOV;
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Abstract: The lack of a neuropathic pain biomarker poses a significant challenge in analgesic development. Diode Laser fiber selective stimulation (DLss) triggers neurogenic axon reflex flare, revealing fiber-specific and selective stimulation of silent, C mechano-insensitive (CMi) fibers in humans, monkeys, and pigs. CMi fibers play a major role in peripheral neuropathic (PN) patients' chronic pain. Additionally, CMi fibers are less denervated compared to polymodal fibers in painful PN, and sensitized, possibly explaining "normal" C pain thresholds in diabetic PN patients with loss of heat sensitivity under DLss. DLss also effectively differentiated post-chemotherapy patients with painful PN from those without pain, and served as a response biomarker for estimation of analgesic efficacy of sodium ion channels blockers in monkey studies. Methods and Study Design: DLss is conducted using Lass-10M (LasMed, Mountain View, CA), and the axon reflex flare response was recorded by Speckle Imager (Perimed, Sweden). 10 healthy volunteers demonstrated high reliability of DLss, <5% variance. 3 PN participants tested with both placebo and active patches. Our ongoing study evaluates DLss as a response biomarker for analgesic efficacy in PN pain patients, using a topical lidocaine patch (TZlido) in a double blind crossover study. A total of 44 PN participants (VAS ≥ 3) will be tested over 5 visits with DLss before, between and after each interval - baseline visit with neuropathy phenotype, then 7 day lead-in, then 7 days of active/placebo patch (12 hrs/day), then 7-day washout, then 7 days of active/placebo patch followed by re-assessment. Phenotypic characterization includes standardized neuropathy examination, skin biopsy, and conduction velocity tests. Painful PN participants report neuropathic pain using the Visual Analog Scale (VAS) daily. Results: For initial participants, the lidocaine patch significantly increased DLss pain and detection thresholds, suppressing axon reflex flare area and patient VAS compared to the placebo. Conclusion: Preliminary data suggest lidocaine modulation of DLss-induced thresholds and flare correlates with VAS modulation. Further recruiting and participant tests will provide a rigorous evaluation of DLss-based response combined biomarker.

Disclosures: M.I. Nemenov: None.

Poster

PSTR275: Primary Sensory Neurons Pain and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR275.21/C134

Topic: D.01. Somatosensation – Pain and Itch

Support: Supported by a grant from the Interdisciplinary Center for Clinical Research within the faculty of Medicine at the RWTH Aachen University"

Title: Different Coding Mechanisms In Humans For Evoked Pain Stimuli Versus Spontaneous Neuropathic Pain

Authors: A. FIEBIG[1], A. TROGLIO[2], A. MAXION[3], M. SCHMELZ[4], E. JØRUM[5], ***B. NAMER**[6];
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Abstract: The neuropathic pain syndrome small fiber neuropathy (SFN) is characterized by a change of somatosensation in opposite directions: intense burning pain sometimes worsened by heat and often contra-intuitively combined with increased thresholds for evoked heat pain. Treatment of pain in SFN is insufficient and there might be individual factors rendering peripheral nerve fiber dysfunction. We examined the nociceptive C-fiber discharges in two patients with SFN (SFN1 and 2) with different variants of the voltage-gated sodium channel Nav 1.9. via microneurography. For that, a thin needle electrode was placed into the superficial peroneal nerve to record extracellularly action potentials from single C-fibers. Action potentials were evoked by regular electrical stimulation in the skin of the receptive field at the back of the foot. Discharges to electrical stimulation, mechanical stimulation, and heat stimuli, as well as pathological spontaneous activity, were assessed. The nerve fibers were classified according to activity-dependent conduction velocity slowing and their mechanical responsiveness into mechano-sensitive (CM, 1a, polymodal) and mechano-insensitive (CMi,1b, sleeping, silent) nociceptors. We observed in SFN1 with a higher neuropathic pain level more spontaneously active (SA) nociceptors than in SFN2 with a lower level of neuropathic pain. The heat pain thresholds were elevated in both patients, but at >50°C for SFN1 was more elevated than for SFN2 at 47.6°C. Predominantly in SFN1, we observed lower discharge rates and frequencies of CM-nociceptors to a heat ramp stimulus compared to healthy controls. Only in SFN 2 CMi-nociceptors exhibited high discharge rates and frequencies in a burst-like fashion suddenly during the heat ramp. In both patients warming the skin increased pain and SA of C-nociceptors. Off-label treatment with the sodium channel modulator lacosamide alleviated pain only in SFN1 with a parallel decrease in SA and a more normal heat pain threshold of 48.5°C. The enigma of warmth-increased pain with simultaneously reduced heat pain sensation might be explained by warmth-induced SA in CMi and the decreased ability of CM to code acute heat. This suggests that the mechanisms and nociceptor classes involved in spontaneous neuropathic pain are different from those mediating acute evoked pain. Our study shows further that distinct differences in discharge patterns in peripheral nociceptors correlate to effectiveness in pharmaceutical treatment. The approach to identifying stratifying parameters in nociceptor discharges might open a way for stratified or even personalized treatment.

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Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.01/C135

Topic: D.01. Somatosensation – Pain and Itch

Support: CIHR grant FDN-148413
Canada Research Chair in Neurophysiopharmacology of Chronic Pain
Scholarship from Fonds de recherche du Québec en Santé

Title: Improving Pain Relief: Advances in the Design of Contulakin-G Analogs Inspired by Nature's Analgesic Arsenal

Authors: *A. LANOIE[1], S. DIARRA[3], J. DALLAGNOL[3], É. CARON-DUVAL[2], L. THÉROUX[2], E. BREAULT[2], M.-A. DANSEREAU[2], I. BROCHU[2], A. MURZA[2], J.-M. LONGPRE[2], P.-L. BOUDREAULT[2], D. CHATENET[3], P. SARRET[1];
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Abstract: The biologically active fragment of neurotensin, NT(8-13), induces non-opioid analgesia by activating NTS1 and NTS2 receptors. Contulakin-G, a 16-amino acid conopeptide O-glycosylated at Thr^{10} from *Conus geographus*, shares a C-terminal sequence with neurotensin and exhibits potent analgesia upon intrathecal (i.t.) administration in preclinical pain models. Although it binds to NTS1 and NTS2 receptors, systemic injection of contulakin-G does not induce analgesia. To enhance blood-brain barrier (BBB) penetration, enzymatic stability and analgesic effectiveness, we report here on the design, synthesis and structure-activity relationship of a novel class of contulakin-G analogs in which the Thr^{10} disaccharide residue has been replaced by lipophilic moieties, such as Glu-cycloheptylamide or Glu-memantide or by amino acids bearing charged, polar, hydrophobic, or aromatic functional groups. All these new derivatives were first characterized for their binding affinity, receptor selectivity and metabolic stability in plasma. The analgesic properties of contulakin-G and its analogs were then assessed by i.t. administration in male *Sprague-Dawley* rats in acute (tail-flick), tonic (formalin test), postoperative (Brennan paw incision) and neuropathic (Chronic Constriction Injury) pain models. Some of the analogs were more effective than contulakin-G in reducing acute pain, with equal efficacy in tonic and postoperative pain models. Interestingly, contulakin-G and the derivative bearing a Glu-cycloheptylamide residue in position 10 were effective in reducing mechanical hypersensitivity induced by chronic neuropathic pain after i.t. injection, a pain

condition resistant to opioids. We also found that the analgesic effect of contulakin-G was not blocked by naloxone, supporting the idea of an opioidergic-independent analgesia. Similarly, co-administration of the NTS1/NTS2 antagonist SR142948A with contulakin-G significantly reversed the analgesic effects of contulakin-G in the formalin pain test. Finally, the hypotensive effects assessed after intravenous injection revealed that all analogs induced a significant drop in blood pressure. In conclusion, our findings highlight the development of new, more easily synthesized contulakin-G analogs with preserved analgesic effectiveness in various preclinical pain models. Future research should explore their BBB permeability and analgesic actions after systemic delivery. In addition, we are seeking to develop more selective NTS2 agonists to minimize the hypotensive effects of NTS1 analogs and to identify the role of amino acids 1-9 by generating truncated derivatives.

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Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.02/C136

Topic: D.01. Somatosensation – Pain and Itch

Support: NIDCR grant DE022129

Title: Fentanyl induced orofacial pain in a rat model

Authors: L. NGUYEN, M. UMORIN, F. TAO, *P. R. KRAMER;
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Abstract: Opioid-induced hyperalgesia is a serious side effect in patients chronically taking opioids. In opioid-induced hyperalgesia, opioid use is paradoxically associated with an increase in nociceptive hypersensitivity. This phenomenon has been reproducibly seen in both humans and animals. To date, there is no consensus treatment and the only reliable treatment is to reduce or eliminate the use of opioids. Currently opioid prescriptions are fundamental to clinical practice. Thus, there is an urgent need to develop an appropriate approach for management. A recent study completed at Texas A&M in Dr. Feng Tao's lab indicated fentanyl induced hyperalgesia in mice within the face and paw lasts for at least 21 days. Our study added to the current knowledge by characterizing opioid-induced hyperalgesia and pain in the face and paw of a rat. Methods included performing sensory testing using von Frey filaments and an affective-motivational test of the face and/or paw 24 hours after injecting a 100 µl of a 60 µg/kg solution of fentanyl. Affective-motivational testing was completed using a conflict avoidance paradigm.

Fentanyl effects on memory and locomotion were determined using memory and rotarod tests. Results indicate a significant increase in both sensory and affective-motivational aspects of pain after daily injections of fentanyl starting about 3 days after the first fentanyl injection. The increased response lasted for at least two weeks after daily injections of fentanyl. Memory and locomotion were not significantly impaired by the fentanyl at the time that sensory and affective-motivational testing. In conclusion, increased pain is observed in the face and paw of the rat after daily injections of fentanyl resulting in a model for the testing drugs that could treat opioid-induced hyperalgesia.

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Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.03/C137

Topic: D.01. Somatosensation – Pain and Itch

Title: Role of orexin receptors within the dentate gyrus in antinociception induced by chemical stimulation of the lateral hypothalamus in animal models of acute and chronic pain

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Abstract: Pain is a complex experience consisting of sensory, affective-motivational, and cognitive dimensions. Hence, identifying the multiple neural pathways subserving these functional aspects is a valuable task. The role of the dentate gyrus (DG) as a relay station of neocortical afferents in the hippocampal formation (HF) in the mediation of antinociceptive responses induced by lateral hypothalamus (LH)-stimulation in different animal models of pain is still a matter of controversy. Adult male Wistar rats weighing 220-250 g were unilaterally implanted with two separate cannulae into the LH and DG. Intra-DG administration of the orexin-1 receptor (OX1R) antagonist, SB334867, or the orexin-2 receptor (OX2R) antagonist TCS OX2 29 was performed just 5 min before intra-LH carbachol microinjection. Animals then underwent the tail-flick test as a model of acute pain and the formalin test using injection into the plantar surface of the hind paw as a model of persistent pain. The results showed that OX1R and OX2R antagonists dose-dependently decreased the antinociceptive effects of carbachol in both tail-flick and formalin tests. In addition, the results obtained from the tail-flick test demonstrated the more prominent role of OX1R in the DG in carbachol-induced antinociception compared to that of OX2Rs in this region. According to the formalin test results, the preventive effect of SB334867 or TCS OX2 29 on carbachol-induced antinociception was approximately equal in both the early and late phases of formalin nociception. The pain modulatory role of the

orexinergic system through a neural pathway from the LH to the DG region suggests an alternative approach to developing more efficient therapeutic agents in the clinical setting.

Disclosures: A. Haghparast: None.

Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.04/C138

Topic: D.01. Somatosensation – Pain and Itch

Support: R01NS113965
R01NS105715

Title: Afferent derived T-cell mobilizing chemokine, CCl27a, regulates muscle hypersensitivity after repetitive ischemia with reperfusion injury

Authors: *G. M. TRIPATHI[1], L. F. QUEME[3,1], M. QUIJAS[1], K. PROPSOM[1], K. KELLERMAN[1], M. P. JANKOWSKI[1,2,4];
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Abstract: Mechanisms of muscle pain development involve both peripheral and central nervous system changes in addition to immune modulation of cellular activity. Ischemic myalgia is a unique type of muscle pain due to transient reduction of the blood supply to a part of the body followed by reperfusion injury (I/R) and is found in disorders such as sickle cell anemia, peripheral vascular disease or complex regional pain syndrome. Our model of prolonged ischemic myalgia which utilizes a repeated I/R injury to the forelimb has been shown to alter primary afferent function and immune signaling pathways. One factor that was found to be expressed in muscle afferents and could be contributing to I/R-related pain is C-C motif chemokine ligand 27a (Ccl27a). This chemokine acts through positive regulation of T-cell chemotaxis and actin cytoskeleton reorganization. To test whether Ccl27a was involved in I/R-related hypersensitivity, we performed a brachial artery occlusion with reperfusion in mice, followed by a second I/R 7 days later to develop prolonged pain-like behaviors. We demonstrated that Ccl27a is enhanced in DRG neurons after repeated I/R and this regulates the infiltration of CD8 and CD25+ T-cells to the muscles. AAV-mediated inhibition of Ccl27a in sensory afferents not only blocked T-cell recruitment to the injured muscles, but also inhibited spontaneous pain-like behaviors after repeated I/R. This data may support a role for nociceptor derived Ccl27a in immune responses in the muscle that regulate I/R-related hypersensitivity.

Results could provide evidence for the development of novel treatment strategies for patients with ischemic myalgia.

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Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.05/C139

Topic: D.01. Somatosensation – Pain and Itch

Title: Pharmacological evaluation of the early and late phases of the mono-iodoacetate (MIA)-induced osteoarthritic joint pain model in rats using an automated dynamic weight bearing system

Authors: *H. RASHID, J. XU, J. BAYOL, S. YANG, S. BEHESHTAEIN, K. APAYART, H. BURSTON, Y. MAMANE;
NuChem Sci. Inc, Saint-Laurent, QC, Canada

Abstract: Osteoarthritic (OA) knee joint pain affects millions of aged people world-wide, often leading to disability and eventual joint replacement. The rat mono-iodoacetate (MIA)-induced osteoarthritic joint pain model has been recognized as one of the most translatable OA pain models. The model is extensively used by researchers to evaluate novel therapeutics targeted for chronic joint pain. In the present study, we have utilized the automated dynamic weight bearing (DWB) system from Bioseb to pharmacologically validate the early and late phase of the MIA-induced OA joint pain model in rats. To induce the model, a single intra-articular injection of MIA into the right knee joint of rats was performed on Day 1. Joint pain evaluation was performed from Day 2 to Day 26. As a glycolytic inhibitor, MIA causes cartilage damage and induces monoarthritic joint pain. To assess joint pain, the advanced automated DWB-2 system was used. To assess joint swelling, joint diameter was measured using a caliper. Synovial fluids were also collected for proinflammatory cytokines evaluation. Effects of two analgesic drugs, naproxen and dexamethasone were evaluated in the model. In the first experiment, we assessed the dose-response effects of MIA (2 mg and 3 mg) in inducing joint pain. Intra-articular saline injection didn’t cause any weight bearing changes or any joint swelling in the rats. On the other hand, significant weight bearing deficit in the injected limb and swelling of the injected joint were observed with both 2 mg and 3 mg of MIA. Joint swelling and weight bearing deficits were MIA dose dependent. Weight bearing deficits in MIA-injected limb showed a biphasic pattern with an early and a late phase. Joint pain reactions were more pronounced between Day 2-7 and Day 22-26 with relatively lower pain levels in between. Joint swelling was more pronounced in the first week after MIA injection. Afterwards, it mostly resolved with a small window in the

later phase. In a follow-up experiment to examine effects of analgesic drugs, 3 mg MIA was chosen to induce the model based on higher window and lower variability compared with 2 mg MIA model. Effects of the drugs were assessed in early and late phase of the model. In the early phase, both naproxen and dexamethasone significantly attenuated joint pain and joint swelling in the MIA rats. In the late phase, only dexamethasone attenuated the joint pain as well as joint swelling. These results suggest that the early phase of the MIA model is NSAID-sensitive while the late phase is less sensitive to NSAIDs. Currently, cytokines analysis in synovial fluids is being performed to further evaluate the inflammatory nature of the two phases of the rat MIA model.

Disclosures: H. Rashid: A. Employment/Salary (full or part-time):; NuChem Sciences Inc. **J. Xu:** A. Employment/Salary (full or part-time):; NuChem Sciences Inc. **J. Bayol:** A. Employment/Salary (full or part-time):; NuChem Sciences Inc. **S. Yang:** A. Employment/Salary (full or part-time):; NuChem Sciences Inc. **S. Beheshtaein:** A. Employment/Salary (full or part-time):; NuChem Sciences Inc. **K. apayart:** A. Employment/Salary (full or part-time):; NuChem Sciences Inc. **H. Burston:** A. Employment/Salary (full or part-time):; NuChem Sciences Inc. **Y. mamane:** A. Employment/Salary (full or part-time):; NuChem Sciences Inc.

Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.06/C140

Topic: D.01. Somatosensation – Pain and Itch

Support: FY23 USU VPR Intramural Award, VPR-75-12975

Title: Phenotypic characterization of pain-related behaviors in a transgenic mouse model of rheumatoid arthritis

Authors: *K. CASTELL[1], M. CAMPANILE[1], J. PAMPALONE[1], I. LUCKI[2], C. A. BROWNE[2];
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[2]Pharmacol. & Mol. Therapeut., Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD

Abstract: Rheumatoid arthritis (RA) is an autoimmune disease that causes pain and inflammation in the joints of approximately 26 million Americans. Behavioral Risk Factor Surveillance System (BRFSS) data reported that 11.6% and 17.3% of male and female veterans (aged 18-44 years) were diagnosed with RA relative to non-veteran male (6.9%) and female (9.8%) subjects. This disease is progressive and the symptoms, such as pain and joint dysfunction, worsen over time. Current pharmacological options for RA-associated pain are limited, with no medications approved to limit or slow the progression of joint damage. The

present study characterized the onset of symptoms in a transgenic (TG) murine model of RA, with the ultimate goal of using this model to screen novel therapeutics to reduce RA-associated pain and attenuate disease progression. The RA model was developed through insertion of the human transgene for Tumor Necrosis Factor-α (TNF-α), a pro-inflammatory cytokine associated with arthritis in humans, into mice with a C57BL/6NTac background, (B6.Cg-Tg(TNF)#Xen, Taconic Biosciences). To characterize the phenotype, 6 male and 6 female TG mice and their age-matched wildtype (WT) littermates were screened in several behavioral tests from 10-18 weeks of age. Bodyweight and scores for arthritis symptom severity (limb swelling and other physical symptoms) were recorded twice weekly, with mechanical hypersensitivity, grip strength, and gait screened once weekly. Genotype X Time interactions were evaluated with mixed-effects models and Sidak's multiple comparisons where appropriate. Genotype differences in escalating arthritis scores ($F(8,176) = 41.00$, $p<0.0001$) and diminished bodyweight gain ($F(8,176)=10.47$, $p<0.0001$) were apparent starting at 10 weeks. Emerging over time, TG mice displayed significantly higher levels of mechanical hypersensitivity ($F(1,22)=59.78$, $p<0.0001$; from 12 weeks of age), decreased grip strength ($F(8,176) = 4.945$, $p<0.0001$; from 14 weeks old), and gait dysfunction across several parameters (pronounced dysfunction at 18 weeks old). The results show a strong phenotype of RA-associated dysfunction with progressive onset making this a useful rodent model to investigate novel pharmacotherapies to treat RA-associated pain.**Disclaimer:** The content and conclusions do not necessarily represent the official position or policy of the Uniformed Services University of the Health Sciences, the Department of Defense, or the U.S. Government.The authors declare no conflicts of interest.**Acknowledgements:** We are grateful to the USU Preclinical Behavioral and Modeling core for the use of the center's equipment.

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Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

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Program #/Poster #: PSTR276.07/C141

Topic: D.01. Somatosensation – Pain and Itch

Support: MSCA ITN Grant 955684

Title: Insights into Paclitaxel-Induced Neuropathic Pain: The Endocannabinoid System as a Therapeutic Target

Authors: *C. DI MARINO[1,3,4,5], A. M. DIEGO[1,6,4,5], M. REDMOND[1,4,5], M. HOPKINS[1,4,5], E. BERROCOSO[3,7,8], M. M. ROCHE[2,4,5], D. P. FINN[1,4,5];
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Abstract: Paclitaxel (PTX) is one the most used chemotherapeutic agents, however, it can induce chronic neuropathic pain in up to 80% of patients. Evidence suggests that the prevalence of chemotherapy-induced neuropathic pain is higher in women compared to men. The neurobiological mechanisms and the systems underlying this sex difference are poorly understood, but a good candidate may be the endocannabinoid system, given that it exhibits sexual dimorphism and plays a key role in the regulation of pain.The aim of the present study was to characterise the effects of acute administration of endocannabinoid system modulators in rats of both sexes expressing PTX-induced neuropathic pain-related behaviour.One hundred male and female Sprague-Dawley rats (N=10 per group) received intraperitoneal injections of Paclitaxel (2 mg/kg in 1:1:18 ethanol:cremophor:saline) or vehicle (VEH) on four alternate days.Pain-related tests (Von Frey and Acetone Drop) were used to assess sensitivity to mechanical and cold stimuli pre-PTX and over days 7-21 post-first PTX administration. Additionally, anxiety-related tests (Elevated Plus Maze and Open Field) were performed on day 16.On day 21, animals received acute i.p. injections of either vehicle, URB597 (FAAH inhibitor, 1mg/kg), SB366791 (TRPV1 antagonist, 1mg/kg) or AA-5-HT (FAAH inhibitor and TRPV1 antagonist, 5mg/kg). Pain-related tests were performed 30 minutes after the injection; euthanasia occurred immediately after. Brain and spinal cord were gross-dissected for measurement of levels of endocannabinoids (anandamide - AEA, and 2-arachidonoyl glycerol -2-AG) and related N-acylethanolamines (N-oleoylethanolamide - OEA and N-palmitoylethanolamide - PEA) by Liquid Chromatography Coupled to Tandem Mass Spectrometry (LC-MS/MS). Data were analysed using either Kruskal-Wallis followed by Mann-Whitney U post-hoc test with Bonferroni-Holm corrections, or Two-way ANOVA followed by Tukey HSD (Honest Significant Difference) post-hoc test for multiple comparisons.
PTX-induced hypersensitivity to mechanical and cold stimuli was evident at each time point analysed. Acute injections of URB597, SB366791, and AA-5-HT attenuated PTX-induced mechanical hypersensitivity in both sexes. However, only AA-5-HT attenuated the cold hypersensitivity induced by PTX treatment in both sexes. In PTX-treated rats, the FAAH inhibitor increased levels of PEA in the Spinal Cord, Periaqueductal Grey (PAG), Rostral Ventromedial Medulla, and Prefrontal Cortex (PFC), and increased levels of OEA in the PAG and PFC.These results suggest that the endocannabinoid system may be a viable therapeutic target for PTX-induced neuropathic pain.

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Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.08/C142

Topic: D.01. Somatosensation – Pain and Itch

Support: DoD W81XWH2010911
VA I01RX001475

Title: Effects of Immunosuppression after Limb Trauma on Nociceptive, Cognitive and Anxiety-Related Outcomes in Mice

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Abstract: Chronic pain is a common and problematic consequence of injuries with few proven methods for prevention or treatment. In addition to pain, functional limitations and neuropsychiatric changes such as cognitive impairment and anxiety worsen outcomes. Our objective was to determine whether inhibiting activation of the adaptive immune response after limb fracture would reduce pain, functional loss, memory changes and anxiety. These experiments used a murine tibial fracture/cast immobilization model that develops these adverse outcomes. Adaptive immunity was blocked using the immunosuppressant FK506 beginning at the time of fracture. The administration of FK506 reduced mechanical allodynia and hindlimb unweighting for weeks after cast removal as well as non-evoked pain. Moreover, FK506 reduced working memory loss in the Y-maze assay although loss of object recognition memory was not improved. Experiments using a zero-maze showed that FK506 prevents development of the anxiety phenotype. Mice placed on running wheels after cast removal showed impaired performance over the following 2 weeks, and this was not improved with FK506 administration. Experiments done in parallel groups showed that FK506 treatment blocked IgM accumulation in the skin of the fractured limbs, and that hippocampal enhancement of MMP8 expression, a metalloproteinase associated with neuroplastic changes after injuries, was completely blocked. Taken together our results show that blocking the adaptive immune response after limb trauma reduces the severity of pain-related outcomes, and improves some neuropsychiatric changes. The early control of pain-supporting immune system activation may, therefore, improve pain, cognitive and anxiety outcomes after injuries.

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Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.09/C143

Topic: D.01. Somatosensation – Pain and Itch

Title: Behavioral, biomarker, and imaging characterization of the mouse spared nerve injury model

Authors: A.-M. KARKKAINEN, R. IMMONEN, L. RAUHALA, ***A. SHATILLO**, S. BÄCK; Charles River Discovery Res. Services, Kuopio, Finland

Abstract: Animal models are important for increasing our understanding of the mechanism of neuropathic pain and for the discovery and development of novel therapies for pain management. This study aim was to assess behavioral, biomarker, and imaging readouts in the mouse spared nerve injury (SNI) model of neuropathic pain to be used in the future efficacy studies of putative therapies. Along with the classical assays, we employed a novel central readout, functional ultrasound imaging (fUS) to measure sensory responses to peripheral stimulation. Functional US is a non-invasive and sensitive neuroimaging tool with excellent spatio-temporal resolution, highly suitable for characterization of pain models.

Spared nerve injury was induced in two cohorts of C57Bl6/J mice according to the method described by Decosterd and Woolf with small modifications. Briefly, a small piece of the tibial and common peroneal nerves distal and close to sciatic nerve furcation was dissected. Control animals were sham-operated leaving the nerves intact. The first cohort of sham and SNI mice was followed for one week post-SNI. Short-term tactile allodynia was assessed with electronic von Frey (evF). Plasma was collected at 24 h, 72 h, and 7 days post-SNI for measurement of neurofilament light chain (NfL) levels with Quanterix Simoa. At endpoint 7 days post-SNI a skin punch biopsy sample was collected from the ipsilateral paw and skin intraepidermal nerve fiber (IENF) endings were evaluated using PGP9.5 immunohistochemistry. The second cohort of SNI mice were followed for two weeks to assess tactile and cool allodynia (acetone cooling test) and subjected to fUS imaging with bilateral hind paw pinprick and mechanical whiskers stimulations.

In the first cohort of mice, in the tactile allodynia (assessed by evF at baseline, 72 h and 7 days post-SNI) assay, mean paw withdrawal threshold (PWT) was decreased by almost 60% starting from 72 h post-SNI. The NfL levels peaked at 24 h, showing a gradual decrease, but remaining highly significant at the 72-h and 7-day timepoints, without reaching baseline levels. Post-surgery plasma NfL levels of sham-operated mice did not differ from the baseline (pre-surgery) level. The SNI mice displayed a decrease in IENF counts as compared to the sham mice. The second cohort of SNI mice showed robust and highly significant mechanical and cool allodynia at 2 weeks post-SNI as well as decreased neurovascular response to pin prick stimulation of the ipsilateral hindpaw in fUS imaging.

The mouse SNI model displays robust changes in all readouts applied in this study, warranting their use in the future drug discovery efficacy studies of novel therapies or mechanisms in neuropathic pain.

Disclosures: A. Karkkainen: A. Employment/Salary (full or part-time):; Charles River Discovery Research Services. **R. Immonen:** A. Employment/Salary (full or part-time):; Charles River Discovery Research Services. **L. Rauhala:** A. Employment/Salary (full or part-time):;

Charles River Discovery Research Services. **A. Shatillo:** A. Employment/Salary (full or part-time):; Charles River Discovery Research Services. **S. Bäck:** A. Employment/Salary (full or part-time):; Charles River Discovery Research Services.

Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.10/C144

Topic: D.01. Somatosensation – Pain and Itch

Support: NINDS – R01NS123070
NIH – R25NS130965

Title: Characterizing cold hypersensitivity in the relapsing-remitting experimental autoimmune encephalomyelitis mouse model

Authors: *K. T. KUKLINSKI, M. K. MADASU, R. AL-HASANI;
Dept. of Anesthesiol., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Approximately one third of patients who have been diagnosed with multiple sclerosis (MS) will develop hypersensitivity to cold temperatures. Despite the relatively high frequency of this symptom's occurrence, little is known about the neurochemical mechanisms that underlie this altered sensation. Recent studies, however, have implicated peripheral activation of the kappa opioid receptors (KOR) in cold hypersensitivity. To determine whether the KOR plays a role in cold allodynia associated with MS, we used the relapsing-remitting experimental autoimmune encephalomyelitis mouse model to induce an MS-like pathology. We selected this disease model over other MS-related models because it causes a robust disease state that does not immediately inhibit motor activity. Once the disease state has been induced, the body weight and experimental autoimmune encephalomyelitis score of each animal is assessed daily to monitor health. Cold hypersensitivity was assessed weekly using a cold plantar assay at either 3 or 10°C. Additionally, mechanical hypersensitivity was assessed using a von Frey assay. Through these tests, we show that diseased mice are made more sensitive to both cold and mechanical stimuli when compared to control mice. Sections of lumbar spinal cord were stained with Luxol Fast Blue and Cresyl Violet to determine whether differential myelination was associated with induction of a worsened disease state. Further investigation of kappa opioid receptor signaling and its role in cold hypersensitivity could result in the development of novel therapeutics which specifically target this system to alleviate pain and discomfort.

Disclosures: K.T. Kuklinski: None. **M.K. Madasu:** None. **R. Al-Hasani:** None.

Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.11/C145

Topic: D.01. Somatosensation – Pain and Itch

Support: Compass Pathways PhD funding
University of Reading strategic PhD funding

Title: The psychedelic drug psilocybin has anti-nociceptive effects in neuropathic pain

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Abstract: Interest in "psychedelic" drugs such as psilocybin, has grown over the past decade, particularly in addressing cognitive disorders such as depression. Recently, there has been a surge of interest in exploring the effects of psilocybin on chronic pain. While there are a limited number of small scale case studies supporting its efficacy in conditions such as lower back pain, migraines, cluster headaches, and fibromyalgia, the current research aims to provide pre-clinical evidence to establish an anti-nociceptive effect of psilocybin on chronic pain, specifically in a mouse model of peripheral nerve injury. This is of particular interest due to increased effort towards finding alternative treatments to opioids for the relief of pain states.The aim of this research is to firstly evaluate psilocybin's ability to alleviate pain-associated behaviours in a mouse model of neuropathic pain induced by peripheral nerve injury, and secondly, to explore the effect of psilocybin when used alongside a ‘gold standard’ pain therapeutic. The Spared Nerve Injury (SNI) model of peripheral neuropathy was induced in male 8-12 week old mice. This model produces robust mechanical and thermal hypersensitivity which last for several weeks. Psilocybin doses of 0.3mg/kg or 1mg/kg were administered intraperitoneally upon reaching peak hypersensitivity. A variety of behavioural tests were used to assess pain-like behavioural responses including tests for mechanical sensitivity and temperature sensitivity. Finally, gabapentin, a commonly used neuropathic pain treatment, was administered following psilocybin treatment.A single injection of psilocybin induced a substantial and enduring reduction in mechanical sensitivity, as evidenced by a notable increase in the 50% withdrawal threshold. Psilocybin-treated mice exhibited a significant decrease in cold hypersensitivity, manifested by reduced paw licking and biting time in response to acetone application, as well as significantly greater time spent on the cold plate in the thermal place preference test. Furthermore, there was an improved and prolonged anti-nociceptive effect of gabapentin in mice pre-treated with psilocybin. Our findings provide strong pre-clinical evidence of the anti-nociceptive effects of psilocybin in a model of neuropathic pain. Ongoing investigations show a potential for psilocybin to also prime the nervous system to potentiate the effect of gabapentin, a gold standard treatment for chronic pain.

Disclosures: T. Askey: 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.; part funding of my PhD by psilocybin biotech Compass Pathways. **M. Maiarú:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; grant funded by Compass pathways. **G. Stephens:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; grant funded by Compass Pathways.

Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.12/C146

Topic: D.01. Somatosensation – Pain and Itch

Support: Wakayama Medical University Young Researcher Support Grant, 2023

Title: Analgesic Effects of Intra-arterial Infusion of Imipenem/Cilastatin Sodium in a Rat Model of Knee Osteoarthritis.

Authors: *Y. MATSUYAMA[1,2], M. YAMANAKA[3], W. TANIGUCHI[3], N. NISHIO[3], H. TAMAI[3], T. UENO[3], R. MIYAKE[3], T. NAKATSUKA[4], H. YAMADA[3];
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Abstract: Introduction: Knee osteoarthritis (KOA) is a prevalent disease among the elderly. Its primary symptom, knee pain, can severely limit physical activity. Recent research has suggested that neovessels associated with nerve fibers may serve as pain sources in musculoskeletal disorders. Transcatheter arterial micro-embolization using imipenem/cilastatin sodium (IPM/CS) was introduced as a method of pain relief in KOA. However, the precise mechanism of its analgesic effects remains unclear. This study aims to assess the analgesic effects of intra-arterial embolization using IPM/CS in a rat model of KOA through behavioral assessments and *in vivo* patch-clamp techniques. Methods:KOA Induction: KOA was induced in male Sprague-Dawley rats by intra-articular injection of sodium monoiodoacetate (MIA). The control group received saline (Sham). Histopathological changes in their knees were assessed using the OARSI scoring system. Intra-Vessel IPM/CS Administration: IPM/CS was injected directly into the femoral artery or vein with a microscope. Histopathological examination was used to confirm the presence of embolic particles. Pressure Application Measurement (PAM): The mechanical pain threshold across the knee joint was assessed(The limb withdrawal threshold: LWT). *In Vivo*

Patch-Clamp Recording: Whole-cell patch-clamp techniques were used to record spontaneous excitatory postsynaptic currents (sEPSCs) in the spinal cord dorsal horn. The data were recorded in the L4 medullary segment level where pain information from the knee joint is considered to be input. Results:Histopathological Findings: The OARSI score was higher in KOA group than sham group. IPM/CS particles were detected inside the synovial arteries of KOA rats, confirming successful embolization. PAM: Mechanical pain threshold tests showed that intra-arterial IPM/CS significantly improved the LWT ratio in KOA rats. Intravenous IPM/CS infusion did not yield similar results. *In Vivo* Patch-Clamp Recording: The frequency of sEPSCs in KOA rats decreased significantly following intra-arterial IPM/CS administration compared to KOA rats receiving saline. Discussion:The analgesic effects of TAME with IPM/CS were demonstrated by the reduction of spontaneous sEPSCs and the increased pain threshold in KOA rats. Histopathological findings confirmed that IPM/CS particles embolized the synovial arteries. Thus, intra-arterial IPM/CS administration potentially reduces the number of vascular endothelial cells and inflammatory mediators, leading to reduced pain perception. It is anticipated that further investigation into the pathological changes occurring peripherally is necessary.

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Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.13/C147

Topic: D.01. Somatosensation – Pain and Itch

Support: NSFC grant

Title: An epilepsy-associated BK mutation induces mechanical hyperalgesia through the activity of the GABA neurons in mice

Authors: *R. ZHANG[1], S. YANG[2], X. WANG[2], Y. ZHANG[2], M. TANG[3], J. XU[2];
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Abstract: BK (Slo1) channels are wildly expressed in sensory neurons and mediate both inflammatory and neuropathic pain in mice. However, underlying the mechanism and the corresponding therapeutics remain obscure. In this study, we utilized a conditional genetic mouse model carrying human BK-N999S, an epilepsy-associated BK gain-of-function (GOF) mutation

identified in patients, to investigate the neuronal mechanism of BK involved in the pathogenesis of pain. We found that the mice carrying BK-N999S mutation, manifest the clinical features of absence epilepsy-like behaviors, and exert a significantly increased mechanical hyperalgesia when subjected to Von-Frey or Randall-Selitto tests. Conditional genetic expression of N999S to GAD-positive neurons ($BK^{N999S/N999S}$ flox-Gad-Cre) in mice caused an even higher sensitivity in response to mechanical stimuli, but not to either thermal or cold stimuli. In contrast, conditional genetic expression of this mutation to Glu-positive neurons ($BK^{N999S/N999S}$ flox-Glu-Cre) in mice showed a significantly decreased mechanical pain behavior, and no changes observed in thermal or cold pain perception. These results suggested that BK channel mediates mechanical hyperalgesia by enhancing the activity of the GABA neurons in mice. Further experiments showed that intraperitoneal injection (i.p.) of paxilline, a BK selective blocker, effectively reverted/alleviated mechanical hyperalgesia induced by genetic expression of BK-N999S to Gad-positive neurons ($BK^{N999S/N999S}$-flox-Gad-Cre). Our study thus uncovered a neuronal mechanism of BK GOF involved in mechanical hyperalgesia in absence epilepsy mutant mice, and suggested that BK inhibition is a promising therapeutic strategy for mitigating BK GOF-induced neurological disorders.

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Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.14/C148

Topic: D.01. Somatosensation – Pain and Itch

Support: Howard Hughes Medical Institute Hanna H. Gray Fellowship
NINDS R35
Defense Advanced Research Projects Agency (DARPA)

Title: Proteomic analysis of the pain neuroaxis in neuropathic and inflammatory pain states

Authors: *B. AHANONU[1], N. KROGAN[2], A. I. BASBAUM[3];
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Abstract: Background & Aims
Pain is a critical, complex, multidimensional percept that initiates appropriate protective behaviors by integrating incoming sensory information with ongoing brain states. At the molecular level, altered protein expression can initiate and maintain pain and are key targets for analgesics. Although transcriptomes are informative, they miss many molecular changes—*e.g.*,

post-translational modifications and protein-protein interactions—and growing evidence indicates protein and gene expression can be discordant. To enable insights into post-injury molecular changes and identify novel therapeutic targets, here we used advances in proteomics to study the pain generating neuroaxis.

Methods

We assembled murine whole tissue proteomes from dorsal root ganglia (DRG), dorsal (DH) and ventral horns of the spinal cord, and multiple brain regions in inflammatory or partial sciatic nerve injury pain models. All injured mice used for downstream analysis displayed mechanical hypersensitivity. To improve dataset quality and size, we optimized tissue processing, analyzed samples with high-sensitivity mass spectrometry, and used a well-validated analysis pipeline that identified 4 to over 6 thousand proteins per sample.

Results

Clustering analysis revealed high intra-tissue proteome overlap and distinct peripheral and central nervous system proteomes. Inflammatory and nerve injury induced hundreds of significantly up- or down-regulated proteins across all tissues. Comparing our DRG and DH proteomes to transcriptomes revealed a correlation between protein abundance and gene expression. Yet—and consistent with prior studies in cell cultures, animal models, and humans—only a small number of genes/proteins were significantly modulated in both transcript and protein. To broaden the candidate of pain-modulated proteins and identify more hits, which we term pain predicting proteins (PPP), we used network propagation approaches and protein interaction networks that identified PPPs implicated in chronic pain.

Conclusions

These proteomes provide a molecular resource across multiple tissues, time points, and pain models. Our datasets' breadth and analysis can help identify the evolution of the proteome, from acute to chronic pain, as well as molecular pathways engaged and modulated across pain conditions. Importantly, the network approaches suggest that existing and new pain-related datasets can be further mined to identify novel targets. This ability to collect and analyze high-quality proteomes will allow the field to gain new insights into the molecular mechanisms of pain and potentially identify novel targets for pain therapy.

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Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.15/C149

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant NS126987

Title: Glia and perineuronal nets in the Prrxl1 model of chronic pain

Authors: *E. N. WILLERSON[1,2], J. M. LUTCHMAN[3], G. LI[3], E. EICHLER[3], K. WILAMOWSKY[4], J. ZAR[4], O. ZAUROV[4], G. CATALDO[3,5], J. C. BRUMBERG[1,6,2,5]; [1]Psychology, The Grad. Ctr., CUNY, New York City, NY; [2]Psychology, Queens College, CUNY, Flushing, NY; [3]Psychology, Queens Col., CUNY, Flushing, NY; [4]Neurosci. Major, Queens Col., CUNY, Flushing, NY; [5]Neuroscience Major, Queens College, CUNY, Flushing, NY; [6]Biology, The Graduate Center, CUNY, New York City, NY

Abstract: Prrxl1 is a paired homeodomain transcription factor responsible for the development of patterning in the trigeminal system and in the spinal cord dorsal horn (DH). In mice, knockout (KO) of this gene results in complete loss of "barrelette" pattern formation - trigeminal somatotopic representation of the whisker pad - in the principal sensory nucleus (PrV) of the trigeminal, and partial disruption of barrelettes in the spinal trigeminal nucleus (SpV). In the spinal cord, KO results in disruption of peripheral afferents - primarily small-diameter c-fibers - finding their targets in the DH. Behaviorally, this results in a mouse which experiences simultaneous hypoalgesia to the body and chronic hyperalgesia to the face. We confirmed this using thermal and mechanical stimulation, as well as using the mouse facial grimace scale. A new assay was developed to induce itch or orofacial pain in mice, used here as a behavioral comparison point for Prrxl1 KOs. Further behavioral data also indicates that Prrxl1 KOs show altered grooming, feeding, and exploration habits. It was previously known that Prrxl1 KOs experience neuronal loss in both the trigeminal system and DH, but here we show that these mice experience changes to glia and perineuronal nets as well, when compared with wildtype controls. Microglia are the resident macrophages of the central nervous system, by staining tissue with ionized calcium binding adaptor molecule 1 (Iba-1), we show that these glia take on an aberrant "activated" morphology in both the trigeminal PrV and SpV of Prrxl1 KO mice. This state has been strongly correlated with upregulation of phagocytosis, neurotoxicity, and disruption of extracellular matrix structures such as the perineuronal net (PNN). The PNN is a web-like structure which typically surrounds parvalbumin+ GABAergic interneurons, and it is known for inhibiting plasticity. PNNs were stained using wisteria floribunda agglutinin (WFA), and here we show that Prrxl1 KO mice have significantly fewer PNNs in the PrV. We also found within-subject differences for both microglia and PNNs across the neighboring trigeminal nuclei, in both Prrx1 KOs and controls. In sum the Prrxl1 KO mice can serve as an innate model of chronic orofacial pain.

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Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.16/C150

Topic: D.01. Somatosensation – Pain and Itch

Support: NS123057
DA03064
T32 DA024628

Title: Inhibition of GABA-aminotransferase (GABA-AT) with OV329 reverses pathological nociception in rodents without abuse liability

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Abstract: An imbalance between the excitatory neurotransmitter glutamate and inhibitory neurotransmitter GABA is implicated in central sensitization prompting the exploration of strategies to restore balance in neuronal excitability for analgesic purposes. Current drugs targeting GABA reuptake inhibition hold therapeutic potential, but are often limited by side effects. Our investigation focuses on inhibiting GABA metabolism via GABA-AT inhibition as an alternative analgesic approach, aiming to elevate endogenous GABA levels without adverse effects. The next-generation GABA-AT inhibitor OV329, known for its superior efficiency in inactivating GABA-AT compared to vigabatrin, has shown promise as an anticonvulsant in preclinical studies. We aimed to assess if OV329 could function as a broad-spectrum analgesic in preclinical models of inflammatory and neuropathic pain. Neuropathic pain was induced in C57BL/6J mice by paclitaxel, while inflammatory nociception was generated by unilateral injection of CFA into the hind paw. Mechanical and cold hypersensitivity were assessed using an electronic von Frey and acetone. Liquid chromatography/mass spectrometry (LC/MS) was employed to measure GABA and glutamate levels. Locomotor activity and motor coordination were evaluated using an activity meter and rotarod test. Behavioral assays included a conditioned place preference assay to assess reward or aversion induced by OV329 and an intravenous drug self-administration paradigm to determine its reinforcing properties compared to morphine. Intrathecal administration of OV329 suppressed paclitaxel-induced allodynia without developing tolerance or motor effects, while increasing GABA and reducing glutamate levels in the lumbar spinal cord. OV329 effectively attenuated CFA-induced inflammatory nociception comparable to morphine, yet without inducing rewarding effects or self-administration behavior. Acute administration of OV329 did not impair motor function or produce motor ataxia, distinguishing it from the first-generation GABA-AT inhibitor vigabatrin. Overall, OV329 exhibited antinociceptive efficacy in preclinical models of CIPN and inflammatory pain, alongside a favorable safety profile at therapeutic doses. These findings suggest that targeting GABA-AT with OV329 may offer a novel analgesic mechanism with a more favorable therapeutic spectrum through enzyme regulation.

Disclosures: L. Assis: None.

Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

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Support: NIDDK K01DK114395-06
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University of Pittsburgh Start-Up funds to KR and BKT

Title: Mast cells and VEGFR2 contribute to pelvic tactile allodynia in a non-invasive mouse model of endometriosis

Authors: *S. ACHARYA, P. PRASOON, B. K. TAYLOR, K. ROMAN;
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Abstract: Endometriosis (EM) impacts approximately 4 million women in the United States (Agarwal et al., 2019). EM lesions are infiltrated with mast cells (MCs) (Fonseca et al., 2023; Matsuzaki et al., 1998) and have elevated expression of vascular endothelial growth factor (VEGF) (Rein et al., 2010) and its cognate receptor VEGFR2 (Steinthorsdottir et al., 2016). We adopted a non-invasive mouse model of EM to test the hypothesis that MC-mediated release of VEGF drives pelvic tactile allodynia and treatments that either stabilize MCs or inhibit VEGFR2 reverse pelvic tactile allodynia. Briefly, female C57BL/6J donor mice (6-weeks old) received an injection of estradiol benzoate (10µg), and 4 days later, each uterine horn was excised and placed in Hank's Balanced Salt Solution (HBSS) and minced. Recipient mice received an intraperitoneal (i.p.) injection of HBSS (500µl; "Shams") or HBSS+donor mice minced uterine horn (500µl; "EM mice"). To assess the development of pelvic tactile allodynia, von Frey (vF) filaments were applied to the suprapubic region before (baseline) and 28 days after tissue injection. Our first experiment evaluated the effect of the MC stabilizer β-nicotinamide mononucleotide (NMN) on mechanical hypersensitivity. In Shams, saline nor NMN (150mg/kg) changed mechanical thresholds. In EM mice, NMN but not saline reversed pelvic tactile allodynia ($p<0.05$) from 9 to 36 hrs. In a separate cohort, we determined the effect of the VEGFR2 inhibitor SKLB1002 (SB) on mechanical hypersensitivity. In Shams, saline nor SB (100mg/kg) altered mechanical hypersensitivity. In EM mice, SB but not saline blunted pelvic tactile allodynia ($p<0.05$) from 60 to 90 min. Next, we conducted a dose-response study in which we intrauterinely (i.u.) infused saline or VEGF (0.001-1pg) in mice without EM on days 1, 4, and 7 and vF tested for 56 days. We demonstrated that i.u. saline or 0.001-0.01pg VEGF does not induce mechanical hypersensitivity. VEGF at 0.1pg caused mechanical hypersensitivity for 14 days ($p<0.05$). VEGF at 1pg induced mechanical hypersensitivity for up to 56 days ($p<0.05$). Thus, we investigated the effect of the MC stabilizer ketotifen fumarate (Keto) on VEGF-

induced hypersensitivity. We i.u. infused saline or VEGF (1pg) and then i.u. infused saline or Keto (10mg/kg) on day 56. Data showed that mechanical thresholds remain unchanged in controls. However, Keto but not saline reversed VEGF-induced pelvic tactile allodynia from 9 to 18 hrs ($p<0.05$). In summary, 1) stabilizing MCs or inhibiting VEGFR2 alleviates pelvic tactile allodynia in EM mice, and 2) intrauterine VEGF dose-dependently elicits pelvic tactile allodynia that is reversible with blunting of MC activity.

Disclosures: S. Acharya: None. **P. Prasoon:** None. **B.K. Taylor:** None. **K. Roman:** None.

Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.18/C152

Topic: D.01. Somatosensation – Pain and Itch

Support: NIDCR Grant DE022129

Title: Spontaneous orofacial pain determination in undisturbed rats for an artificial neural network model

Authors: *M. UMORIN[1], D. LE[2], B. KRUG[2], S. VASIREDDY[2], P. KRAMER[1];
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Abstract: Determining spontaneous painful state in free-moving, undisturbed rodents is an important technique in pain research. To measure spontaneous pain responses 10-20 one year-old female rats were injected with either CFA or formalin into whisker pad. The rats injected with CFA were video recorded 24 hours after injection during the light phase for one hour. The rats injected with formalin were video recorded for one hour immediately following injection. Rearing, face wiping, licking, body wiping, stretching or sniffing, and being still were scored from the videos for bout frequency, activity duration as fraction of total observation time and average activity bout duration. After application of CFA the average bout duration for face wiping increased. After application of either CFA or formalin bout frequency for rearing decreased, time fraction for rearing decreased and bout frequency for being still has decreased. In contrast, the average bout duration for licking increased in both CFA and formalin injected rats. Results indicate that various behavior patterns can be used to determine spontaneous pain for inflammatory models of orofacial pain. Future studies will analyze how long these changes can be measured, the effect of measuring in the dark phase, how increased measurement time effects the results, the effect of sex and measurements in different models including neuropathic pain models. The results of this study will be used to train an artificial neural network model to

recognize different activities from a video for automatic determination of pain from undisturbed rats.

Disclosures: M. Umorin: None. **D. Le:** None. **B. Krug:** None. **S. Vasireddy:** None. **P. Kramer:** None.

Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.19/C153

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH 1R01NS132483-01A1

Title: Social Defeat Stress induces chronic pain in juvenile mice involving TLR4 activation in DRG

Authors: *A. PANICHKINA[1], J. B. LEMES[2], T. L. YAKSH[3];
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Abstract: ABSTRACT: Social defeat stress induces chronic pain in juvenile mice involving TLR4 activation in dorsal root ganglia.
Chronic stress is a risk factor for chronic pain development in humans. Studies in rodents using social defeat stress (SDS) demonstrated to trigger social avoidance behavior and chronic allodynia in mice subjected to chronic stress, and this was associated with neuraxial pain signaling changes. Toll-like receptor 4 (TLR4) has been involved in neuropathic and inflammatory pain phenotypes by provoking inflammatory pathway activation and neuronal sensitization at the spinal and supraspinal levels, however, our understanding of the role of neuraxial TLR4 in pain processing caused by chronic stress remains to be elucidated. Recent work has suggested that SDS leads to increased TLR4 expression in prefrontal cortex and microglial activation (PMID: 24331544).
Our present work study aims to investigate participation of spinal and dorsal root ganglion (DRG) TLR4 receptors in promoting behavioral augmentation of nociceptive behaviors during and after SDS in male and female mice. To do so, C57BL/6 mice (4-5 weeks) were subjected to 6 sessions of SDS during 10 days, consisting of 10 minutes of physical contact with the aggressor (CD1 retired male breeder). After each session, mice are kept in the same cage separated by a plexiglass divider, allowing constant sensorial contact between the aggressor and the defeated mice. The mechanical threshold of the hind paws was measured using the von Frey filament, and thermal withdrawal was measured using the Hargreaves method. Dorsal root ganglia (DRG L3-L5), spinal cord, and brain were collected for flow cytometry and

immunohistochemistry analysis.
The behavioral data demonstrated mechanical allodynia in male and female juvenile mice subjected to 6 SDS sessions compared to the control group, which lasted up to 10 days after the SDS protocol. Thermal hyperalgesia was detected one day after the last session (day 11 of protocol) in male and female defeated mice, but in male defeated mice up to 10 days after the SDS protocol. On day 22 of the protocol, flow cytometry revealed significant differences in TLR4 activity in the DRG cells of male and female defeated mice, more specifically TRPV1+ (c-fibers neuron) and in CD11B+ (macrophages) cells. No changes were observed in the spinal cord.
Together, these results suggest that TLR4 receptors expressed in the DRG participate in the chronic pain development induced by the social defeat stress in juvenile male and female mice. Ongoing work is extending these findings to SDS-induced cortical activation.

Disclosures: A. Panichkina: None. **J.B. Lemes:** None. **T.L. Yaksh:** None.

Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.20/C154

Topic: D.01. Somatosensation – Pain and Itch

Support: R37 NS108278-05
R01 NS070711-14

Title: Episodic pain following heat exposure in Fabry disease reveals a novel regulatory mechanism for TRPA1.

Authors: *J. D. ENDERS[1], E. PRODOEHL[2], A. SRIRAM[2], B. DHARANIKOTA[2], C. L. STUCKY[3];
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Abstract: Fabry disease is the most common lysosomal storage disorder, arising from X-linked mutations in α-galactosidase A (*GLA*) and subsequent accumulation of glycosphingolipids in tissues. Patients with Fabry disease develop painful small-fiber neuropathy punctuated by intractable episodic pain evoked by exercise, fever, and environmental heat. Our prior work in a *Gla*-knockout (Fabry) rat model has demonstrated development of pain-like behaviors with concomitant sensitization of transient potential receptor cation channel ankyrin-containing 1 (TRPA1) in the dorsal root ganglia (DRG) neurons in Fabry rats. Here, we demonstrate that transient exposure to heat (40ºC for 30 minutes) elicits episodes of transient-yet-robust

mechanical hypersensitivity in young, pre-neuropathic Fabry rats. Additionally, the number of DRG neurons responsive to the TRPA1 agonist mustard oil increases following exposure to heat in Fabry, but not wildtype, rats. Cellular resilience to heat is transcriptionally dependent on heat shock factor 1 (HSF1), which coordinates expression of the chaperone component heat shock proteins (HSPs). To assess whether behavioral and neuronal sensitization on heat exposure is due to impaired heat shock response in Fabry disease, we assessed nuclear translocation of HSF1 in Fabry DRG neurons following heat exposure. While HSF1 robustly trafficked to the nucleus of wildtype DRG neurons following heat exposure, HSF1 remained cytoplasmic in Fabry DRG neurons even following heat exposure. Moreover, HSP70- and HSP90-family transcripts were reduced in Fabry DRG neurons both at baseline and following heat exposure. We further determined that HSP70 negatively regulates TRPA1, as preincubation with VER-155008, an HSP70 inhibitor, increased the number of mustard oil-responsive DRG neurons at baseline, which was further exacerbated by heat, in naïve DRG neurons. Together this work establishes the Fabry rat as a model to study episodic pain in Fabry disease, describes mechanisms contributing to impaired heat tolerance in Fabry DRG neurons, and identifies HSP70 as a potent regulator of TRPA1 function.

Disclosures: J.D. Enders: None. **E. Prodoehl:** None. **A. Sriram:** None. **B. Dharanikota:** None. **C.L. Stucky:** None.

Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.21/C155

Topic: D.01. Somatosensation – Pain and Itch

Support: R01NS097880

Title: Open Field Two-Texture Preference Test Does Not Detect Neuropathic Pain-Like Behavior After Unilateral C5 Spinal Cord Injury In Mice

Authors: *M. LYTTLE[1], D. JAFFE[1], D. FREEMAN[2], A. C. LEPORE[1], M. R. DETLOFF[2]; [1]Neurosci., Thomas Jefferson Univ., Philadelphia, PA; [2]Drexel Univ., Philadelphia, PA

Abstract: One of the major complications often associated with a spinal cord injury (SCI) is the development of neuropathic pain. While well-established methods including von Frey testing and the mechanical conflict avoidance system are often used to assess SCI-induced neuropathic pain, these methods have been shown to have numerous limitations, including experimenter bias and long periods of active testing. As such, we set out to develop an open field, two-texture preference assay to detect neuropathic pain-like behaviors following a unilateral C5 SCI in mice. To do so, we modified the open field chamber by introducing both a rough and a smooth texture

to the floor of the chamber. We hypothesized that the rough surface would elicit neuropathic pain like behavior as measured through a reduced total time spent and total distance traveled on the rough surface compared to the smooth surface in mice with other neuropathic pain-like behaviors. However, testing revealed that both at baseline and post-injury, mice spent more time and traveled a greater distance on the rough surface compared to the smooth surface. Additionally, this test did not show any significant correlations with the von Frey test or the mechanical conflict avoidance system. While this novel assay may be able to provide information pertaining to other components of functional recovery, this test is not able to discriminate neuropathic pain-like behaviors following a SCI.

Disclosures: M. Lyttle: None. **D. Jaffe:** None. **D. Freeman:** None. **A.C. Lepore:** None. **M.R. Detloff:** None.

Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.22/Web Only

Topic: D.01. Somatosensation – Pain and Itch

Support: Subvención SIP 20240205
CF-2023-I-822

Title: Antinociceptive effect of Nbenzylpiperidinyl4 flurobenzamide in adult zebrafish (Danio rerio)

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Abstract: Studies on zebrafish have demonstrated nociceptive responses to various stimuli, including thermal, mechanical, and chemical stimuli. For example, exposing zebrafish to a noxious temperature or pinching their tails can elicit observable behaviors indicative of nociception, such as increased swimming activity, erratic movements, or rubbing the affected area against objects. In this study, we use acetic acid as an algesic stimulus to evaluate the possible participation of the sigma-one receptor (S1R) in nociception. N-(benzylpiperidinyl)-4-fluorobenzamide (LMH-2) is a new compound synthesized as an antagonist of S1R; it has a high affinity (Ki = 6.0 nM). In this research, fish were immersed in LMH-2 (0.5-2 µM) for 1 h before acetic acid injection (1% i.p., 20 µL), The number of quadrants of a glass box was counted for 30 min, and locomotor activity was quantified as

nociceptive behavior. The results showed that LMH-2 presents an antinociceptive effect in a concentration-dependent manner on the behavior characterized by the fish movement, significantly ($p > 0.05$) like those of tramadol used as a positive control. In addition, when the fish were immersed in PRE0-84 (2 μM), an agonist of S1R, the nociception increased significantly concerning the basal behavior of acetic acid. Furthermore, PRE-084 was combined with LMH-2, and fish immersed 24 h previous acetic acid showed that PRE-084 prevented the antinociceptive effect of LMH-2. These results suggest that S1R is participating in inflammatory pain in the zebrafish.

Disclosures: D. Morales Galindo: None. **M. Deciga-Campos:** None. **G. Navarrete-Vazquez:** None.

Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.23/C156

Topic: D.01. Somatosensation – Pain and Itch

Support: USAMRDC CP190070
NHMRC Fellowship (ID: 2027008)

Title: Persistent sleep disturbance enhances and maintains specific pain behaviors and systemic BDNF levels after intervertebral disc injury

Authors: *M. F. BARBE[1], L. S. STONE[2], M. G. VAN DER BAS[3], E. R. MCGONAGLE[4], P. W. HODGES[5], D. M. KLYNE[6];
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Abstract: Poor sleep coexists in up to 88% of chronic pain cases, such as with low back pain, which is the most common type of chronic pain. We hypothesize that persistent sleep disturbance enhances and maintains post-injury pain. We examined pain related behaviors across 9 wks in rats that received lumbar intervertebral disc (IVD) injury. Thereafter, sleep disturbance (SD) was enforced within 3 days of the surgically induced injury and maintained for 9 wks. Forty-one Sprague-Dawley adult female rats were used: 21 received punctures of L4 and L5 IVDs (DP rats), 20 were sham control (C) rats. Animals were anesthetized with isoflurane prior to surgery. Meloxicam (2 mg/kg body wt) was provided one day prior to, day of, and 3 days post-surgery.

Topical lidocaine was administered before and for 3 days post-surgery. DP and C rats were evenly randomized to receive undisturbed or disturbed sleep (SD) (4 groups, n=10-11/ group) for 9 wks post-injury. Persistent sleep disturbance enhanced aversion to cold (12o and 14oC) temperatures in DP+SD rats during place preference testing, compared to DP rats, with the latter showing similar cold sensitivity as uninjured C rats (with or without SD). Both DP and DP+SD rats showed local (lower back) sensitivity to pressure at 9 wks post-injury, compared to C groups; DP+SD rats also showed local sensitivity at wk 6. DP and DP+SD rats showed similar remote (upper thigh) sensitivity to pressure in wks 3-9, each enhanced relative to C groups. DP and DP+SD rats displayed reduced positive social interactions with a novel adult female rat at 3 wks, compared to C groups; yet only DP+SD rats showed increases in negative social interactions (e.g., aggression) at wks 3 and 6. Serum BDNF levels were higher at 9 wks in DP+SD rats, compared to DP rats, and lower in DP rats, compared to C groups. In contrast, serum IL-6 levels were lower in both SD groups (C+SD and DP+SD), compared to rats without SD, and correlated strongly with lower back sensitivity. Serum TNFalpha levels increased similarly in DP and DP+SD rats, compared to both C groups. In summary, persistent sleep disturbance resulted in late stage (9 wks post-injury) aversion to noxious cold temperatures in disc punctured (DP+SD) rats (DP alone rats did not show such aversion), enhanced and persistent abnormal behaviors (including aggression), increased BDNF and decreased IL-6 serum levels at late stage. Findings suggest that sleep disturbance facilitates pain and the transition to chronicity after IVD injury, but not in the absence of injury. Key neuroimmune mechanisms appear to underly these relationships.

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Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.24/C157

Topic: D.01. Somatosensation – Pain and Itch

Support: DA009158
DA047858

Title: Exploring the link between negative emotional states and vulnerability to opioid misuse in a mouse model of neuropathic pain

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Abstract: Neuropathic pain is a form of maladaptive plasticity associated with neurobiological adaptation in brain reward pathways. Pain relief produces negative reinforcement through activation of the mesolimbic reward circuit. Pathological pain may produce negative affective states, anhedonia and motivational states that facilitate drug-seeking and/or pain relief. Pain-induced transformations in reward processing enhances motivational salience to pain, analgesia and related cues, affecting sensitivity to opioids and non-opioid analgesics. Here, we tested the hypothesis that mice subjected to a traumatic nerve injury sparing the tibial nerve (SNIt) would produce negative affective states that impact opioid addictive behaviors. We used a mouse model of traumatic neuropathic nerve injury where the tibial branch of the sciatic nerve is spared, and the peroneal and sural branches are ligated and cut (SNIt). By employing i.v. self-administration in mice, we studied the effects of SNIt in morphine self-administration, relapse-like behavior, and motivation to work for morphine infusions. We also examined whether SNIt would affect oral oxycodone consumption and seeking in a two-bottle choice (TBC) paradigm. We also studied how SNIt influences food self-administration and the motivation to work for food rewards. Affective states were measured using the light/dark box, elevated plus maze, open field, marble burying, sucrose preference, nest building, chocolate preference, and forced swim test. Comparisons were made with sham-operated and naive groups. SNIt, sham-operated and naive groups exhibited similar levels of i.v. morphine intake on fixed ratio 1 (FR1), FR2 and FR3 schedules. Likewise, no differences were found in the motivation to work for morphine infusions. All groups displayed similar motivation to work for natural rewards in the food self-administration task. Overall, SNIt mice tended to consume less oxycodone than the other groups in the TBC paradigm, whereas no distinctions were detected between groups in the oxycodone-seeking test after forced abstinence. Strikingly, no differences were observed between SNIt, sham-operated and naive groups in affective states measured in the light/dark box, elevated plus maze, open field, marble burying, sucrose preference, nest building, chocolate preference, or forced swim test. Robust mechanical allodynia was observed in SNIt but not sham-operated or naive groups throughout the observation interval. Our results suggest that traumatic neuropathic nerve injury in the SNIt model did not reliably increase negative affective states or opioid vulnerability in male mice.

Disclosures: I. Oliva: None.

Poster

PSTR276: Pain Models: Analgesics, Behavior, Opioids, and Beyond

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR276.25/C158

Topic: D.01. Somatosensation – Pain and Itch

Support: PAPIIT IN219720
PAPIIT IN204023
CONAHCYT CF-2023-I-654

Title: A novel model of chronic neuropathic orofacial pain for assessing pain phenotypes and its neural substrates

Authors: *C. MONTES-ANGELES, R. ANDRADE GONZALEZ, I. O. PEREZ-MARTINEZ; Natl. Autonomous Univ. of Mexico, Mexico City, Mexico

Abstract: Chronic neuropathic orofacial pain (CNOP) can be produced by surgical interventions, and it is detrimental for the quality of life of who suffer from it. The development of an experimental model similar to its clinical features is necessary for a precise approach to the study of its neurobiological basis. We propose the mental nerve compression injury as a useful model for studying different phenomena that occur in the development of such disease, from behavior for identifying phenotypes, to analyze facial expression, as well as to assess the neural substrates involved. Wild-type (WT) mice went through mental nerve compression injury to induce CNOP, and were assessed with von Frey test, for nociceptive threshold on day 3 to week 14. To determine the role of nucleus accumbens (NAc) dopamine-receptor-expressing neurons (DRn), D1- and D2R-Cre mice were infected with a genetically engineered caspase in NAc, causing specific ablation of such neurons. Mechanical sensitivity was tested before and after it, and after nerve injury. For facial expression analysis, WT mice were face-recorded in a head-fixed system during the onset of mechanical stimulation, at -1, 4 and 7 days after nerve injury; electrophysiological recordings were performed in anterior cingulate cortex (ACC). In vglut2-ires-cre mice ACC neurons were ablated in a Caspase-dependent manner. Videos were analyzed with an artificial-vision tool; firing rate z-score was calculated and analyzed with a Generalized Lineal Model to identify neurons modulated by the pain facial response. We found that injured WT mice showed mechanical hypersensitivity during the first weeks. Mice were classified in high and low threshold (HT and LT), being most of them HT mice. Most HT mice recovered from mechanical hypersensitivity, whereas most LT mice remained hypersensitive. NAc DRn ablation decreased the percentage of HT mice and increased the time of hypersensitivity recovery, suggesting this population participates in nociceptive threshold profiles and recovery capacity. The painful facial response is dependent on the stimuli force and it is exacerbated by the mental nerve lesion. GLM analysis detected a neural population modulated by the pain facial response for both stimuli, that increased its firing rate after the stimuli onset. ACC neural ablation showed no effect on the baseline facial response but abolished the changes observed in the context of neuropathic pain.This way, we found that mental nerve compression injury produces two chronic pain development phenotypes, is functional for the analysis of facial expression through artificial vision tools, and for the assessment of the neural structures involved.

Disclosures: C. Montes-Angeles: None. **R. Andrade Gonzalez:** None. **I.O. Perez-Martinez:** None.

Poster

PSTR277: Thalamic and Cortical Processing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR277.01/C159

Topic: D.02. Somatosensation – Touch

Support: NIH Grant R00 NS119739

Title: Primary somatosensory cortex is required for normal perception of movement direction

Authors: Y. LEE, R. QI, *A. J. EMANUEL;
Cell Biol., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: The somatosensory system enables us to recognize objects, discern textures, and integrate sensorimotor feedback by extracting relevant features of the tactile world. One such feature is the direction that a stimulus moves across the skin. While neural tuning to direction has been described within the primary somatosensory cortex (S1), we do not know whether this region is required for perception of this tactile feature. Therefore, we designed an operant training paradigm to require head- and paw-restrained mice to report the direction of movement across the glabrous (non-hairy) skin of the mouse forepaw and then employed optogenetic silencing approaches to evaluate the contributions of three cortical regions to the perception of tactile movement direction. We trained VGAT-ChR2 transgenic mice to perform a go/no-go operant task for discrimination of the direction (proximal to distal vs. distal to proximal) of a light brushing stimulus across the glabrous skin of the left forepaw. Once the mice reached a criterion performance of discrimination index (d') $\geq$ 2.0, we directed focused laser light (445 nm, 40 Hz pulses, 5 mW average power) to one of three cortical regions (contralateral to the paw stimulus) or a non-brain control area throughout the duration of the stimulus presentation on each trial. Optogenetic silencing of forepaw S1, but not hindpaw S1 or posterior parietal cortex, resulted in decreased performance in VGAT-ChR2 mice compared to interleaved control trials (d's of 2.24 ± 0.16 [mean ± SEM] and 1.10 ± 0.19 for control and forepaw S1 locations, respectively). Therefore, activity in forepaw S1 is required for mice to reliably report the direction of tactile stimuli.

Disclosures: Y. Lee: None. **R. qi:** None. **A.J. Emanuel:** None.

Poster

PSTR277: Thalamic and Cortical Processing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR277.02/C160

Topic: D.02. Somatosensation – Touch

Support: NINDS BRAIN R01NS104928
NINDS BRAIN RF1NS128896
NINDS R21 NS112783
National Science Foundation Graduate Research Fellowship
James H. Gilliam Fellowships for Advanced Study program

Title: The timescales of layer 6 corticothalamic neuron activity in the recruitment of cortico-cortical and cortico-thalamo-cortical pathways

Authors: *S. RUSSO, E. DIMWAMWA, G. B. STANLEY;
Georgia Inst. of Technol., Atlanta, GA

Abstract: Layer 6 corticothalamic (L6CT) neurons are an excitatory neuron type with projections to both cortex and thalamus. L6CT neurons have been reported to have facilitatory and suppressive effects on cortical and thalamic neurons depending on their rate of activation and synchronization. We hypothesize that the facilitatory or suppressive functional effects of L6CT neurons on the cortex, and whether they are mediated by intracortical or cortico-thalamo-cortical pathways, depend on the detailed timecourse of L6CT activation. To characterize the temporal patterns induced by L6CT activation, we optogenetically activated L6CT neurons in NTSR1-cre mice selectively expressing channelrhodopsin-2 in L6CT neurons. Leveraging the Vibrissal pathway of awake, head-fixed mice, we presented LED inputs consisting of either ramp-and-hold or pulsatile stimuli while recording neuronal activity in the barrel cortex, the ventral posteromedial nucleus (VPm), and the reticular nucleus (TRN) of thalamus using silicon probes. First, we hypothesized that time-dependent dynamics of L6CT neuron activity may influence the effect of L6CT inputs on cortical and thalamic neurons. To test this hypothesis, we measured the effect of prolonged L6CT neuron activation and found activity-dependent increases and decreases in the firing rates of different layers and cell types over time. Using dimensionality reduction, we simplified this complex pattern to two components: one slowly changing component that is represented in all units, and one rapidly changing component that is mainly represented in the thalamus and middle cortical layers, suggestive of differential recruitment of the cortico-cortical vs cortico-thalamo-cortical pathways. We next asked whether the activation timescales of L6CT neurons would shift the relative contribution of the cortico-cortical versus cortico-thalamo-cortical pathways in shaping cortical activity. To answer this question, we compared the activity across cortical layers induced by L6CT activation on different timescales. Brief L6CT activation recruits a biphasic firing-and-silence pattern throughout the cortical column; conversely, prolonged L6CT activation primarily results in a sustained reduction of cortical firing. Taken together, our data suggests that different temporal dynamics of L6CT neuron activation modulate cortex through a complex interplay between cortico-cortical and cortico-thalamo-cortical pathways. This relates to the emerging evidence indicating that L6CT neurons are activated in a variety of behavioral contexts that occur on different timescales and suggests a flexible role of L6CT neurons across contexts.

Disclosures: S. Russo: None. **E. Dimwamwa:** None. **G.B. Stanley:** None.

Poster

PSTR277: Thalamic and Cortical Processing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR277.03/D1

Topic: D.02. Somatosensation – Touch

Support: NIH Grant AG065290
Neurodegeneration Consortium

Title: Cholinergic synaptic modulation of the thalamic reticular nucleus in adult mice

Authors: *N. RIVERA-RAMIREZ[1], R. JAGIRDAR[2], J. R. CAMPBELL[2], M. SILVA-PÉREZ[2], J. CHIN[2], M. BEIERLEIN[1];
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Abstract: The thalamic reticular nucleus (TRN) plays a crucial role in regulating sleep, attention, and sensory processing by mediating inhibition in other thalamic nuclei. TRN displays a core-shell organization, with neurons in each region displaying distinct molecular, cellular, and functional properties. Neuromodulators such as Acetylcholine (ACh) are thought to play important roles in the regulation of TRN activity. However, the source of cholinergic afferents mediating release and the cell-type specificity of cholinergic signaling in the TRN have not been explored. To address these questions, we activated cholinergic afferents in the presence of antagonists for fast glutamatergic and GABAergic synaptic transmission in thalamic slices from 3-4-month-old mice. We found that stimulus-evoked ACh release led to biphasic excitatory-inhibitory (E-I) postsynaptic responses in both TRN core and shell neurons, with an initial short-latency α4β2 nicotinic receptor (nAChR) EPSC followed by a long-lasting inhibitory postsynaptic current (IPSC) mediated by muscarinic ACh receptors (mAChRs). In current clamp, ACh release led to a brief increase and a prolonged pause in TRN firing, indicating that cholinergic inputs to TRN neurons act primarily inhibitory. Interestingly, we found that the relative contribution of the muscarinic component to the E-I response was more prominent in shell neurons. Selective blockade of either M2 or M4 mAChRs led to a reduction of muscarinic IPSC, while the M2/M4 antagonist AF-DX 384 completely eliminated them, indicating a contribution of both M2 and M4 mAChRs to the postsynaptic response. In contrast, M2 but not M4 antagonist reduced autoinhibition, suggesting the exclusive participation of M2 receptors in regulating release. To determine the source of neurons involved in rapid ACh release, we injected AAV9-DIO-channelrhodopsin-2 into either the basal forebrain (BF) or the pedunculopontine nucleus (PPT) of the brainstem of choline acetyltransferase (ChAT)-cre mice. We found that optogenetic activation of cholinergic inputs from either BF or PPT led to biphasic E-I postsynaptic responses in the TRN, with BF afferents generating responses in a larger fraction of postsynaptic neurons. Taken together, our results indicate that cholinergic afferents

from both BF and PPT generate powerful responses in the TRN, which differ in a target-dependent manner.

Disclosures: N. Rivera-Ramirez: None. **R. Jagirdar:** None. **J.R. Campbell:** None. **M. Silva-Pérez:** None. **J. Chin:** None. **M. Beierlein:** None.

Poster

PSTR277: Thalamic and Cortical Processing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR277.04/D2

Topic: D.02. Somatosensation – Touch

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Title: A recurrent cortical circuit triggers somatosensory perception

Authors: *Y. OISI[1], Y. ATSUMI[1], Y. SAITO[1], T. SUZUKI[1], S. KATO[2], K. KOBAYASHI[2], K. KOBAYASHI[3], M. MURAYAMA[1];
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Abstract: Understanding how perception arises in the brain is one of the fundamental challenges for neuroscience. Several theories of perception emphasize the important role of recurrent circuits consisting of feedforward (FF) and feedback (FB) projections. However, it remains unclear how such interactions contribute to perception due to methodological limitations in manipulating circuits precisely in time and space in primate research. We have previously reported a recurrent circuit consisting of cortical projections between the primary somatosensory cortex (S1) and the secondary motor cortex (M2) in mice (Manita et al., *Neuron* 2015). This circuit was activated by somatosensory stimulation in the order S1→M2→S1. Furthermore, M2→S1 FB inputs can trigger dendritic spikes and burst firing in S1 neurons. Based on these findings, we hypothesized that the M2→S1 FB inputs to S1 contribute to somatosensory perception. Here, we tested this hypothesis by combining recording and manipulation of neural activity in stimulus detection task in mice. We trained thirsty mice to report the detection of a somatosensory stimuli by liking to obtain water reward. We then measured the perceptual

threshold in individual mice and investigated how the threshold changes with circuit manipulations. First, we found that S1 and M2 lesions and optogenetic inhibition of each area significantly increased the threshold, indicating impaired perception. Pathway-specific optogenetic inhibition of both the S1→M2 FF and M2→S1 FB inputs also impaired perception. These results suggest that the S1-M2 recurrent circuit contributes to perception. Therefore, we determined the effect of optogenetic inhibition of M2→S1 FB inputs in S1 by electrophysiology. Optogenetic inhibition attenuated the S1 neural activity, suggesting that amplification of S1 neural activity via M2→S1 FB inputs is implicated in perception. Next, we tested whether activation of either S1→M2 FF or M2→S1 FB projections is sufficient for somatosensory perception. Pathway-specific optogenetic activation of both S1→M2 FF and S1→M2 FB projections was able to induce illusory somatosensory perception. Finally, we investigated the role of each pathway in perception. Pathway-specific activation of M2→S1 FB inputs with pharmacological M2 inactivation was able to induce illusory perception. In contrast, activation of the S1→M2 FB inputs with pharmacological S1 inactivation impaired illusory perception. These results support our hypothesis that somatosensory perception requires S1 activity that is evoked by M2→S1 FB inputs.

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Poster

PSTR277: Thalamic and Cortical Processing

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Program #/Poster #: PSTR277.05/D3

Topic: D.02. Somatosensation – Touch

Support: NIH Grant R21NS125594

Title: Cortical dynamics in forelimb S1 and M1 evoked by brief photostimulation of the mouse's hand

Authors: *D. PIÑA NOVO, M. GAO, J. M. BARRETT, G. M. SHEPHERD;
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Abstract: The synaptic circuits and spiking dynamics in primary somatosensory (S1) and motor (M1) areas are fundamental to sensorimotor integration in cortex. The ascending sensorimotor loop through these areas is important for conveying tactile and other somatosensory signals to influence output along corticospinal and other descending motor pathways. Recent studies have characterized synaptic connectivity along the ascending pathway through mouse hand/forelimb S1 and M1 (Yamawaki et al., 2021, eLife). Here, using linear arrays to record simultaneous spiking activity in S1 and M1, we investigated the peripherally evoked spiking dynamics in these

two cortical areas. The brief (5 ms) optogenetic stimulation of sensory afferents in the hand of awake transgenic mice evoked short-latency barrages of activity appearing first in S1 and then in M1. The corticocortical latencies (from S1 to M1) were short. However, the estimated net propagation speed for S1-to-M1 was vastly slower than for hand-to-S1 signaling. Compared to S1, M1 responses were attenuated in amplitude and slightly shorter in duration. The main early sensory responses were followed by a prolonged period of suppressed activity, and an ensuing "rebound" response. These characterizations provide quantitative measures of spiking dynamics of cortical activity along the hand/forelimb-related transcortical loop. They also support a conceptual model in which ascending somatosensory signals rapidly reach S1 via high-speed subcortical circuits, generate a characteristic self-limited barrage of activity, and slowly propagate to M1 via densely polysynaptic cortical circuits, generating a similar but delayed and attenuated profile of activity.

Disclosures: D. Piña Novo: None. **M. Gao:** None. **J.M. Barrett:** None. **G.M. Shepherd:** None.

Poster

PSTR277: Thalamic and Cortical Processing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR277.06/D4

Topic: D.02. Somatosensation – Touch

Support: Eunice Kennedy Schriver National Institute of Child Health & Human Development, NIH Grant R01HD094588

Title: The ventral posterior lateral (VPL) thalamic nucleus serves as a substrate for delayed lower jaw-to-forepaw reorganization in the posterior forepaw barrel subfield (FBS) in rat primary somatosensory cortex (SI)

Authors: *R. WATERS[1], L. WANG[1], J. W. TSAO[2], A. L. DE JONGH CURRY[3];
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Abstract: Introduction: Forelimb amputation in rats leads to immediate and delayed reorganization in the forepaw barrel subfield (FBS). Lower jaw-to-forepaw reorganization occurs immediately in the anterior FBS, where the lower jaw input is sourced from the adjacent lower jaw barrel subfield (LJBSF). In contrast, the delayed reorganization, occurring more than 6 weeks after deafferentation, affects both anterior and posterior FBS, as well as the former forepaw representation in the ventral posterior lateral (VPL) thalamic nucleus. It remains unclear whether the VPL acts as a subcortical source for the new lower jaw input in chronic forelimb deafferented rats. Here we provide evidence that VPL contributes to the new lower jaw input in the posterior FBS in rats with chronic forelimb deafferentation.

Methods: Anesthetized rats, 6 weeks after forelimb deafferentation, were used to identify lower jaw responses in the former forepaw representation in VPL and FBS. A carbon fiber electrode was inserted into the former digit representation in VPL to record single and multi-unit responses. Mechanical and electrical stimulation was applied to the lower jaw skin surface to evoke responses in VPL; the electrode was then fixed in place. Stimulation of the lower jaw evoked responses in the FBS. Microstimulation of the recording site in VPL was used to examine connectivity with the FBS. Once established, the VPL recording site was ablated and connectivity to the FBS was reexamined. IGOR-Pro software processed signals. Electrolytic lesions recovered recording/stimulating sites in VPL and FBS.
Results: 1) Neurons within the former digit representation in both VPL and FBS exhibit burst-like patterns in forelimb deafferented rats. 2) All sites previously representing digits in both VPL and FBS show responses to lower jaw stimulation. 3) Connectivity between the VPL and FBS remains unchanged after forelimb deafferentation. 4) Ablating recording sites in VPL only abolishes lower jaw responses in the posterior FBS.
Conclusions: Our findings indicate that forepaw VPL provides a source for the new lower jaw input in posterior FBS in chronic deafferented rats. Whether VPL also serves as a source of new lower jaw responses in anterior FBS remains to be determined.

Disclosures: R. Waters: None. **L. Wang:** None. **J.W. Tsao:** None. **A.L. De Jongh Curry:** None.

Poster

PSTR277: Thalamic and Cortical Processing

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Program #/Poster #: PSTR277.07/D5

Topic: D.02. Somatosensation – Touch

Support: 1R01NS092894-01
1R01NS124222-01

Title: Somatosensory neuroprosthetic performance assessment in rodents: optimized somatosensory thalamic microstimulation

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Abstract: In somatosensory neuroprosthetics, patientsoften report that sensations from intracortical and peripheral microstimulation(MiSt) feel unnatural. This issue is compounded in animal models, where it ischallenging to evaluate the perceived naturalness of these sensations. Toaddress this, we developed a novel behavioral task using a 2D rat roboticmanipulandum (RRM) and a precision 3D robotic tactor capable of applyingvariable force levels to the paw. Our

methodology involves a two-alternativeforced-choice (2AFC) task where rats are presented with skin indentations onthe thenar pad, chosen for its strong slowly adapting sensory receptive field,ranging from 500 to 2000μm in 50μm increments. We vary the initial stimulus byup to ±100%, chosen from a uniform distribution, and analyze whether the ratperceived an increase or decrease in indentation depth using a manipulandumpush/pull reaching movement with the paw not receiving the somatosensorystimulus to determine a psychometric response surface.Subsequently, in a spatial search task, eachtrial begins with the RRM position randomized within a 2x2 cm workspace, and aforce field which corresponds to tactile stimulus intensity also centered on arandom point in the workspace. The touch intensity is defined as theindentation depth in the paw which is not actuating the RRM. The field willfall off in intensity from its center point at a rate which gives an equalamount of perceptibility change for each unit of distance from the centerpoint; this rate is determined by the data gathered in the 2AFC task. The taskrequires the rat to move the RRM and search for the maximum of the force fieldwhere it must then hold the RRM for 800ms to receive a reward. A viscous forcefield instantiated by the RRM is used to keep the rats from making ballisticfeedforward reaches rather than searching while making feedback drivenmovements.Tests were conducted on 4 Male Lewis ratsimplanted with 64 S1 channels and 64 thalamic channels in the forepawrepresentations of both regions on the ipsilateral side to the hand whichmanipulates the RRM. All protocols were approved by the University of Houston'sIACUC.Following the spatial search tasks with realtouch feedback, the real stimulation provided by the tactor will be replaced byintrathalamic MiSt. This stimulation is directed via model predictive control(MPC) to drive the cortex to its appropriate state depending on what the realstimulus intensity should be given the current position of the RRM in a lowlatency (>10ms), closed loop manner. We propose that comparing taskperformance under the real and artificial stimulus paradigms will serve as anindicator of the naturalistic quality of MiSt.

Disclosures: W.A. Walker: None. **L. Dickey:** None. **B. See:** None. **J.T. Francis:** None.

Poster

PSTR277: Thalamic and Cortical Processing

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Program #/Poster #: PSTR277.08/D6

Topic: D.02. Somatosensation – Touch

Support: Eunice Kennedy Schriver National Institute of Child Health & Human Development, NIH Grant R01HD094588

Title: Lower jaw-to-forepaw reorganization in ventral posterior lateral thalamic nucleus (VPL) following forelimb deafferentation

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Abstract: Introduction: We reported rapid and delayed lower jaw-to-forepaw reorganization in the rat forepaw barrel subfield (FBS) of somatosensory cortex following forelimb deafferentation. Rapid reorganization occurs only in the anterior FBS. The neighboring lower jaw barrel subfield (LJBSF) projects to the anterior FBS and reportly serves as an immediate input source. However, neurons in the posterior FBS begin responding to lower jaw input six or more weeks after forelimb deafferentation, but their source is unknown. While the ventral posterior lateral thalamic nucleus (VPL) projects to the entire FBS, VPL neurons in intact forelimb rats do not respond to lower jaw stimulation. However, other studies in monkeys have shown that VPL neurons become responsive to lower jaw input months or years after forelimb deafferentation. If similar reorganization occurs in rat VPL post-deafferentation, VPL could serve as a new source of lower jaw input in the posterior FBS.
Methods: We used anesthetized rats to investigate lower jaw reorganization in VPL both in normal rats and rats six weeks or more post-forelimb amputation. Single and multi-unit responses were recorded in VPL neurons using a carbon fiber electrode. To examine input to VPL, mechanical and electrical stimulation were applied to the skin surface of the forepaw and lower jaw. Signal processing was conducted using IGOR-Pro software. Electrolytic lesions were used to recover penetration sites.
Results: 1) Neurons within the forepaw representation in VPL exhibit irregular firing patterns in intact forelimb rats, whereas in amputated rats (>6 weeks post-amputation), these neurons display burst-like firing patterns. 2) Neurons within the forepaw representation in VPL respond exclusively to input from the forepaw in intact rats. 3) In forelimb amputated rats (>6 weeks post-amputation), VPL neurons throughout the former forepaw representation respond to new input from the lower jaw.
Conclusions: Our findings suggest that the forepaw VPL serves as a source for the new lower jaw input in the FBS in chronic deafferented rats. However, whether VPL is the original source of this delayed lower jaw-to-forepaw reorganization in FBS requires further investigation.

Disclosures: L. Wang: None. **A.L. De Jongh Curry:** None. **J.W. Tsao:** None. **R.S. Waters:** None.

Poster

PSTR277: Thalamic and Cortical Processing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR277.09/D7

Topic: D.02. Somatosensation – Touch

Support: NIH R21 MH123906
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Title: Divergent plasticity in thalamocortical synaptic strength in S1 and S2 during sensory learning

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Abstract: Synaptic plasticity at neocortical synapses is thought to underlie learning. During tactile learning, changes in the activity of both primary and secondary somatosensory cortex (S1 and S2) have been associated with task expertise. Indeed, S1 and S2 are interconnected by both direct excitatory connections and also indirectly, through higher-order thalamus (posterior-medial thalamus; POm), and POm is itself important for learning. Because previous electrophysiological analyses have identified pathway-specific synaptic potentiation at POm synapses in S1, we hypothesized that this plasticity might also occur in S2, further enhancing activity initiated by this thalamocortical loop. Using pathway-specific stimulation of POm afferents as well as fluorescence-based anatomical techniques, we characterized learning-related changes in the strength of POm synapses onto layer 2/3 pyramidal (Pyr) neurons in both S1 and S2. To selectively label excitatory synapses, we virally expressed Citrine-PSD95.FingR in Drd3-Cre transgenic mice and tdTomato in POm axons. Using high-resolution confocal microscopy and digital reconstructions in fixed tissue, we aligned POm axons with PSD95 puncta. Synapse size was evaluated as animals learned a sensory association task where a gentle airpuff to the facial whiskers predicted a water reward during home-cage training. During the first two days of training, we identified a gradual and significant increase in the size of POm-associated synapses on the L2/3 tufts in S1, where basal synapses were unaffected. Notably, overall PSD95 puncta size that broadly represents all excitatory inputs onto S1 L2/3 Pyrs was not altered. In contrast, in S2 POm-associated synapses onto L2/3 Pyr neurons showed a progressive and significant reduction in size during training. Overall PSD95 puncta size representing all excitatory inputs onto L2/3 Pyr in S2 decreased. Pathway-specific ChR2-mediated analysis of quantal EPSCs from POm afferents corroborated anatomical findings in both S1 and S2. All changes in POm-synapse size were renormalized after longer period of training, when animals had learned the task. Thus, higher-order thalamocortical inputs are differentially regulated depending on target area, and enhanced interactions between S1 and S2 may not depend upon indirect connectivity from POm thalamus. Future studies will investigate the roles of these divergent synaptic changes on transcortical information flow during learning.

Disclosures: A. Ray: None. **J.A. Christian:** None. **A.L. Barth:** None.

Poster

PSTR277: Thalamic and Cortical Processing

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Title: Establishing multiscale measures of myelin in the rat sensorimotor cortex

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Abstract: The advent of noninvasive neuroimaging has reignited interest in myelin mapping to understand brain growth and development. Despite controversy over the historical use of myelin to map the brain, the ability for contemporary magnetic resonance imaging (MRI) to measure the movement of water has reignited demand for multiscale measures of myelin content. For instance, qualitative histological maps of primary somatosensory cortex (S1), primary motor cortex (M1), and parietal association cortex (PtA) have been established, but not yet deployed to track neurobiological growth. In this project, we acquired histology and in-vivo MRI datasets of the brain from adult mice and rats. Specifically, we used a standardized Gallyas silver method to visualize myelin, and an adjacent series was stained with thionin for Nissl, in 40µm coronal histological brain sections. On the 9.4T small animal bore scanner, we used ultrashort echo-time T1 mapping for anatomical features and diffusion imaging to infer white matter-gray matter boundary, as well as T2, T2* and magnetization transfer. In the current report, we present our histological results along with preliminary MRI showing the more than 30% change in myelin density that defines the border between M1/S1 and PtA/S1 in the rat. In particular, we demonstrate S1 can be identified and delineated along its dorsal border by its differential columnar myelin content relative to adjacent M1 or PtA. Our work establishes the quantitative differential cortical myelin content of S1 across multiple scales of organization, from histology through to MRI, enabling the estimation of native functional brain organization across individuals noninvasively without behavioral assessment. The capability of a more detailed and sensitive method to detect specific functional brain areas provides a powerful tool to track brain growth and development, as well the impact of disease to enable more individualized and therefore effective treatment plans for patients suffering from myelin degradation conditions.

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Poster

PSTR277: Thalamic and Cortical Processing

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Topic: D.02. Somatosensation – Touch

Support: NIH U24EB028998
NSF 1904444-1042C
NYS DOH01-C38328GG-3450000

Title: Thalamocortical Circuit Topology and Dynamics Shape Angular Tuning Responses in a Multiscale Model of the Mouse Whisker Pathway

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Abstract: Angular tuning is a key feature of the rodent whisker pathway, characterized by increased activity of groups of neurons to a specific whisker deflection angle (Minnery, 2003). This response can be observed in the brainstem, thalamus, and the primary somatosensory cortex (S1), providing a mechanism to trace the flow of information in this pathway, and test the dynamic interactions that shape thalamic signals relayed to the cortex. The nuclei involved in the thalamocortical communication within this pathway are the thalamic barreloids in the ventral posteromedial (VPM) nucleus, the thalamic reticular nucleus (TRN), and the corticothalamic projecting (CT) neurons in S1. The VPM is responsible for forwarding brainstem information to S1, while the TRN and CT provide inhibitory and excitatory feedback signals, respectively, which can shape the activity of the VPM neurons. Detailed reconstructions of neuronal projections uncovered new pathways of information flow and integration across cortical and thalamic regions (Lam & Sherman, 2005, 2006, 2010, 2011; Shepherd & Yamawaki, 2021). These studies provided a series of possible circuit configurations that could be involved in shaping the response of the thalamic neurons, improving thalamocortical communication. With this in mind, in this study we developed a detailed multiscale mechanistic model of the mouse whisker pathway, using the NetPyNE tool and the NEURON simulator. Our goal was to investigate how network topology and activity influence the observed angular tuning response in the mouse thalamus. We characterized the network based on the angular tuning response of thalamic neurons to different whisker deflection angles and evaluated the contribution of different network arrangements that have been proposed by experimental studies, such as open-

loop vs closed-loop projections (Lam & Sherman, 2005). Our preliminary results show that no single factor is responsible for the preservation of the angular tuning response. Instead, our findings suggest that a combination of network topology and dynamic interactions with TRN and CT feedback enhances the activity of thalamic relay neurons aligned with the deflection angle while inhibiting activity in misaligned neurons. Our results also point towards a widespread projection of TRN neurons at the barreloid level, instead of focal and closed-loop inhibitory feedback to specific cells. Combined, these mechanisms could act as a filter of information at the thalamic level, improving the efficiency of thalamocortical communication.

Disclosures: J. Moreira: None. **F.S. Borges:** None. **S. Dura-Bernal:** None.

Poster

PSTR277: Thalamic and Cortical Processing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR277.12/D10

Topic: D.02. Somatosensation – Touch

Title: Investigating chronic pain following forelimb deafferentation using the rat grimace scale

Authors: N. NEMATI[1], ***A. DE JONGH CURRY**[1], L. WANG[2], J. W. TSAO[3], R. S. WATERS[2,4];
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Abstract: Introduction: A leading mechanism underlying phantom limb pain (PLP), a chronic neuropathic pain that manifests post-amputation, is functional reorganization of sensorimotor cortices and subcortical nuclei. Cortical and subcortical reorganization subsequent to forelimb amputation in rat has been documented, but the presence or extent of chronic pain associated with amputation in rat has not been explored. The rat grimace scale (RGS), previously used to quantify pain levels in acute and chronic pain conditions, may be a useful indicator of chronic pain following amputation. As an initial step for chronic pain detection in the rat amputation model, the purpose of this study is to investigate if forelimb-amputated rats display behaviors indicative of increases in negative affective states.
Methods: Adult Sprague Dawley rats, 8 with forelimb amputation, 4 sham-operated, and 4 unoperated (control), were observed individually by the same, single observer twice per week for 1 week before and 6 weeks after surgery to score facial behaviors using RGS during 10-min sessions. Specific behaviors (action units, AUs) scored were changes in orbital tightening, whisker position, ear curling, and nose flattening. AUs were assigned a score of 0, 1, or 2 to indicate severity of each AU change once per minute and averaged to create an RGS sample score. The 10 RGS sample scores were then averaged to create an RGS session score.

Results: Significant differences were found between average session RGS scores per week for amputee and control subjects for all post-surgery weeks (Kruskal-Wallis, $p<0.05$). Changes in overall average pre-surgery and post-surgery RGS score for the amputee, sham, and control groups were 0.57, 0.25, and 0.10, respectively, with significant differences between amputee and control groups and between amputee and sham groups (Mann-Whitney, $p<0.05$).
Conclusion: Change in RGS scores from pre- to post-surgery was significantly higher in the amputee group compared to control. Results indicated that a threshold change of 0.3 in pre-to-post surgery RGS scores may differentiate amputee from control and sham subjects over a 6-week period, but further studies are needed to confirm that RGS is a robust indicator of negative affective states or chronic PLP in amputee rats.

Disclosures: N. Nemati: None. **A. De Jongh Curry:** None. **L. Wang:** None. **J.W. Tsao:** None. **R.S. Waters:** None.

Poster

PSTR277: Thalamic and Cortical Processing

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Program #/Poster #: PSTR277.13/Web Only

Topic: D.02. Somatosensation – Touch

Support: NIH grant R01NS129982

Title: Probing the extra-hand surround with laminar recordings in monkey area 3b

Authors: *J. L. REED[1], H. X. QI[1], A. BASTOS[2], J. H. KAAS[1];
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Abstract: Despite the importance of hand use in primates, little is known about how receptive field (RF) surrounds are generated in neurons in primary somatosensory cortex area 3b that appear to integrate touch information from both hands. When the excitatory RF is on one hand, modulation to touch on both hands could be called the 'very far' suppressive surround or the 'extra-hand' suppressive surround. Callosal connections are mostly absent in the primate area 3b hand representation, and the connections differ by layer. Thus, laminar response patterns will guide our future inactivation studies to localize sources of extra-hand surrounds in area 3b. We expect that supragranular layers have extra-hand surrounds, while we predict that layer 4 neurons will have near surrounds, and infragranular layers will have near and far surrounds, and weak or absent extra-hand surrounds. We use multielectrode arrays to record from 3b in adult owl monkeys, squirrel monkeys, and galagos, which all have the area 3b hand represented on the cortical surface. Our previous studies recorded layer 3 neurons with planar arrays. In owl monkey cortex, we recorded from layers 2 through 6 using linear arrays (NeuroNexus) with 16 contacts, 100 micron spacing. Cortical depth and RF mapping guided array placement. Neuronal

activity and local field potentials (LFPs) were recorded at 40kHz and 1kHz, respectively (Plexon). Data processing in MATLAB included average firing rates, peak magnitudes and latencies of averaged LFPs, and Current Source Density (CSD) in response to tactile stimulation. For stimulation on the hand contralateral to 3b, an early current sink corresponds to layer 4. We used CSD and multiunit activity for cortical depth estimates, and after data collection, microlesions are delivered at selected sites. Electrolytic lesions are used to reconstruct laminar locations from brain sections processed for co-registering architectural boundaries. Two-sample Kolmogorov-Smirnov tests of the distributions and t tests paired for the same contact in two stimulus conditions indicated significant suppression for bimanual stimulation compared to a single digit (on the contralateral hand). This was true for matched and nonmatched digits compared to the single digit condition, but no difference was detected between matched and nonmatched bimanual stimulation. Initial analyses indicate suppression follows our predictions but occurs across all cortical layers. Lack of somatotopic specificity suggests widespread feedback connections drive suppression, while the suppressive effect of simultaneous stimulation found across all layers suggests that feedforward sources require investigation.

Disclosures: J.L. Reed: None. **H.X. Qi:** None. **A. Bastos:** None. **J.H. Kaas:** None.

Poster

PSTR277: Thalamic and Cortical Processing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR277.14/D11

Topic: D.02. Somatosensation – Touch

Title: Perceptual decision-making under uncertainty and surprise

Authors: *D. DOGAN[1], A. REZEIKA[2], T. CELIKEL[1];
[1]Georgia Inst. of Technol., ATLANTA, GA; [2]Donders Inst. for Brain, Cognition and Behavior, Nijmegen, Netherlands

Abstract: Perceptual decision-making is influenced by the interplay between stimulus predictability and temporal uncertainty. However, the effects of these mechanisms on sensory information processing are not fully understood. In this study, we investigated how such factors impact perceptual thresholds for tactile sensitivity using focused ultrasound stimulation (FUS). FUS uses low-intensity focused ultrasound waves to evoke haptic sensation by exerting an acoustic radiation force, displacing mechanosensitive receptors on cell membranes. Its high spatial resolution, non-contact nature of force delivery, and spatiotemporal control over the soundwave parameters make it an ideal method to quantitatively study the sense of touch. The study was conducted with human subjects (N = 21, age group: 18-24) instructed to respond if they had felt a vibration on their fingertip under varying conditions, testing for the effects of stimulus predictability (priming), temporal uncertainty, and stimulus surprise measured by

sensory (probability of success) and motor (response time) metrics. Our results show that temporal uncertainty, but not stimulus predictability, results in reduced tactile sensitivity and increases the perceptual thresholds. In contrast, stimulus surprise has no significant effect on perceptual thresholds. Surprisingly, the top-down regulation of the perceptual threshold for touch detection during stimulus uncertainty and temporal predictability does not change reaction times, although sensory evidence itself is inversely correlated with the reaction time. Distributed surface EEG recordings across the cerebral cortex showed a parieto-frontal circuit that encodes stimulus predictability in a context- and time-varying manner. These results suggest that uncertainty in time affects the brain's ability to combine sensory information, which leads to higher perceptual thresholds and have implications for how we understand the neural processes that make perceptual decisions.

Disclosures: D. Dogan: None. **A. Rezeika:** None. **T. Celikel:** None.

Poster

PSTR277: Thalamic and Cortical Processing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR277.15/D12

Topic: D.02. Somatosensation – Touch

Title: Ultrasonic touch: using focused low-intensity ultrasonic pulses to remotely evoke sense of touch

Authors: S. PRIDGON, D. DOGAN, ***T. CELIKEL**;
Georgia Inst. of Technol., Atlanta, GA

Abstract: The sense of touch is a complex and multifaceted sensory experience that plays a crucial role in our daily lives. While the neural mechanisms underlying the sense of touch are not yet fully understood, recent advances in neurostimulation techniques have opened up new avenues for studying the peripheral (sensory) and central (neural) determinants of how we perceive and process the tactile world. In this study, we introduce the use of low-intensity focused ultrasound stimulation (FUS) as a novel tool to study haptic touch. FUS is a non-invasive and painless technique that uses high-frequency sound waves to remotely stimulate the skin and evoke a sense of touch. It allows for precise control over the intensity, duration, frequency, and spatial location of the stimulation. We demonstrate the feasibility of using FUS to elicit tactile sensations in humans (N = 21, age group: 18-21) and explore the relationship between stimulus amplitude and duration in the perception of touch using a combinatorial approach. Our experiments revealed that the perceptual threshold for punctate stimuli was independent of stimulus duration (tested between 1-100 ms) across a broad range of durations (3.9-100 ms) and showed that the minimum duration of touch required for the detection of the stimulus was 3.9 ms. Overall, our study demonstrates the potential of FUS as a novel and

innovative approach to studying the sense of touch and suggests that the brain's ability to detect touch is not influenced by the duration of the punctate stimulus.

Disclosures: S. Pridgon: None. **D. Dogan:** None. **T. Celikel:** None.

Poster

PSTR277: Thalamic and Cortical Processing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR277.16/D13

Topic: D.02. Somatosensation – Touch

Support: R01NS129059 from NIH / NINDS

Title: Thalamocortical alterations underlie tonic pressure pain in both chronic pain patients and pain free controls

Authors: *M. CANNISTRA[1], V. SACCA[2], Q. KONG[3], S. REDDY[3], M. ZHU[4], A. URSITTI[4], B. SIEGEL[5], Y. LIU[4], W. LI[6], J. KONG[3];
[1]Massachusets Gen. Hosp., Charlestown, MA; [2]Psychiatry, Massachusetts Gen. Hosp., Charlestown, MA; [3]Massachusetts Gen. Hosp., Charlestown, MA; [4]Massachussetts Gen. Hosp., Charlestown, MA; [5]Psychiatry, Massachussetts Gen. Hosp., Charlestown, MA; [6]Dept. of Psychiatry, UTHealth Houston, Houston, TX

Abstract: Purpose: Pain is a highly debilitating condition. To date, the pathophysiology of pain remains poorly understood, hindering the development of effective pain treatment. The thalamocortical (TC) circuitry is known to be involved in pain perception as well as the genesis and maintenance of chronic pain. The aim of this study was to investigate the alternation of thalamocortical (TC) connectivity of chronic low back pain during tonic pressure pain, no pain pressure and resting state as compared to no-pain controls. **Methods:** MRI scans were applied using a 3T Simons Scanner**.** 23 chronic low back pain (cLBP) patients and 27 pain free control were included in the data analysis. The 6-minute MRI scans were applied during tonic pressure pain (with an individualized average pain intensity of 4 using a 0-10 visual analog scale) and during no-pain pressure in random order. All stimuli were applied on left leg. A resting state fMRI scan was also applied at the beginning of the scan. Data analysis was performed using the CONN toolbox. Three thalamic subdivisions—ventral posterolateral thalamus (VPL), mediodorsal thalamus (MD), and the motor thalamus subregion (MThal) —associated with somatosensory, limbic circuity and motor were used as seeds for seed-based rsFC analysis. A threshold of $p < 0.005$ at voxel-wise level and $p < 0.05$ at cluster level (FDR corrected)**Results:** During tonic pressure pain, cLBP patients (compared to pain free control) are associated with greater VPL functional connectivity (FC) in the bilateral hippocampus and amygdala and left parahippocampal gyrus. During the no-pain pressure, cLBP (compared to pain free controls) is

associated with decreased connectivity between MThal and precentral gyri (primary motor cortex, M1) as well as right middle frontal gyrus and right inferior frontal gyrus. During the resting state, cLBP (compared to pain free controls) is associated with increased connectivity between MThal and M1 (right precentral gyri) as well as bilateral post cingulate gyrus, right precuneus and right middle cingulate gyrus. **Conclusion:** These results support our hypothesis of TC circuit dysfunction in cLBP. Specifically, cLBP is associated with different TC alternation during different conditions (pressure pain, no pain pressure and resting), our finding may shed light on our understanding of pathophysiology of cLBP.

Disclosures: M. Cannistra: None. **V. Sacca:** None. **Q. Kong:** None. **S. Reddy:** None. **M. Zhu:** None. **A. Ursitti:** None. **B. Siegel:** None. **Y. Liu:** None. **W. Li:** None. **J. Kong:** Other; holds equity in two startup companies (MNT, BTT), a patent on applying neuromodulation and a pending patent application on video guided imagery peripheral stimulation but declares no conflict of inter.

Poster

PSTR277: Thalamic and Cortical Processing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR277.17/D14

Topic: D.02. Somatosensation – Touch

Support: NSERC Discovery Grant

Title: Prefrontal contributions to relevancy-based facilitation post-concussion: A TMS study

Authors: *K. DOLMAN, S. MUGHAL, S. K. MEEHAN, R. STAINES;
Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Throughout our daily lives, the central nervous system must process an abundance of incoming sensory information from the environment. To prevent higher cortical areas from becoming overloaded with incoming sensory information, a mechanism known as sensory gating (SG) acts to selectively facilitate, or attenuate somatosensory information based on task demands. SG can be modulated by attention, resulting in the facilitation of sensory information that is goal-relevant (relevancy-based facilitation). Past work from our lab found that individuals with a history of concussion display a delay in the facilitation of relevant sensory information to guide movement compared to healthy controls. Of interest, this delay was found to occur at two timepoints reflective of distinct stages of sensory processing. The current work aimed to investigate this delay by probing the contribution of the dorsolateral prefrontal cortex (DLPFC), an area of the brain known to play a role in the modulation of incoming sensory information as well as being particularly vulnerable to concussive forces. To test this, somatosensory-evoked potentials (SEPs) were elicited via median nerve stimulation while participants performed a task

that manipulated their focus of attention toward or away from proprioceptive feedback. Participants performed this task before and after the administration of intermittent theta burst stimulation (iTBS) or continuous theta burst stimulation (cTBS) over the DLPFC, which occurred at separate experimental sessions at least 7 days apart. SEP data replicated past work, showing a differential delay in relevancy-based facilitation that occurred at either the P50-N70 or the N70-P100. Preliminary results reveal that modulating DLPFC activity via cTBS and iTBS alters the pattern of facilitation of relevant sensory information. Given that frontal brain areas are particularly vulnerable to concussive forces, these results suggest a critical role of the DLPFC in the neuronal network underpinning relevancy-based facilitation.

Disclosures: K. Dolman: None. **S. Mughal:** None. **S.K. Meehan:** None. **R. Staines:** None.

Poster

PSTR277: Thalamic and Cortical Processing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR277.18/D15

Topic: D.02. Somatosensation – Touch

Support: NINDS grant UH3 NS107714
NINDS grant R35 NS122333

Title: Modulating Perceptions: The Impact of Directed Attention on ICMS of the Primary Somatosensory Cortex

Authors: *A. H. ALAMRI[1], N. D. SHELCHKOVA[1], N. G. HATSOPOULOS[1], C. M. GREENSPON[2];
[1]Univ. of Chicago, Chicago, IL; [2]Dept. of Organismal Biol. & Anat., Univ. of Chicago, Chicago, IL

Abstract: Sensory modulation plays a critical role in perception by filtering and prioritizing sensory information, crucial for flexible behavior. While the role and mechanisms of attention have been extensively studied in intact sensory systems, the extent to which attention modulates the properties of neural responses to intracortical microstimulation (ICMS) in impaired systems remains unclear. This study investigates the impact of directed attention on the perception of ICMS in the primary somatosensory cortex (S1). In this study, we utilized ICMS with a single human participant, who had four Utah arrays implanted: two in S1 and two in the primary motor cortex (M1), to investigate the role of attention on S1 and its connections with motor cortex. Results show that attention influences the detection threshold of ICMS, particularly with respect to stimulus amplitude. Stimuli at or below the detection threshold were more readily detected when attention was focused on the stimulated digit. Conversely, stimuli above the detection threshold were detected more often when attention was directed away from the area of

stimulation, suggesting an increased responsiveness to unexpected stimuli. Next, we introduced a modification to the task which required the subjects to detect changes in stimulation intensity, starting from a non-zero baseline. This modification is designed to mitigate confounding effects from transient activity triggered by stimulation onset. Finally, we are investigating the effects of directed attention on the communication space between S1 and M1.

Disclosures: A.H. Alamri: None. **N.D. Shelchkova:** None. **N.G. Hatsopoulos:** F. Consulting Fees (e.g., advisory boards); BlackRock Microsystems. **C.M. Greenspon:** None.

Poster

PSTR277: Thalamic and Cortical Processing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR277.19/

Topic: D.02. Somatosensation – Touch

Support: Alchemist Brain to X (B2X) Project funded by Ministry of Trade, Industry and Energy (20012355, NTIS: 1415181023)

Title: Functional Characteristics of High-Gamma Activity in Human Cortex

Authors: *S. RYUN[1], C. CHUNG[2];
[1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Seoul Natl. Univesity, Seoul, Korea, Republic of

Abstract: High-gamma (HG) activity is a well-known neurophysiological activity observed in various cortical regions. However, its mechanisms and functional roles are unclear. Specifically, although the relationship between theta-gamma activities is relatively well-established in some cortical areas including the hippocampus, it is unclear whether this relationship and functional characteristics are consistent across various cortical regions. Here, we investigate this issue using phase-amplitude coupling and effective connectivity analysis. In this study, we first confirm the coupling between theta-HG activities and then evaluate the information flow between them. Twelve patients with drug-resistent epilepsy underwent electrocorticography (ECoG) grid or depth electrode insertion surgery. Eight patients with electrodes located on the primary somatosensory cortex (S1) participated in the texture stimulation task, and four patients with electrodes located in the hippocampus performed a memory encoding task. We calculated theta-HG phase amplitude comodulation (PAC) using ECoG signals from the S1 and hippocampus during texture stimulation and memory encoding periods, respectively. To evaluate information flow between theta activity and HG envelope, we calculated partial directed coherence (PDC) between them. For significance testing, we created surrogate datasets using the multiple partitioning method for PAC and the fast Fourier transform (FFT)-surrogate method (phase randomization) for PDC. We then performed the simulation 1000 times and extracted the z-scores or p-values of each condition. We found that HG activities are phase-locked to the theta

oscillations both in the S1 and hippocampus during tasks. The information flow between theta and HG activities showed distinct differences in S1 and hippocampus. That is, in the hippocampus, the theta oscillation drove the hippocampal HG activity. On the other hand, in the S1, the fluctuation of the HG envelope drove the theta oscillatory activity, and these results were consistent across patients. Interestingly, these directional differences in information flow were also observed across other cortical regions. In this study, we showed that the relationship between theta and HG activities varies across cortical regions. Specifically, results from the S1 area indicated that S1 HG activity during stimulation is not induced by slow oscillatory activity such as theta oscillation but may be driven directly by external inputs. Therefore, we suggest that HG activities have different characteristics depending on cortical function and region.

Disclosures: S. Ryun: None. **C. Chung:** None.

Poster

PSTR277: Thalamic and Cortical Processing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR277.20/D16

Topic: D.02. Somatosensation – Touch

Support: EU grant Horizon 2020 No 860949

Title: Tactile integration in the mechanoreceptors

Authors: *N. RAULT[1], F. ZELDENRUST[2], T. CELIKEL[3];
[1]Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ., Netherlands, Netherlands; [2]Radboud Univ., Nijmegen, Netherlands; [3]Dept. of Neurophysiol., Georgia Inst. of Technol., Atlanta, GA

Abstract: The sense of touch is vital for interacting with the environment and navigating our surroundings. Despite their importance, the functional properties of mechanoreceptors, the sensory receptors responsible for detecting touch, remain poorly understood. To address this question, in this study, we present a computational model based on adaptive exponential integrate and fire neurons (Adex) fitted with “Covariance Matrices Advanced evolution strategy” (CMAes) to electrophysiological recordings of mechanoreceptors. Our results reveal that the three mechanoreceptor types (Rapidly Adapting (RA), Slowly Adapting Type 1 (SA1), and Slowly Adapting Type 2 (SA2)), observed in [1], each have distinct filtering properties that together define the tactile system’s initial resolution for touch detection. We demonstrate that each receptor type plays a unique role in processing punctate tactile stimuli with varying amplitude and duration. SA1 serves as a temporal integrator, RA functions as a binary encoder (discriminator) for stimulus amplitude, and SA2 encodes both time and amplitude dimensions. Furthermore, we show that integrating activity from heterogeneous mechanoreceptors is

necessary for precise stimulus detection. These fundamental insights offer a novel mechanistic insight into tactile perception and propose testable hypotheses on the neural basis of tactile perception in the sensory periphery.

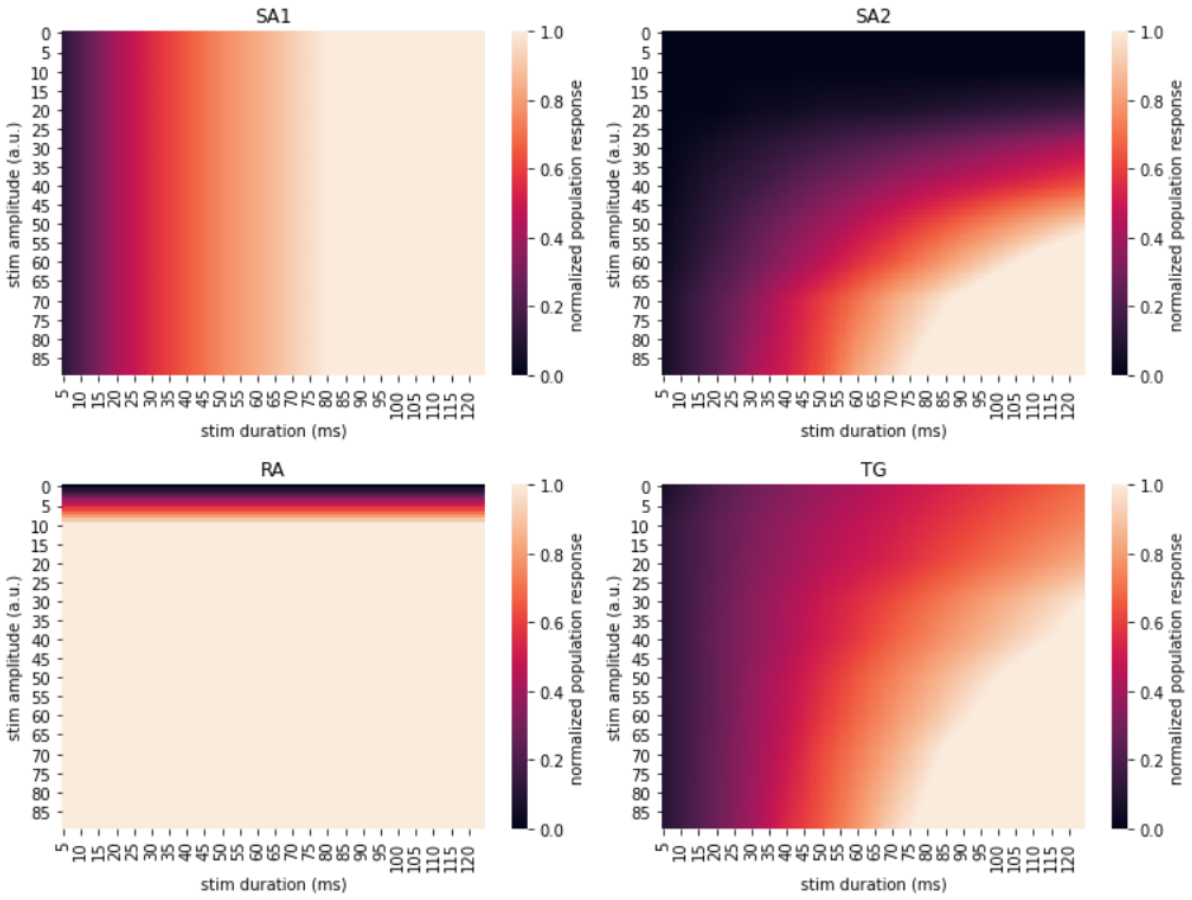

Figure. Response properties of mechanoreceptors and trigeminal (TG) neurons. Color bars represent the normalized population response. The firing rates are normalized within the mechanoreceptor and TG populations.

[1]Sonekatsu, M., Yamada, H., & Gu, J. G. (2020). Pressure-clamped single-fiber recording technique: A new recording method for studying sensory receptors. *Molecular Pain, 16*, 174480692092785. https://doi.org/10.1177/1744806920927852

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Poster

PSTR277: Thalamic and Cortical Processing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR277.21/D17

Topic: D.02. Somatosensation – Touch

Support: 1R01NS092894-01
1R01NS124222-01

Title: Somatosensory neuroprostheses: optimized intrathalamic microstimulation utilizing deep learning

Authors: *B. SEE[1], L. DICKEY[1], W. WALKER[2], J. T. FRANCIS[3];
[1]Biomed. Engin., Univ. of Houston, Houston, TX; [2]Univ. of Houston, Houston, TX; [3]BME, Univ. of Houston, Sugar Land, TX

Abstract: Despite major advances in decoding motor neural activity for the operation of robotic limbs, patient use of these assistive systems has been limited by their lack of sensory feedback, which is necessary for even simple everyday tasks. Somatosensory prostheses utilizing electrical stimulation of sensory areas corresponding to the somatotopic representation of the limb could provide actionable sensory feedback. A major challenge with this approach is that electrical stimulation often results in the generation of paresthesias, consisting of tingling, burning, shocking, and other abnormal sensations. The generation of paresthesias is partially caused by the activity evoked by electrical stimulation not sufficiently matching the natural activity resulting from touch. Our group has previously demonstrated a method to address this deficiency, using model predictive control for stimulation in the forelimb representation in the Ventral Posterolateral (VPL) thalamus based on resulting primary somatosensory cortical (S1) activity from focal natural touch stimuli. Intrathalamic Microstimulation (ITMS) optimized by this method produced cortical Local Field Potentials (LFP) more similar to that resulting from natural touch. To further improve this methodology, nonlinear models such as artificial neural networks (ANNs) can be used to relate VPL and S1 activity to the touch stimulus input space. In this work we implant Lewis rats in the VPL thalamus and cortex with multichannel NeuroNexus silicon arrays, and use a CNN-RNN model to relate natural touch activity from the VPL to S1 in order to find a basis set of activity that can be used to model activity contained in the somatotopy of both regions. After freezing the weights of this model, additional input and hidden layers are added to the model relating the forelimb touch input space to the cortical activity. Using this model to implement the controller better accounts for the non-linearities inherent in the thalamocortical somatosensory system, as compared to naturalistic touch by quantitative measures including mean squared error, Mahalanobis distance, and touch location classifier accuracy.

Disclosures: B. See: None. **L. Dickey:** None. **W. Walker:** None. **J.T. Francis:** None.

Poster

PSTR277: Thalamic and Cortical Processing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR277.22/D18

Topic: D.02. Somatosensation – Touch

Title: Volumetric analysis of thalamic nuclei and cerebral hemispheres in the nine-banded armadillo (Dasypus novemcinctus)

Authors: M. UGARTE, O. FARNSWORTH, L. MILAM, *J. PADBERG;
Univ. of Central Arkansas, Conway, AR

Abstract: In order to identify features of neural organization that may be common across mammalian clades, we are conducting a series of studies examining the brain of a representative species of Xenarthra, a relatively early branch of the mammalian lineage. The nine-banded armadillo (Dasypus novemcinctus) is the only extant xenarthran species found in North America. This species is known to have extensive pyriform cortex, a relatively large region of auditory cortex, and a relatively small region of visual cortex. In order to better quantify the extent of sensory structures, we are performing a volumetric analysis of the cerebral hemispheres and thalamic nuclei in this species using confocal microscopy and Nikon Elements AR software. In two cases, fixed whole brain specimens were sectioned at 50**μ**;m in the coronal plane and stained for cytochrome oxidase histochemistry (CO), cytoarchitecture (Nissl), and immunocytochemistry using antibodies to calbindin (Cb), parvalbumin (PV), and nonphosphorylated neurofilament protein (SMI-32). Sections were then mounted and each series of sections was scanned and imported into the software. Boundaries of structures were outlined manually by three independent observers, and the resultant areal data were converted into volumes using section thickness. Preliminary results indicate no significant left/right asymmetries in volumes of hemispheres or thalamic nuclei. Ongoing efforts will examine volumetric proportions of thalamic nuclei, hippocampus, pyriform cortex, and cerebral hemispheres with respect to whole brain and white matter volumes in this species. These analyses will help delineate specializations in the brain of the armadillo and will further our understanding of xenarthran neural organization in comparison to members of other mammalian clades.

Disclosures: M. Ugarte: None. **O. Farnsworth:** None. **L. Milam:** None. **J. Padberg:** None.

Poster

PSTR278: Olfaction: From Physiology to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR278.01/D21

Topic: D.03. The Chemical Senses

Support: Chen Senior Postdoctoral Fellowship
T&C CHEN CENTER FOR SYSTEMS NEUROSCIENCE AWARDS

Title: The Neural Language of Love: Decoding Optimal Mate-searching in C. elegans

Authors: *X. WAN, P. W. STERNBERG;
Caltech, Pasadena, CA

Abstract: *C. elegans* hermaphrodites/females emit volatile cues that broadcast their reproductive status and readiness for mating; males must locate the potential mate in a complex environment. We investigated the response of *C. elegans* males to volatile sex pheromone, a highly selected behavior that provides an opportunity to elucidate mechanisms of efficient navigation as well as sex-specific behavior. We found that the sex pheromone chemoreceptor SRD-1 exhibits sexually dimorphic expression in both head and tail regions, suggesting that there are spatially separated detectors for pheromone. Pan-neuronal imaging shows SRD-1-dependent sex-specific neuronal activation with only male AWA neurons and male-specific PHD neurons activated by pheromone. The tail located PHD neurons are required for difficult but not simple navigation tasks. By observing trajectories of individual males, we observed that males employ multiple navigation strategies—orthokinesis, klinokinesis, klinotaxis, and tropotaxis—in response to pheromonal cues, enriching our understanding of behavioral complexity driven by olfactory stimulus. We computationally modeled aspects of the head versus tail signaling and find that it is relatively straightforward to match key experimental observations with a few parameters that capture the most relevant system properties. *C. elegans* offers a compelling demonstration of an efficient olfactory mechanism. Fine-tuning the expression of a single receptor allows for effective tropotaxis-mediated spatial navigation while simultaneously facilitating the execution of distinct sex-specific behaviors. This research lays the groundwork for future studies in complex systems and could transform our understanding of sexually dimorphic traits from receptor expression to neural circuit dynamics.

Disclosures: X. wan: None. **P.W. Sternberg:** None.

Poster

PSTR278: Olfaction: From Physiology to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR278.02/D22

Topic: D.03. The Chemical Senses

Support: NIH Grant F99/K00 5K00NS118741-04
NSF NeuroNex Award #2014217

Title: Chemosensory encoding by tangential inputs to the Drosophila navigation center

Authors: *K. M. NUNEZ[1], J. FREED[1], K. VAN HASSEL[1], S. MUBARAK HUSSAIN[2], K. NAGEL[1];
[1]Neurosci. Inst., New York Univ. Langone Hlth., New York, NY; [2]Biol., Univ. of New Mexico, Albuquerque, NM

Abstract: Like most animals, *Drosophila melanogaster* uses chemosensory information to guide navigation and foraging. Tangential inputs to the fan-shaped body are anatomically poised to provide chemosensory input to the fly navigation center and a small number of these have been shown to respond to select odorants and tastants. However, how chemosensory information is encoded across the diverse tangential input population of ~150 connectomically-defined neuron types is unknown. Here we set out to characterize chemosensory representations across the population of tangential inputs and to link these representations to behavior. First, we used wide-field calcium imaging to survey responses of developmental classes of tangential neurons to six odorants of varying valences. We found that most populations showed broad responses to odors with substantial variation across flies. These data suggest that chemosensory information is represented by a population code across the array of tangential inputs and strongly modulated by behavioral state or experience. Next, we performed whole-cell electrophysiology from ventral tangential inputs derived from a single developmental class and recorded responses to both odorants and tastants. We found that neurons targeting the same layers of the fan-shaped body showed similar temporal response profiles but distinct chemo-sensory tuning. A subset of these neurons showed pronounced changes in intrinsic properties during the course of recording— from tonic firing where sensory information was transmitted to burst firing where sensory information was blocked— providing a potential substrate for response differences observed across flies. Recording from a more specific driver line revealed more uniform chemo-sensory tuning suggesting that diverse tangential inputs to each layer are genetically-defined. In ongoing work, we plan to record from several specific classes of tangential neurons derived from multiple developmental lineages, and to compare sensory tuning to the effects of optogenetic activation and inactivation on navigation and foraging behavior. The expected output of this work is a first map of how tangential inputs to the fly navigation center transform chemosensory information into behavior.

Disclosures: K.M. Nunez: None. **J. Freed:** None. **K. van Hassel:** None. **S. Mubarak Hussain:** None. **K. Nagel:** None.

Poster

PSTR278: Olfaction: From Physiology to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR278.03/D23

Topic: D.03. The Chemical Senses

Support: ONR Grant GR0019433

Title: Creating an apparatus for high throughput extracellular recording with on-demand configuration in free-behaving locust

Authors: *F. DENG[1], Q. COQUEREL[2], R. CHANDAK[3], B. RAMAN[3];
[1]Washington Univ. in St Louis, RICHMOND HEIGHTS, MO; [2]Washington Univ., Saint Louis, MO; [3]Biomed. Engin., Washington Univ. In St. Louis, Saint Louis, MO

Abstract: <META NAME="author" CONTENT="菲阳 邓">Methods to record neural signals from multiple circuits and for long periods of time in behaving animals is an open challenge in neuroscience. Here we present a method using custom-built, flexible multielectrode and multicenter arrays for recording odor-evoked neural and behavioral responses in locusts (Schistocerca americana). By combining a twisted-wire fabrication method with polydimethylsiloxane (PDMS) as a structural agent, we devised a process that allows manufactured electrodes to be flexible and conform to various shapes, facilitating high-throughput recording from multiple brain regions with varying topologies. We used this approach to demonstrate that we could record from multiple neural centers: antennal lobe, mushroom body, and central complex simultaneously and for multiple days. Further, by integrating minimally invasive surgical technique to expose the brain, we successfully recorded locusts that were freely behaving in a trackball-based olfactory VR. We further recorded neural signals while conditioning free-moving locusts with positive (sugar water) and negative (electric shock) rewards. Our results indicate that variances in odor-evoked responses of appetitive odorants (conducive for classical conditioning) and non-appetitive odorants (not conducive for classical conditioning) were not uniformly distributed but confined to distinct neural manifolds. In sum, our results establish a proof-of-concept methodology for achieving higher success rate, neural yield, longevity, and adaptability to monitoring neural response during various innate and acquired behavioral responses.

Disclosures: F. Deng: None. **Q. Coquerel:** None. **R. Chandak:** None. **B. Raman:** None.

Poster

PSTR278: Olfaction: From Physiology to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR278.04/D24

Topic: D.03. The Chemical Senses

Support: NIH Grant 1RF1NS128865

Title: Cellular and functional heterogeneity of interhemispheric connections in the anterior olfactory nucleus

Authors: *L. N. NEWMAN, S. JAYAKUMAR, B. L. LOGEMAN, C. G. DULAC, V. N. MURTHY;
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Abstract: The olfactory system in most mammals begins with two unique air flows separated by a nasal septum. Olfactory signals are then relayed to the brain in two separate olfactory bulbs, from which the brain produces a unitary perception of the olfactory environment. The mechanisms by which the brain combines these separate inputs to obtain perceptual unity remain unknown. The anterior olfactory nucleus (AON) is the earliest olfactory cortical area to project contralaterally, making it an excellent candidate for the combination of bilateral olfactory information. The AON is also implicated in social behavior and olfactory memory. However, the specific cell types involved in the interhemispheric AON connection are unknown, as are the functional consequences of contralateral input to the AON. Using anterograde and retrograde anatomical tracing, we show that contralaterally-projecting AON neurons are glutamatergic (VGLUT1-positive) but not GABAergic (VGAT-positive) (N=3 VGLUT1-cre mice, N=5 VGAT-cre mice), making synapses with both VGLUT1 and VGAT-positive neurons (N=3 VGLUT1-cre mice, N=3 VGAT-cre mice). Preliminary results from single nucleus RNA sequencing of the AON reveal 27 distinct neuronal clusters, 6 of which are VGLUT1-positive putative pyramidal cells, providing candidate populations to investigate the mechanisms of inter-AON signaling. Optogenetic stimulation of contralaterally-projecting AON axons during whole-cell recording of AON cells resulted in EPSPs with a connection probability of 0.46 (n=13 cells). This work will contribute not just to our understanding of the AON, but also to the processing done by the olfactory cortex. Insights from the cellular and functional diversity within the AON will guide future research on the role of the AON in olfactory-related disorders. This work is sponsored by NIH grant 1RF1NS128865

Disclosures: L.N. Newman: None. **S. Jayakumar:** None. **B.L. Logeman:** None. **C.G. Dulac:** None. **V.N. Murthy:** None.

Poster

PSTR278: Olfaction: From Physiology to Behavior

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Program #/Poster #: PSTR278.05/D25

Topic: D.03. The Chemical Senses

Support: NIH/NINDS RF1 NS128975
NIH/NINDS R24 NS098536

Title: Olfactory bulb activity and active sniffing during naturalistic foraging in freely moving mice

Authors: *J. A. SMITH[1], K. A. BOLDING[3], J. TAI[4], I. G. DAVISON[2];
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Abstract: Understanding the critical role of the olfactory system in guiding naturalistic foraging behaviors promises to provide fundamental insights into sensory perception and ecological adaptations. Mice rely heavily on their olfactory senses to navigate complex environments and locate potential food sources. Although odor-evoked activity has been intensively studied in head-fixed animals, little is known about the dynamic sensory signals acquired by freely moving animals when actively sampling their environment. To address the gap in knowledge about real-time olfactory sensory-motor strategies, we engineered a novel head-mounted miniscope with an expanded field of view allowing us to bilaterally image glomerular activity in the main olfactory bulb (MOB). MOB imaging in freely moving animals revealed that sensory information was largely confined to a relatively small distance within 10 cm of the odor source. Average glomerular activation in the MOB increased as animals approached odor sources, allowing us to map well-studied concentration-dependent coding onto spatial distance measures. Interestingly, glomerular activity often showed directional tuning near the odor source, and these signals appear to inform future turning behavior. Odor-evoked activity was often temporally sparse, suggesting animals only obtain sensory information on a subset of sniff samples. To directly relate sniffing activity to behavior and neural activity, we implanted thermistors to track sniffing during foraging, which reveals that sensory sampling has a complex relationship between movement speed and directional changes. Currently, we are integrating sniff measurements and MOB miniscope imaging to test how active olfactory sampling strategies relate to both sensory-driven activity in the MOB and ongoing moment-to-moment navigational decisions.

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Poster

PSTR278: Olfaction: From Physiology to Behavior

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Program #/Poster #: PSTR278.06/D26

Topic: D.03. The Chemical Senses

Support: NIH R01 DC006666-19

Title: Optogenetic Inhibition of Gustatory Cortex Disrupts Taste-Potentiated Odor Associations

Authors: *A. SURENDRAN[1], I. GOLDSTEIN[1], K. MAIGLER[2], T. GRAY[2], D. B. KATZ[3];
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Abstract: Though olfactory experience is conventionally thought of in terms of orthonasal delivery (i.e., through the nose), rats learn an odor-reward association preference learning task faster when the odor is retronasally experienced (through the mouth; Blankenship et al., Curr Biol., 2019). Our data shows that rats trained after receiving combined (retro and ortho) olfactory exposure (OE) develop a significantly stronger preference for the paired odor than unexposed rats (n=12, 6 per group), suggesting that pre-exposing animals to a combined retro/ortho olfactory stimulus potentiates preference learning. Given that retronasal association preference learning and learning potentiation from taste pre-exposure (Flores et al., Learn Mem., 2018) are both gustatory cortex (GC) dependent, we ask whether the enhancement of this paired odor association learning from combined OE is also dependent on GC. Using the optogenetic inhibitor ArchT to dampen activity in GC during conditioning trials (GCx), we hypothesize that such inhibition would block learning potentiation seen from OE. A complementary demonstration of GC's involvement is seen when we quantify viral expression at the site of the fiber. Preliminary results indicate no significant increase in preference for the paired odor after combined OE during GCx. Furthermore, the ratio of virus expression in GC compared to extracortical areas significantly negatively correlates with the extent of preference learning in OE animals. Together the data suggest that GC must be online for potentiation of combined retro/orthonasal association preference learning.

Disclosures: A. Surendran: None. **I. Goldstein:** None. **K. Maigler:** None. **T. Gray:** None. **D.B. Katz:** None.

Poster

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Program #/Poster #: PSTR278.07/D27

Topic: D.03. The Chemical Senses

Support: NIH Grant R01NS123903

Title: Olfactory bulb local field potentials track breathing rhythms at multiple timescales

Authors: S. RAFILSON, *M. SMEAR;
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Abstract: Odors carry useful navigational and episodic information, but most of the chemical world cannot be accessed without actively sampling the environment. To optimally orient by olfactory information, the brain must unify odor-driven activity with representations of self-movement and context. Studies in other sensory modalities demonstrate that contextual signals are common in primary sensory areas, and it has long been known that olfactory bulb (OB) local field potentials (LFP) are coupled with behavior. Our lab has found that individual olfactory bulb

neurons track the long-timescale rhythmic structure of breathing, in the absence of experimenter applied stimuli or tasks. To better understand the coupled rhythms of breath and OB population activity dynamics, we analyzed local field potentials. During free movement, respiration is rhythmically organized into discrete states lasting minutes, whereas these states are not apparent during head fixation. In the OB, low frequency LFP oscillations correlate with sniff frequency and LFP waveforms in multiple frequency bands are aligned to inhalation. Interestingly, we observe a differential modulation of alignment in LFPs with varying sniff frequencies: alignment in high frequency LFPs strengthens as sniff frequency increases, whereas alignment in low frequency LFPs diminishes. Thus, OB LFP tracks information about timing and frequency of the respiratory cycle, and the alignment may be associated with behavioral state and odor information. We propose that these contextual signals, particularly those dependent on active sampling, facilitate the incorporation of olfactory information into cognitive maps of self and environment.

Disclosures: S. Rafilson: None. **M. Smear:** None.

Poster

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Location: MCP Hall A

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Program #/Poster #: PSTR278.08/D28

Topic: D.03. The Chemical Senses

Title: Dynamics of granule cell circuits in zebrafish olfactory bulb throughout development and learning

Authors: *N. TEMIZ[1,2], B. HU[1,2], R. W. FRIEDRICH[1,2];
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Abstract: The olfactory bulb (OB) at early developmental stages predominantly comprises mitral cells and superficial interneurons while deep interneurons, particularly granule cells, are lacking. This early circuitry is functional and can perform canonical computations including a decorrelation of overlapping odor representations. Granule cells emerge later in development and eventually become the most abundant cell type in the adult OB, raising the question of how they contribute to information processing. We used zebrafish to examine how the cellular composition and odor evoked population activity changes during development. We found a steep increase in the number of GABAergic neurons 3 weeks postfertilization, coinciding with the emergence of top-down projections from telencephalic area Dp, the zebrafish homolog of piriform cortex, as revealed by viral tracing. Using two photon calcium imaging we found that odor evoked activity patterns evolved with development, which included an increase in response amplitudes, and increase in tuning sharpness of excitatory neurons, and more pronounced pattern decorrelation. To explore the effects of learning on odor representations we devised a semi-

automated training apparatus for olfactory associative conditioning of group housed zebrafish in their home tanks. We found that training increased the amplitude of responses to trained and other odors, enhanced pattern decorrelation, and increased the discriminability of odor representations by a simple classifier. These results show that the development of the deep interneuron network coincides with a refinement of odor representations in the OB. Moreover, our results are consistent with the hypothesis that deep interneurons contribute to learning related modifications of odor processing, which may involve the integration of information conveyed by sensory input and top-down projections.

Disclosures: N. Temiz: None. **B. Hu:** None. **R.W. Friedrich:** None.

Poster

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Topic: D.03. The Chemical Senses

Support: NIH R01DC016289
NTT Research

Title: Cortical encoding of odor mixtures in an olfactory cocktail party task

Authors: *N. XIA, V. N. MURTHY;
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Abstract: The sensory world is noisy and cluttered, creating a challenge to extract relevant information. It is unclear how animal brains process complex, high-dimensional sensory signals efficiently to suit different contexts and behavioral needs. We address this question by studying how mice encode the information about complex odor mixtures in an olfactory cocktail party task. In this Go/NoGo task, mice smell odor mixtures with up to 16 components (with a palette of 65535 distinct stimuli in total) and decide which one of the two target odors is present in the mixtures. We improved the training curriculum to achieve task learning by mice in five days. We have recorded the activity of mitral/tufted cells in the olfactory bulb, and neurons in the piriform cortex in naïve mice, mice under training and expert mice. In preliminary data, we find that average neuronal responses in piriform cortex to the Go odor mixtures increased as mice learned the association (44, 38, 57, 56, 53, 70 neurons across 6 days of recording). We then used an optimal linear classifier to assess the discriminability of piriform population activity to Go and NoGo odor mixtures across the course of learning. We found that the learned animal has much higher discriminability than the naïve animal (19.6% for the naïve animal, 52.6% for the expert animal). This increase is significant starting at day 2 of training and keeps growing even as mice are trained with increasingly difficult curriculum, with more complex mixtures (71.8%, 76.9%,

100%, 31.4% for day 2,3,4,5, respectively), with the only exception being day 5 which has the most difficult mixture curriculum. We found that the increase in discriminability cannot be explained by the increase in average neuronal activity, ruling out a trivial nonspecific scaling effect. We also tested the discriminability of piriform population to the number of components in the mixtures and found that the discriminability is higher in the expert animal than in the naïve animal (17.5% for the naïve animal, 52.4% for the expert animal). We are extending the analysis to more animals, while also expanding it, to illuminate the mechanism of complex odor mixtures encoding in the olfactory bulb and piriform cortex.

Disclosures: N. Xia: None. **V.N. Murthy:** None.

Poster

PSTR278: Olfaction: From Physiology to Behavior

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Program #/Poster #: PSTR278.10/D30

Topic: D.03. The Chemical Senses

Support: R01DC021213 (NIDCD/NIH)
R01DC017985 (NIDCD/NIH)

Title: Taurine-conjugated bile acids activate distinct vomeronasal neurons and drive aversive behaviors in mice

Authors: *V. MANOHARAN, J. WANG, J. P. MEEKS;
Neurosci., Univ. of Rochester, Rochester, NY

Abstract: The rodent accessory olfactory system (AOS) detects environmental chemosignals and guides several social and non-social behaviors. Fecal bile acids have been found to activate neurons in the AOS, potentially serving as mammalian pheromones and kairomones. Using live volumetric Ca^{2+} imaging, we screened several classes of naturally occurring bile acids for their capacity to activate peripheral vomeronasal sensory neurons (VSNs). We found that taurine-conjugated bile acids, including taurine-conjugates of cholic acid, deoxycholic acid, lithocholic acid, and chenodeoxycholic acid (TCA, TDCA, TLCA, TCDCA, respectively) activate large VSN populations that do not respond to unconjugated (CA, CDCA, DCA, LCA), glycine-conjugated (GCA, GDCA, GLCA, GCDCA), or keto-conjugated (7-keto DCA, 12-keto DCA, 7-keto LCA) bile acids. A minority of VSNs activated by taurine-conjugated bile acids were also sensitive to sulfated steroids. Among the taurine-conjugates, tauro-deoxycholic acid (TDCA) displayed particularly strong potency, activating many VSNs at sub-micromolar concentrations. Exposing mice *in vivo* to mouse fecal extracts 'spiked' with TDCA – which is normally undetectable in mouse feces – elicited stress-associated behaviors in mice in a non-social context (avoidance, digging, grooming, etc.), supporting the hypothesis that TDCA may function as a

mouse kairomone. These studies establish taurine-conjugated bile acids as a novel class of potent vomeronasal ligands driving aversive behaviors in mice.

Disclosures: V. Manoharan: None. **J. Wang:** None. **J.P. Meeks:** None.

Poster

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Topic: D.03. The Chemical Senses

Support: NSF award #2021795

Title: Serotonergic modulation of odor-evoked neural and behavioral responses in solitary and gregarious locusts.

Authors: *Y. BESSONOVA[1], J. KELLEY[2], B. RAMAN[3];
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Abstract: Phenotypic plasticity is the ability of an organism of the same genotype to exhibit different phenotypic characteristics, like behavior and morphological traits, based on environmental conditions. Locusts are one of the examples of such organisms. Depending on the social environment, locusts can exist as solitary grasshoppers or gregarious locust swarms. The phenotypic phase of locusts (solitary vs gregarious) is not a constant and the release of serotonin has been implicated to play a significant role in phase change in locusts. In particular, the increase in serotonin levels in the thoracic ganglion causes solitary locusts to undergo the transition into gregarious ones. On the other hand, the increase of serotonin in the brain is linked to the conversion of gregarious locusts into solitary grasshoppers. How sensory processing is altered in a phenotypic phase-dependent manner to support different behavioral responses to the same stimuli is not fully understood. We began by examining the appetitive behavioral responses of locusts (*Schistocerca americana*), to a panel of diverse odorants, before and after serotonin injections into the brain or thoracic ganglion. We examined how the odor-evoked behavioral responses were altered depending on the site of serotonin injection and the phenotypic state of locusts. Serotonin injection in the head of gregarious locusts altered behavioral responses in an odor-specific fashion. In contrast, serotonin injection into the head/thoracic ganglion of solitary locusts resulted in a homogenous increase or decrease in behavioral responses depending on the site of exogenous serotonin injection. Electrophysiological recordings from early olfactory circuits revealed increases in odor-evoked neural activity for most odorants. Using a linear statistical model, we reveal a simple mapping between the non-specific changes in neural responses to odor-specific changes in behavioral outcomes.

Disclosures: Y. Bessonova: None. **J. Kelley:** None. **B. Raman:** None.

Poster

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Topic: D.03. The Chemical Senses

Support: U19NS112953
U19NS107464

Title: Behaviorally relevant features of the neural code in olfactory bulb

Authors: *S. KARIMIMEHR[1,2], S. CEBALLO[3], M. KARADAS[3], D. RINBERG[3,4,5];
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Abstract: Odor stimuli evoke spatiotemporal patterns of activity at the glomerular level in the olfactory bulb. The relationship between patterns of neural activity and perceptual similarities among sensory stimuli remains debatable. In this study, we designed an experiment using the 2-alternative-forced-choice (2AFC) paradigm to measure the generalization ability of mice in precise odor discriminations. This task allowed us to smoothly vary the spatiotemporal patterns of activity using three-component odor mixtures to identify the relevant features of neural activity that drive behavioral discriminations. We trained mice to discriminate a specific mixture of three odors (referred to as the "Target") from a range of different odor stimuli (referred to as "Non-Targets"). In subsequent probe trials, we manipulated the mixture's composition to test the mice's ability to generalize their response. We then employed two-photon Ca2+ imaging to measure the neural activity in mice expressing the fast calcium indicator GCaMP6f in presynaptic glomeruli. Based on these recordings, we aim to identify the critical aspects of neural activity that influence behavior. Our preliminary data indicate that this correlation exists within the early temporal window of the sniff cycle, and changes in the odor sets suggest that the relevant glomeruli for the task are determined by the task demand. These findings provide valuable insights into how the olfactory system represents and distinguishes between odor mixtures, highlighting the importance of the order of neural activity and the significance of temporal dynamics in encoding these mixtures.

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Poster

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Topic: D.03. The Chemical Senses

Support: U19NS112953
U19NS104650

Title: The geometry of odor representations and their transformation in the peripheral olfactory system

Authors: *J. S. HARVEY[1], J. V. GILL[1], M. KARADAS[1], R. REISER[1], D. RINBERG[1,2]; [1]Neurosci. Inst., NYU Langone Hlth., New York, NY; [2]Center for Neural Science, New York University, New York, NY

Abstract: The olfactory landscape is uniquely complex and varied, with animals having to grapple with an overwhelming chemical diversity of volatiles as they seek to sense kin, food, threats and mates at a distance. It remains unclear how the olfactory system represents stimuli as a function of their chemical composition and concentration, and which features shape the geometry of representations as they are transformed from sniff to smell.
To address this question, we collected 1-photon calcium imaging data in the peripheral olfactory system of the mouse. We recorded olfactory sensory neurons in olfactory bulb glomeruli for a large panel of chemically diverse odorants, across a broad dynamic range from threshold to saturation of response. We look at how the odor-evoked responses evolve as temporal sequences, and how the features of odorant identity and concentration are represented. Comparing data from multiple animals, we also evaluate how conserved the manifold of neural activity is across individuals for representing odor identity and concentration.
We additionally compare these results to data for the same odor set collected in mitral cells with 2-photon calcium imaging, to better understand how odor representations are actively shaped and transformed as they propagate through the peripheral olfactory system. Using our data, we build a statistical model for how olfactory geometric relationships are transformed from inputs to outputs by the circuitry of the olfactory bulb.

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Poster

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Topic: D.03. The Chemical Senses

Support: U19NS112953
U19NS104650
Leon Levy Scholarship in Neuroscience

Title: The geometry and role of sequential activity in sensory processing and perceptual generalization

Authors: *J. GILL[1], M. KARADAS[2], S. SHOHAM[3,4,5], D. RINBERG[6,7,8];
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Abstract: Animals encode sensory stimuli with precisely timed activity across modalities. For example, mice can rapidly recognize odors, independent of their concentration, based on complex spatiotemporal patterns of mitral and tufted cell (MTC) activity in the olfactory bulb. Yet, it remains unknown how sequential MTC activity is organized, and what role sequential activity plays in guiding perception. We performed fast 2-photon calcium imaging of hundreds of MTCs with sub-sniff temporal resolution to a battery of odors. We constructed a space of MTC tuning using the pairwise correlations between MTC odor responses averaged over a single sniff. We then analyzed the propagation of sequences in this space and discovered that sequences originated in a set of similarly tuned neurons and propagated to more distantly tuned neurons, so that the latency of MTC activation was linearly related to distance in tuning space. Further, we found that the early but not the later part of sequences carried concentration invariant information about odor identity. Finally, inspired by the discovery that similarly tuned MTCs are activated sequentially across odors, we propose a role of activity sequences in training the piriform cortex to learn perceptually generalizable odor representations. Like the role of retinal waves in establishing the retinotopic organization of visual processing, MTCs sequentially activated together across odor responses may be responsible for establishing odor cortical maps, even for odors that have never been experienced. These ideas were tested in a proof-of-principle computational model for sequence-based unsupervised training of synapses from MTCs to the piriform cortex, which revealed that sequential activity across the entire sniff permits perceptual generalization for novel odors. The olfactory system provides a tractable model of the proposed principle that sequential activity acts as a scaffold for learning relevant activity manifolds between networks, which is applicable to other information processing circuits beyond olfaction.

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Poster

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Simons Foundation, Simons Society of Fellows Junior Fellow, Grant 965381

Title: Concentration is not intensity: unraveling the neural correlates of odor intensity

Authors: *B. BARRA[1], J. S. HARVEY[1], J. ZHAO[1], R. REISER[1], D. BRANN[2], S. LEWIS[3], R. PELLEGRINO[4], M. SEPPO[5], A. SHERIFF[6], T. TSUKAHARA[7], S. R. DATTA[8], K. M. FRANKS[9], A. FLEISCHMANN[5], A. KOULAKOV[10], C. ZELANO[11], D. RINBERG[1], J. MAINLAND[4];
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Abstract: How the brain represents physical properties of sensory stimuli is a central question in sensory systems neuroscience. In vision, the number of photons translates into brightness; in audition, sound-wave amplitude translates into volume. In olfaction however, how concentration relates to perceived odor intensity is poorly understood. Several studies have shown that changes in odorant concentration are correlated with changes in number of responsive neurons, neural firing rates, temporal shifts in responses relative to inhalation, or overall synchrony of neural responses. However, odorant concentrations are inappropriate proxies for perceived intensity: at similar concentrations some odors evoke strong sensations, while others are barely perceptible. Therefore, it remains unclear which neural phenomena underlie the perception of odor intensity. One major challenge to study the neural encoding of intensity is obtaining perceptual reports and neural recordings from the same animal model. Here, we leveraged a behavioral paradigm in the mouse that allows us to measure which concentrations of an odor pair are intensity-matched. We selected a set of three odors and computed the intensity-matched concentrations for each odor pair. We found the derived intensity-matches from one odor pair both matched to the same concentration of a third odor ($p < 0.05$). We repeated this measure at three different concentration ranges, with errors as low as 1 ppm (standard deviations ranged from 0.15 pm to 10.42 ppm). By pairing neural recordings and the intensity-matching paradigm in the mouse we can study the encoding of odor intensity in a mouse model in an unprecedented fashion. Namely, we asked which neural features are good candidates to encode intensity, i.e. show equivalence at intensity-matched concentrations of different odors. We recorded wide-field one-photon imaging in the olfactory bulb at ten concentrations of each odor, ranging from 0.005% to 10% of

saturated vapor pressure. We then computed response magnitude, response latency and number of activated glomeruli, which have been previously found to correlate with concentration changes. We found that these neural features do not seem to explain intensity equivalence across odors at intensity-matched concentrations. We plan to use machine learning approaches to combine optical recordings and behavioral data and formulate new hypotheses for an intensity code in olfaction.

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Poster

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Program #/Poster #: PSTR278.16/D36

Topic: D.03. The Chemical Senses

Title: The anterior olfactory nucleus is essential for mediating exploratory behaviors induced by novel olfactory stimuli.

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Abstract: When novel stimuli are detected, humans and animals react with orienting and exploratory behaviors driven by sensory input. For example, mice exposed to novel odorants respond with an increase in respiration frequency and pupil dilatation. These spontaneous responses reflect the adaptive value of exploring unfamiliar stimuli in complex environments. How novel odors are discriminated throughout the early olfactory pathway is poorly understood. We used a spontaneous novelty detection paradigm to study how the olfactory system processes novel and familiar odors. We trained mice for four days while familiarizing them with an odor set. Then, we introduced a novel odor set on the fifth day to compare the influence of novel and familiar odors on behavior and neuronal responses. Our data showed stronger neural responses to novel odors in the anterior olfactory nucleus (AON), compared to the Piriform Cortex (PCx). Hence, we hypothesized that the AON, but not the PCx, is crucially involved in detecting stimulus novelty. To verify this, we inhibited these regions with muscimol infusions and

observed respiration and pupil diameter during the spontaneous novelty detection paradigm. We found that inhibiting the AON, but not the PCx, reduced sniffing and pupil dilatation in response to novel odors. Given these results, the subsequent questions remained: a) did AON inhibition selectively influence novelty detection, or odor discrimination in general b) was the effect mediated by AON feedforward or feedback projections. To address these questions, we selectively inhibited the AON neurons that project back to the olfactory bulb (OB) using DREADDs. We found that inhibiting the AON to OB projection caused a reduction in response to novel odors. However, we observed that feedforward projections from the AON to the PCx were also labeled. Therefore, we cannot definitively conclude whether the feedback projection from AON to OB or the feedforward projection from AON to PCx is primarily important for discriminating between novel and familiar odors. We are now inhibiting feedback projections from the PCx to the OB, which will provide insight into the potential involvement of PCx to OB projection in odor discrimination. These approaches will further elucidate the specific contributions of the AON and PCx in olfactory processing and discrimination.

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Poster

PSTR278: Olfaction: From Physiology to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR278.17/D37

Topic: D.03. The Chemical Senses

Support: Institute for Mind and Biology Seed Grant

Title: Sex and circadian effects on odor discrimination behavior in Long Evans rats

Authors: S. DETWILER[1], N. TURKI[2], N. BECTON[3], E. BELL[3], B. J. PRENDERGAST[4,5], *L. M. KAY[4,5];
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Abstract: Many sources of unknown and often uncontrolled variability can impact or impede progress in understanding cognitive behavior. Here we examine influence of two commonly-neglected variables - sex and time-of-day - on odor discrimination. In one study, we tested adult Long Evans rats in a go/no-go (GNG) paradigm to discriminate 2 odors across several odor sets. In a second study, we tested rats on three 2-alternative choice (TAC) tasks to discriminate 5 odorants across two odor sets. In GNG, female rats sampled odors slightly but significantly

longer than male rats and responded slightly but significantly more slowly to achieve the same performance levels of > 90% after a day or two of training on each test odor set in sessions of 200 trials. In the TAC tasks, female rats sampled a full sniff longer than males, and sampling times did not differ across the three tasks. Response times and performance did not differ by sex in the TAC tasks. Rats are nocturnal animals, but few studies report the precise time of testing, although many report testing during the light phase, when rats are inactive. Testing in a reversed light cycle may be an option, but it limits the use of most sources of light to avoid circadian disruption. To examine the effect of testing at different times of day, in a third study, we trained rats to perform the GNG task around the midpoint of the light phase. Then we examined whether testing four hours earlier or later than the standard test time would affect learning of new odor sets. In unshifted control tests rats learned to discriminate new similar odorants quickly when trained and tested at the typical reward-entrained test time: in 30-minute sessions, female and male rats reached ~65% performance on the first day and > 90% on the second day of training. When shifted four hours earlier or later than the normal test time, learning was severely impaired, and performance was at chance for two successive test days. Taken together the data indicate: (1) performance and variance are comparable across males and females, further justifying inclusion of females in olfactory studies without the need to control for estrous cycle day, (2) females use longer sampling times across multiple tasks in odor discrimination with sampling time differences varying by task category (GNG/TAC), and (3) mismatches between the time-of-day of training and subsequent testing strikingly impair performance of both sexes in a common learning paradigm. It is unlikely that any modulation of performance or variability caused by the estrous cycle exceeds that which already exists in non-cycling males. Proper attention should be paid to balancing and reporting time-of-day of behavioral training and testing.

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Poster

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Topic: D.03. The Chemical Senses

Support: University of Chicago Social Sciences Division and Institute for Mind and Biology Seed Grant

Title: Oscillations in the rat olfactory system reflect cognitive load in discrimination tasks of varying demand

Authors: *H. LI[1,2,3], A. STUART[1,3], J. ZENG[4], N. BECTON[4], E. BELL[4], L. M. KAY[5,3,6]; [1]The Univ. of Chicago, Chicago, IL; [2]Department of Psychology, University of Chicago, Chicago, IL; [3]Institute for Mind and Biology, Chicago, IL; [4]The Col., The Univ. of Chicago, Chicago, IL; [5]Dept. of Psychology, Univ. of Chicago, Chicago, IL; [6]The College, The University of Chicago, Chicago, IL

Abstract: The rat olfactory system is a good model for investigating how sensory and cognitive elements influence learning. Rat Olfactory Bulb (OB) gamma (35-110 Hz) band oscillations of the Local Field Potential (LFP) are elevated during odor sampling in similar-odor (fine) discrimination. Moreover, blocking gamma oscillations in the OB disrupts fine odor discrimination for rats, mice, and honeybees, but not coarse discrimination. We test here the role of some of the cognitive elements driving increased gamma power during odor discrimination tasks. Based on previous results, we hypothesized that cognitive load influences gamma elevation in the OB and that rats can adjust gamma depending on cognitive context. To manipulate cognitive load, we used a variation of a Two-Alternative Choice (TAC) protocol for rats. We achieve higher cognitive load through 1) an increased number of stimuli, some of which are very similar, and 2) a harder task with a low level of predictability (high load, uninformative cues) compared to an easier task with a higher level of predictability (low load using informative context cues telling rats whether a fine or coarse discrimination odor was coming). Male and female rats were implanted with bipolar electrodes in the left OB, anterior piriform cortex, and dorsal hippocampus (dentate gyrus and CA1). We recorded LFPs from these areas while the rats performed the TAC task with informative or non-informative cues. We show that rats can successfully learn and perform this difficult variation of the TAC task. When provided with an informative context cue, rats change their strategies in performing the discrimination task, showing longer sampling times and slightly lower accuracy in overall performance; these rats can manipulate the power of gamma oscillations on a trial-by-trial basis to support fine or coarse odor discrimination. Rats provided with non-informative cues show elevated gamma oscillations throughout and perform slightly better at the less predictable task, with a shorter sampling time. Our results show that gamma elevation in the rat OB reflects better behavioral performance, which can be achieved with different cognitive strategies given the demand of tasks.

Disclosures: H. Li: None. **A. Stuart:** None. **J. Zeng:** None. **N. Becton:** None. **E. Bell:** None. **L.M. Kay:** None.

Poster

PSTR278: Olfaction: From Physiology to Behavior

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Program #/Poster #: PSTR278.19/D39

Topic: D.03. The Chemical Senses

Support: R01DC019405-01A1
NSF GRFP

Title: Olfactory predictive coding under uncertainty

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Abstract: The natural odors we encounter every day are noisy. They comprise complex, volatile mixtures that are spatiotemporally dynamic, face interference from other stimuli, and appear across disparate contexts. Adaptive olfactory behavior thus requires accounting for the noise that arises from these sources of variability. However, we know little about how the olfactory system manages sensory noise to support robust perception. We hypothesize that olfactory noise is regulated in a manner consistent with predictive coding in which sensory information is weighted according to its precision: precise information is amplified, and noisy information is suppressed. Thus, precise olfactory information is prioritized for use in downstream perceptual decisions whereas noisy information is ignored. Neurobiologically, predictive coding hypothesizes that this amplification and suppression is mediated by neuromodulatory centers in the basal forebrain and brainstem that disinhibit (amplify) olfactory neurons carrying precise information and inhibit (suppress) olfactory neurons carrying noisy information. To test these hypotheses, we developed a paradigm under 7T fMRI in which participants learned predictive associations between shapes (circle or square) and odors (pine- or banana-dominant mixtures). Critically, we manipulated both the noisiness of these associations and the noisiness of the odor mixtures, allowing us to examine multiple sources of olfactory noise. With computational modelling, we discovered that participants down-weighted noisy odor information when forming the shape-odor associations. Additionally, analyses of the fMRI data revealed that blood-oxygenation-level-dependent activity in the brainstem and basal forebrain were parametrically modulated by the level of olfactory noise. All together, these results are consistent with our hypotheses that neuromodulation of the olfactory system works to amplify precise information and suppress noisy information in the service of robust olfactory behavior.

Disclosures: S. Lyons: None. **J.A. Gottfried:** None.

Poster

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Program #/Poster #: PSTR278.20/D40

Topic: D.03. The Chemical Senses

Title: Human piriform cortex differentiates odors by identity, valence, and edibility

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Abstract: When a new odor wafts its way into our awareness, how long does it take to recognize? To decide that we like it? Or whether we want to eat it? And how is this evolving olfactory experience reflected in our brain activity? Here, we paired an odor feature rating task with high temporal resolution intracranial recordings to investigate the neural signatures of human olfactory perception. On each trial, participants evaluated a real-world odor (e.g., cheese, dirt, lemon, shampoo) on one of three dimensions: (i) pleasantness, (ii) edibility, or (iii) identity. Participants' ratings reliably differentiated pleasant and unpleasant odors as well as edible and inedible odors. Participants also overwhelmingly endorsed the true label of an odor versus an incorrect foil label. To compare behavior with odor-related brain activity, local field potentials were simultaneously recorded via surgically implanted EEG electrodes (placed as part of treatment for intractable epilepsy). Electrodes in piriform cortex were selected for analysis. We saw pronounced odor-evoked increases in theta band power (in piriform, but not control regions). A set of support vector machine classifiers were trained to decode neural responses based on odor identity, valence, or edibility. Early results demonstrate above-chance decoding on all three dimensions. Future analyses will reveal the nuances of the odor code as it evolves over time.

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Poster

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Title: Investigating the neural and physiological correlates of infant olfaction

Authors: *L. K. SHANAHAN[1], L. B. MITHAL[2], M. MESSINA[3], E. OFFICE[4], S. OTERO[4], P. C. SEED[2], T. KAHNT[5];
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Abstract: Odor perception serves a crucial role early on in human development. For example, newborns already exhibit a preference for their mother's scent, and are soothed by the scent of maternal breastmilk. Despite the ecological relevance of such cues, the neural mechanisms underlying infant olfaction are not well understood. A couple initial functional magnetic resonance imaging (fMRI) studies suggest that odors activate piriform cortex and nearby olfactory brain areas in infants, but more work is needed to further establish these findings, characterize neural responses to specific odors, and incorporate physiological measurements. Here, we presented odors to one-month-old infants (n = 12) while they slept, and we collected nasal airflow and fMRI data during odor exposure. Olfactory stimuli included two appetitive scents (isoamyl acetate and ethyl hexanoate) and two aversive scents (isovaleric acid and cyclopentanethiol). Analysis of fMRI data indicates robust odor-evoked activity in bilateral piriform cortex and thalamus. Interestingly, in piriform cortex, fMRI response magnitude varied significantly across the four odors. Moreover, analysis of nasal airflow data suggests that infants may modulate their inhales based on odor valence, with larger inhales in response to appetitive as compared to aversive odors. Taken together, our findings show that young infants already show strong neural responses to a range of olfactory stimuli, even during sleep, and suggest that nasal airflow may be a viable behavioral metric to assess odor preference in early development.

Disclosures: L.K. Shanahan: None. **T. Kahnt:** None.

Poster

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Topic: D.03. The Chemical Senses

Support: NIH Grant R01EB031080
NIH Grant R01DC019405

Title: Imaging Neural Activity in Human Olfactory Bulb Using an Alternative Functional MRI Method

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Abstract: The olfactory system plays an important role in our daily lives, not only dictating what we smell but also intertwining with emotion, memory, and learning. Anatomically, the olfactory bulb (OB) serves as the entry point to the olfactory cortices, gathering odor information from the olfactory epithelium, and relaying it to higher-order circuits. Despite extensive research in rodent and non-human primate models, our understanding of OB function in humans remains limited due to constraints in imaging techniques.

Functional magnetic resonance imaging (fMRI) using conventional gradient-echo echo-planar imaging (GE-EPI) is a common and powerful method for studying human olfaction. It indirectly detects neural activity by measuring blood-oxygenation-level-dependent (BOLD) activity. However, imaging olfactory-related regions, particularly the OB, poses challenges due to their proximity to the air and tissue interface of ethmoid sinuses, leading to high susceptibility. This unique positioning creates significant signal dropout. The small size of the OB exacerbates this issue, making detection nearly impossible with this method.

Arterial Spin Labeling (ASL) emerges as a promising alternative for fMRI. Unlike conventional BOLD-based fMRI, it directly measures neurovascular coupling by quantifying blood perfusion using magnetically labeled arterial blood water protons, acting as an endogenous tracer. Moreover, ASL shows superior functional sensitivity in regions with low signal-to-noise ratio and high susceptibility, making it ideal for studying the OB and other olfactory-related areas.

In this pilot study, we developed and optimized an ASL protocol specifically tailored for detecting perfusion signals within the OB region. We measured and presented the first report of resting OB blood flow in humans. To examine the OB neural activation patterns during odor delivery, we measured the perfusion signals in a block design experiment and demonstrated a correlation between the signals and odor stimulation. Our findings underscore the feasibility of utilizing ASL to explore neural dynamics in human olfactory-related regions, including the OB and the orbitofrontal cortex, where BOLD fMRI faces challenges, advancing our understanding of the human olfactory system and its underlying mechanisms.

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Poster

PSTR278: Olfaction: From Physiology to Behavior

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Program #/Poster #: PSTR278.23/D43

Topic: D.03. The Chemical Senses

Title: Theta intertrial coherence as a marker of olfactory discrimination ability: Insights from scalp EEG analysis

Authors: *Y. MURAOKA, A. NAKANE, S. SHIMIZU;
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Abstract: Recent advances in brain imaging techniques have greatly contributed to our understanding of olfactory information processing in the human brain. Among these techniques, electroencephalogram (EEG) has been used as a valuable tool due to its high-time resolution, revealing intricate temporal dynamics. However, the modulation of EEG signals in the time-frequency domain and their relationship with olfactory abilities remain unclear. To address this gap, we conducted a comprehensive time-frequency analysis and examined the associations between olfactory abilities and EEG features. We used the previous research dataset (Kato et al. 2022, N = 22) and our dataset (N = 12). These datasets included scalp EEG recordings during several kinds of olfactory stimulations and subjective assessments of the odors used. Additionally, our dataset included olfactory discrimination abilities assessed via the sniffin' discrimination test. Time-frequency analysis of EEG features was conducted using wavelet transform, with event-related spectral perturbations (ERSP) and intertrial coherence (ITC) employed for further analysis. Furthermore, Spearman's rank correlation coefficient was calculated to explore the relationship between individual discrimination abilities and EEG features. Source estimation results obtained through brainstorm3 were also subjected to time-frequency analysis to identify brain regions associated with olfactory abilities. Time-frequency analysis revealed that ERSP and ITC in theta band (4-7 Hz) increase significantly after olfactory stimulation onset in both datasets (Kato et al. dataset: ERSP = 268.8±58.4%, $p < 0.001$, ITC = 0.085±0.038, $p < 0.001$; ERSP = 66.2±17.1%, $p < 0.001$, ITC = 0.10±0.03, $p = 0.004$). Furthermore, theta ITC change after stimulation negatively correlated with olfactory discrimination (rho = 0.71, $p = 0.01$). This correlation was not observed with ERSP (rho = -0.30, $p = 0.34$). Source estimation revealed that ITCs negatively correlated with discrimination ability were localized to the orbitofrontal cortex and left temporal gyrus. These findings show a link between ITC and olfactory discrimination ability, suggesting that individuals with higher discrimination abilities may exhibit distinct neural activity patterns in response to different stimuli. This finding indicates the potential utility of ITC in using neurofeedback to enhance olfactory perception.

Disclosures: Y. Muraoka: A. Employment/Salary (full or part-time):; Nippon Telegraph and Telephone Corporation. **A. Nakane:** A. Employment/Salary (full or part-time):; Nippon Telegraph and Telephone Corporation. **S. Shimizu:** A. Employment/Salary (full or part-time):; Nippon Telegraph and Telephone Corporation.

Poster

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Topic: D.03. The Chemical Senses

Support: R01 DC 018539
R01 DC 018539

Title: Neural basis of sniff response modulation in humans based on odor perceptual information

Authors: *V. SAGAR[1], G. ZHOU[2], G. LANE[1], T. KAHNT[3], C. ZELANO[4];
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Abstract: Olfactory perception is intimately associated with sniffing. Sniffing is widely considered to be an important aspect of the odor perception, eliciting the activity in the olfactory cortex. However, precisely how odor perceptual information modulates sniff responses and the precise neural mechanisms underlying this modulation are less understood. In this study, we analyzed fMRI data from subjects sniffing 160 different odorants over 4320 trials. Our findings show that sniffing patterns vary among perceptually distinct odors and that sniffing could be used to decode the identity of the odorant. We employed representational similarity analysis, revealing that similarities in sniffing patterns correspond to the perceptual qualities of odors. We examined the BOLD responses in the major olfactory areas and found that a subset of sniff-responsive voxels in the amygdala are consistently modulated by percept-related sniffing information in each participant. Our research also highlights temporal disparities between odor perception and sniffing patterns, suggesting that there is an optimal time window during a sniff that can be used to decode information about odor identity. These findings suggest a percept-related neural system of modulating one's breathing in response to chemical stimuli.

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Poster

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Topic: D.03. The Chemical Senses

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NIH grant T32 NS047987

Title: Context Dependent Odor Processing in the Human Brain

Authors: *Q. YANG[1], G. ZHOU[2], V. SAGAR[2], G. LANE[2], N. L. ANDERSON[3], R. BRAGA[2], C. ZELANO[2];
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Abstract: Context-dependent flexibility in odor object coding is a fundamental requirement of human odor processing. In nature, odor objects consist of complex mixtures made up of numerous chemicals, and the same chemicals are often present in a number of different naturally occurring smells. This requires the human brain to analyze not just the chemical features, but also the context in which these stimuli are encountered. How appealing an odor is depends in part on its context. For example, a cheese-like smell from valeric acid may smell appealing when emanating from a food but not so much when from a person. Human primary olfactory cortex is comprised of multiple cortical regions, each receiving monosynaptic input from the olfactory bulb in parallel. These include the anterior olfactory nucleus, olfactory tubercle, piriform cortex, parts of amygdala (the medial amygdala, anterior cortical amygdala, and the pariamygdaloid complex), and entorhinal cortex. This parallel anatomical organization is unique among sensory systems—which typically display serial organization—and its importance is unknown. Recent work in humans has shown that different olfactory areas form distinct networks with the rest of the brain, suggesting that these areas may extract distinct information from identical odor stimuli, possibly depending on the context in which they were encountered. While we know that basic features of odor stimuli can be decoded from responses in many of these regions, their specific functions in processing odors in natural, ecological contexts is unknown. Here we aim to systematically probe the mechanisms underlying context dependent coding flexibility and odor-guided decisions in the human brain. Participants completed a context-dependent odor consideration task where an identical set of odor stimuli were presented under different contexts (food, person, location, and none) through naturalistic, engaging stories. Using high-resolution and high-precision functional neuroimaging, we are examining how responses to stories and odors change in olfactory cortical areas across contexts. Preliminary findings suggest that the medial amygdala shows increased activity in response to odors encountered in the context of a person, compared to identical odors presented in other contexts (permutation test, $z = 2.31$, $p = 0.01$). Representational similarity analysis also indicated that information about the person context is represented in the medial amygdala. Further analyses will probe effects of other contexts and other cortical areas, in order to elucidate how the human olfactory system guides context-dependent decision-making.

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Poster

PSTR279: Hair Cells and the Periphery

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Program #/Poster #: PSTR279.01/D46

Topic: D.05. Auditory and Vestibular Systems

Support: R01DC018566

Title: Genome-wide association study for age-related hearing loss in CFW mice

Authors: *O. POLESSKAYA[1], T. MISSFELDT SANCHES[1], R. CHENG[1], T. ZHOU[2], E. BOUSSATY[2], M. OKAMOTO[1], K.-M. NGUYEN[3], E. DU[2], O. LA MONTE[2], A. A. PALMER[1,4], R. FRIEDMAN[2];
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Abstract: Background. Age-related hearing loss (ARHL) is the most common cause of hearing loss and is one of the most prevalent conditions affecting the elderly globally. ARHL is influenced by environmental and genetic factors. The mouse and human inner ears are functionally and genetically homologous. Investigating the genetic basis of ARHL in an outbred mouse model will lead to a better understanding of the molecular mechanisms of this condition. The goal of this study was to identify genetic loci involved in regulating ARHL. We used Carworth Farms White (CFW) outbred mice, because (1) this strain has variation in the onset and severity of ARHL, and (2) they are an outbred population, which allows mapping complex traits to small genomic regions. Auditory Brainstem Response (ABR) was measured at a range of frequencies in 946 male and female CFW mice at the ages of 1, 6, and 10 months. Results. We obtained genotypes at 4,27 million single nucleotide polymorphisms (SNP) using low-coverage (mean coverage 0.27x) whole-genome sequencing followed by imputation using STITCH. To determine the accuracy of the genotypes we sequenced 8 samples at over 30x coverage and used them to estimate the discordance rate, which was 0.55%. We performed genetic analysis for the ABR thresholds for each frequency at each age, and for the time of onset of deafness for each frequency. The SNP heritability ranged from 0 to 42% for different traits. Genome-wide association analysis identified several regions associated with ARHL that contained potential candidate genes. This work is ongoing, our final target sample size is 2,000 CFW mice. Conclusion. We performed GWAS for ARHL in CFW outbred mice and identified several loci, containing multiple candidate genes. This work helps to identify genetic risk factors for ARHL and to define novel therapeutic targets for the treatment and prevention of ARHL.

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Poster

PSTR279: Hair Cells and the Periphery

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Title: Genetic and environmental contributions to age related hearing loss: Results from the VETSA longitudinal twin study

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Abstract: Over 430 million people worldwide experience disabling hearing loss. Hearing loss becomes more prevalent with age, affecting over 25% of adults older than 65. Hearing loss is known to arise from both genetic and environmental factors. Although the genetic component of hearing loss has been well established, our interest here is in how the genetic contributions to hearing loss change over time. In this study, we report the pure tone hearing thresholds across 250Hz, 500Hz, 1000Hz, 2000Hz, 4,000Hz, and 8,000Hz from over 1,000 male-male twins composed of monozygotic (MZ) and dizygotic (DZ) pairs sampled from the Vietnam Era Twin Study of Aging (VETSA). Twins were tested at an average age of 56 at wave one, at an average age of 62 at wave two, and at an average age of 68 at wave three. Multivariate ACE Cholesky models were used to calculate the percent of variance due to genetic and environmental contributions for each acoustic frequency and time point. A cross-lagged panel model was used to calculate the stability of the genetic contributions across time points. Latent variables were used to estimate components of phenotypic variance: additive genetic variance (A), which captures the cumulative effect of individual genes; common or shared environmental variance (C), which quantifies the environmental factors shared by individuals that contribute to similarities in traits; and unique environmental variance (E), accounting for the environmental influences unique to an individual. Audiograms revealed a typical sloping pattern characteristic of age-related hearing loss at each wave. There was a significant effect of wave on participants' hearing acuity, where hearing acuity was highest in wave one, lower in wave two, and lowest in wave three. For all acoustic frequencies tested, genetics accounted for over 40 percent of the variance in hearing acuity, and the relative contributions of genetics were stable across the three time points. Conversely, the change in hearing acuity over time was more attributable to unique (person-specific) environmental factors than to genetics. These results replicate previous findings that genetic influences are important in determining hearing acuity. The unique contribution of

the present analysis is the demonstration that the contribution of genetic factors to hearing acuity remains consistent over 15 years during this mid- to later-life period. Taken together, these data demonstrate that environmental factors play a larger role in individuals' change in hearing over time, while the level of genetic determinants of hearing acuity remains stable as individuals age.

Disclosures: R.M. O'Leary: None. **A. Wingfield:** None. **M.J. Lyons:** None. **C.E. Franz:** None. **W.S. Kremen:** None.

Poster

PSTR279: Hair Cells and the Periphery

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR279.03/D48

Topic: D.05. Auditory and Vestibular Systems

Support: ICMR-51/02/2022-ANA/BMS

Title: Morphology of the blood-nerve barrier in the developing and aging human cochlear nerve

Authors: *T. JACOB[1], T. ROY[2];
[1]Anat., All India Inst. of Med. Sci., New Delhi, India; [2]Anat., North DMC Med. Col. and Hindu Rao Hosp., New Delhi, India

Abstract: Hearing in humans is a complex neural phenomenon that also forms the basis of speech and language. The development of its parts and aging in them affect psychosocial aspects of human life. The human cochlear nerve carries sound impulses that have been transformed into electrical nerve impulses in the cochlea to the cochlear nuclear complex in the brainstem. The nerve is affected by developmental defects and aging. The blood-nerve-barrier is formed by tight junctions between capillary endothelial cells and foot processes of glial cells like the astroglia and Schwann cells. This barrier prevents toxins and other systemic factors from affecting nerve health and impulse conduction. Here, after obtaining ethical clearance, we studied the elements of the blood-nerve barrier in 8 fetal, and 15 adult specimens of the human cochlear nerve by using transmission electron microscopy (Talos-S, Thermo Fisher Scientific). We identified the central and peripheral parts of the cochlear nerve by the difference between oligodendroglia and Schwann cells. Glial cells formed processes that covered the capillaries intimately. The endothelial cells of the capillaries were connected to each other by tight junctions and they shared their basement membrane with pericytes. In the older specimens, we were able to identify more inclusion bodies, autophagosomes containing lamellar bodies, increased fibrillar content and more heterochromatic nuclei, when compared to those specimens that were derived from younger individuals. The number of tight junctions were also fewer. Hence, with increasing age, there are morphological features indicative of disruption of the blood-nerve barrier. This disruption may be responsible for the decreased hearing acuity that occurs with increasing age.

Disclosures: T. Jacob: None. **T. Roy:** None.

Poster

PSTR279: Hair Cells and the Periphery

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR279.04/D49

Topic: D.05. Auditory and Vestibular Systems

Title: Longitudinal Assessment of Age-Related Hearing Loss in C57BL/6J and 129X/SVJ Mice: Insights from Distortion Product Otoacoustic Emissions and Auditory Brainstem Response

Authors: N. BROWN, G. RICHARD, C. GATEWOOD, T. AUGUSTO, J. WILLETTE, J. WHITE, ***H. JIN**;
Jackson Lab., Bar Harbor, ME

Abstract: C57BL/6 mice are frequently employed as control subjects in studies focusing on hearing loss; however, there exists a paucity of literature concerning the longitudinal assessment of their auditory function. Given the imperative of longitudinal investigations for elucidating age-related hearing loss and the impacts of diverse interventions, our study aimed to monitor age-related hearing loss in C57BL/6J mice (n=10 each for males and females), alongside 129X/SVJ mice (n=10 each for males and females). The mice were assessed at intervals of 7, 10, 15, 25, 30, 40, 52,80 weeks of age. We conducted Distortion Product Otoacoustic Emission (DPOAE) testing with 13-step F1/F2 frequency combinations, encompassing F2 values ranging from 1kHz to 32 kHz, and Auditory Brainstem Response (ABR) testing, utilizing tone bursts at 6, 12, 18, 24, and 30 kHz, as well as clicks. Our study revealed age-dependent decreases in cSNR (Corrected Signal to Noise Ratio) as measured by DPOAE in C57BL/6J mice, with significant differences emerging as early as 40 weeks of age compared to 10 weeks at F2 frequencies of 8.8 kHz, 12.5 kHz, and 19.3 kHz, respectively ($P<0.05$). Conversely, ABR test thresholds, encompassing both tone burst and click stimuli, remained within normal ranges up to the 52-week time point. However, by 52 weeks, ABR thresholds at all test frequencies began to surpass the normal range, indicating progressive hearing loss. By 80 weeks, all mice exhibited deafness with no ABR response, suggesting severe hearing impairment. Meanwhile, both DPOAE and ABR data demonstrated significant hearing loss as early as 10 weeks of age in 129X/SVJ mice. This finding is consistent with the known susceptibility of SVJ mice to early hearing deficits, attributed to the "age-related hearing loss 1" mutation of cadherin 23, which confers hearing deficits from a young age and eventual hearing loss. Notably, we did not find gender differences among these two strains. Our findings suggest that age-dependent hearing loss in C57BL/6 mice commences from outer hair cell dysfunction in the cochlea as early as 40 weeks of age. This study represents the first longitudinal examination of hearing loss in C57BL/6

mice employing DPOAE and ABR measurements, offering a valuable reference for future investigations into murine models of hearing loss.

Disclosures: N. Brown: None. **G. Richard:** None. **C. Gatewood:** None. **T. Augusto:** None. **J. Willette:** None. **J. White:** None. **H. Jin:** None.

Poster

PSTR279: Hair Cells and the Periphery

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR279.05/D50

Topic: D.05. Auditory and Vestibular Systems

Support: NIH/NIDCD Grant DC015790
NIH/NIDCD Grant DC017722

Title: Immune response in the spiral ganglion following cochlear hair cell loss

Authors: *A. M. CARO[1], S. H. GREEN[2];
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Abstract: Spiral ganglion neurons (SGNs) of the cochlea slowly die after neonatal aminoglycoside-induced hair cell loss in rats, resulting in the death of >80% of all SGNs over the course of ~14 weeks. Treatment with anti-inflammatory agents rescues SGNs after hair cell loss, implicating an inflammatory/immune response as a cause of death. Hair cells were ablated in Sprague Dawley (SD) rats by daily injection of the aminoglycoside kanamycin from postnatal day 8 (P8) to P16, resulting in ablation of all cochlear hair cells by P19. Macrophages increase in abundance and activation by P21, well prior to the start of significant SGN death, which is first apparent at P39. Concurrent with the start of SGN death, there is an increase in lymphocytes in the ganglion, including CD4+ helper T cells, CD8+ cytotoxic T cells, and CD161+ NK cells. To determine whether the lymphocyte component of this inflammatory response is causal to SGN death, we evaluated SGN survival after hair cell loss in two immunodeficient rat strains: 1) RNU nude rats lacking T cells (Crl:NIH-Foxn1^{rnu}) and 2) SRG rats lacking T, B, and NK cells (Sprague Dawley-Rag2^{em2hera}Il2rg^{em1hera}/HblCrl). Heterozygous RNU+ rats with a normal T cell compartment (Crl:NIH-Foxn1$^{rnu/+}$) and immunocompetent Sprague Dawley (SD) rats were used as controls. Kanamycin-treated and untreated hearing control rats from each strain/genotype were euthanized at P70 and cochlea were frozen and sectioned for immunofluorescence to detect hair cells (myosin 6/7a), neurons (β3-tubulin), leukocytes (CD45), macrophages (IBA1), T cells (CD4, CD8), B cells (CD19), and NK cells (CD161). We found that SGN survival in RNU nude rats is not significantly different than in RNU+ rats after hair cell ablation, indicating that T cells are not required for SGN death. Similarly, preliminary data suggest that genetic ablation of T, B, and NK cells in the SRG rats does not prevent SGN death. These data indicate that lymphocytes

are not required for SGN death after kanamycin-induced hair cell loss. We also found that macrophage number is significantly increased in the kanamycin treated groups compared to hearing controls for all strains evaluated. Therefore, we hypothesize that macrophages are responsible for SGN death and will test this hypothesis using a macrophage depletion model in kanamycin treated SD rats.

Disclosures: A.M. Caro: None. **S.H. Green:** None.

Poster

PSTR279: Hair Cells and the Periphery

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR279.06/D51

Topic: D.05. Auditory and Vestibular Systems

Support: NIH R15 DC018935

Title: Elevated macrophage density in the spiral ganglion promotes hearing loss and synaptopathy

Authors: *K. SLEIMAN[1], L. K. CLIMER[2], A. REPTAK[1], A. HORNAK[1], W. SESE[1], R. STOFFEL[3], D. D. SIMMONS[1];
[1]Biol., Baylor Univ., Waco, TX; [2]St. Jude Children's Res. Hosp., Memphis, TN; [3]Rice Univ., Houston, TX

Abstract: Acquired sensorineural hearing loss can be due to aging, infection, noise exposure, or ototoxic drugs and is accompanied by increased cochlear inflammation. Sensorineural hearing loss is associated with loss of cochlear hair cells, spiral ganglion (SG) neurons, or the synapses between the two. However, it is not understood how the cochlear immune system is involved. The SG is rich in fractalkine, the primary ligand for CX3CR1-expressing macrophages. It is unknown whether macrophages residing in the SG serve a primarily neuroprotective or neuropathic role. We hypothesize that the initial, resting density of SG-resident macrophages determines the extent of hearing loss and synaptic loss. We have generated a mouse model with progressive, accelerated hearing loss via genetic deletion of the calcium binding protein oncomodulin (OCM KO). Semi-quantitative analysis of the immune marker CD45 and the macrophage and microglia specific marker IBA-1 using immunofluorescence indicates that OCM KO animals have higher levels of inflammation in the SG compared to wildtype mice. Despite this heightened inflammation, their hearing thresholds are comparable. Hearing loss and cochlear inflammation have also been reported after systemic treatment with lipopolysaccharide (LPS). To trigger an acute inflammatory response, OCM KO and wildtype mice were treated intraperitoneally with LPS. Distortion product otoacoustic emissions (DPOAEs) and auditory brainstem responses (ABRs) were recorded before treatment and 1-2 days after treatment. In

general, LPS treatment elevated hearing thresholds for both OCM KO and wildtype mice. However, threshold shifts for OCM KO mice were greater than for wildtype mice. LPS treatment also altered synaptic density below inner hair cells, and increased immune cell counts in Rosenthal's canal. While wildtype mice exhibited extensive SG inflammation after LPS treatment, macrophage density in the SG of OCM KO mice was unaffected by LPS treatment. This differential effect of LPS treatment on the inflammatory status of the ganglion suggests that the initial density of immune cells prior to an acute stimulus influences the extent of synaptic loss and auditory threshold shifts.

Disclosures: K. Sleiman: None. **L.K. Climer:** None. **A. Reptak:** None. **A. Hornak:** None. **W. Sese:** None. **R. Stoffel:** None. **D.D. Simmons:** F. Consulting Fees (e.g., advisory boards); Board of Scientific Counselors, NIDCD.

Poster

PSTR279: Hair Cells and the Periphery

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR279.07/D52

Topic: D.05. Auditory and Vestibular Systems

Support: NIH R01DC01379801A1
VAI01RX003532

Title: Exploring the link between blast-induced hearing loss and the progression of Alzheimer's disease

Authors: *R. SANGALETTI[1], W. M. WALTERS[2], S. WILLIAMS[3], S. RAJGURU[4], N. KERR[5];
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Abstract: Exposure to hazardous noise causes irreversible injury to the structures of the inner ear, leading to changes in hearing and balance function with strong links to age-related cognitive impairment. While the role of noise-induced hearing loss in long-term health consequences, such as progression or development of Alzheimer's Disease (AD) has been suggested, the underlying mechanisms and behavioral and cognitive outcomes or therapeutic solutions to mitigate these changes remain understudied. The goal of this study is to characterize the association between blast exposure, hearing loss, and the progression of AD pathology, and determine the underlying mechanisms. To this aim we acquired wild-type (WT) and 3xTg-AD mice, a well-established experimental model of AD pathology. Mice of 4 months of age were randomly assigned at the

beginning of the study to no blast or blast-exposed groups. Blast injury was carried out in an ecologically valid oxyacetylene gas tube. Functional outcomes and cognitive, affective and anxiety deficits were assessed at different time points using auditory functional tests (auditory brainstem response and cervical vestibular evoked myogenic potential) and behavioral assessments (novel object recognition, water maze, open field) over 3 months. In addition, we performed western-blot and immunohistochemistry analysis to examine expression levels of amyloid beta (Aβ), Tau, and inflammasome proteins in the peripheral auditory system and multiple brain regions including auditory cortex and brainstem after blast exposure. We found that blast injury led to auditory sensorineural cell loss and consequent combination of temporary and permanent hearing and balance impairment. Behavioral analysis revealed that both WT- and 3xTg-blast mice as well as 3xTg sham animals had challenges with the novel object recognition. In open field, 3xTg blast mice exhibited significant increases in fecal boli when compared to WT mice while 3xTg animals (sham and blasted) showed a decrease in overall mean speed, total distance traveled and mobility while time spent at the center of the arena and frequency zone transition center-border increased noticeably. In addition, Aβ, Tau and inflammasome proteins were elevated in cochlea as well as brainstem and cortex of 3xTg mice suggesting an involvement of pyroptosis related mechanisms in the impact of hearing loss on the onset and progression of cognitive decline in AD pathology. Our results suggest a strong correlation between early hearing loss and progression of AD pathology. Preventive and therapeutic strategies aimed at attenuating hearing loss could be beneficial in delaying the onset of Alzheimer's disease.

Disclosures: R. Sangaletti: None. **W.M. Walters:** None. **S. Williams:** None. **S. Rajguru:** None. **N. Kerr:** None.

Poster

PSTR279: Hair Cells and the Periphery

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR279.08/D53

Topic: D.05. Auditory and Vestibular Systems

Support: NIH R01DC01379801A1

Title: Auditory and Vestibular Consequences of Mild Traumatic Brain Injury

Authors: *F. RACITI[1], N. KERR[2], S. RAJGURU[3];
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Abstract: Traumatic brain injury (TBI) represents a public health challenge, with significant and enduring consequences for individuals and communities. While the immediate effects of TBI are

widely acknowledged, its long-term impact extends beyond cognitive and motor functions to encompass a diverse range of sensory dysfunctions often overlooked in clinical assessments. Among these less-explored yet clinically significant outcomes are hearing and balance impairments. The CDC estimates that auditory and vestibular loss affects 8 to 67% of TBI patients, with prevalence largely contingent upon injury severity. In cases of moderate to severe TBI, auditory and vestibular losses typically result from conductive or mixed mechanisms, often secondary to temporal bone fractures. In mild TBI these dysfunctions primarily manifest as sensorineural, stemming from transient or permanent damage to hair cells and the associated neural pathways. Despite hearing and balance impairments being common post-concussive symptoms, there's limited understanding on the temporal dynamics and the pathophysiology of these phenomena. In this work we characterize relevant short- and long-term changes in auditory and vestibular activity in an ecologically valid mTBI model (n=6 male Brown Norway rats, 14-16 weeks). The closed head injury was induced using the accelerated weight drop system, simulating a single controlled weight drop (450g at 1 meter). Threshold values of auditory brainstem responses (ABRs) and cervical vestibular myogenic potentials (cVEMPs) evoked by pure tone bursts at different frequencies (ABR 2, 4, 8, 16, 24, 32 kHz cVEMP 1, 8 kHz) were evaluated prior the injury and monitored up to 28 days post-concussion. Previous studies in mTBI rat models revealed complete recovery of their cognitive and motor functions within three days from the injury. Here we observe the same recovery timeframe for post-concussion changes of cVEMP and ABR responses to low and high frequency stimuli respectively. In contrast, changes in ABRs evoked by low frequency stimuli remain significant until 14 days after mTBI. Notably, mild head trauma does not affect cVEMP responses to 8 kHz stimuli. Overall, these results show the complex dynamics of mTBI-induced auditory and vestibular dysfunctions highlighting the importance of considering sensory outcomes alongside cognitive and motor assessments in TBI research and clinical practice. The detailed characterization of auditory and vestibular functional outcomes in the established model will help delving deeper into the molecular mechanisms driving these sensory dysfunctions, ultimately improving therapeutic interventions for TBI patients.

Disclosures: F. Raciti: None. **N. Kerr:** None. **S. Rajguru:** None.

Poster

PSTR279: Hair Cells and the Periphery

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR279.09/D54

Topic: D.05. Auditory and Vestibular Systems

Title: An AAV-based cochlear hair cell targeted antioxidative gene therapy that prevents and treats oxidative damage induced hearing loss.

Authors: S. CHANDRASEKAR, P. WACKYM, *T. M. MOWERY;
Rutgers Univ., Piscataway, NJ

Abstract: BACKGROUND: Hearing loss affects nearly half a billion people worldwide, with the leading cause in young and aging adults being oxidative damage through recreational and occupational noise exposure or ototoxic medicines. In all of these cases, increased reactive oxygen species leads to oxidative damage of the inner and outer hair cells of the cochlea. When enough oxidative damage has accumulated, the hair cells die; however, it is thought that antioxidative treatments might prevent neuropathy. Currently there are no FDA approved drugs or therapies to prevent or treat noise or ototoxic induced sensorineural hearing loss. Therefore, we have developed an adeno-associated virus (AAV) gene therapy that protects these cells from oxidative damage and death. The therapy is based on the COREHYPOTHESIS that increasing the bioavailability of the antioxidative superoxide dismutase in the cochlear hair cells will prevent oxidative damage associated with noise and ototoxic exposure.
METHODS: Adult (P86) Mongolian gerbils (*Meriones Unguiculatus*)received baseline auditory brainstem response recordings and/or c+VEMPs followed by intra cisterna magna injections of either saline or our AAV SOD1 (intracellular), SOD2 (mitochondrial), or SOD3(extracellular) gene therapy. After three weeks of trans gene expression, animals were exposed to 1) one week of noise exposure (110dB SPL 2hrs/day) to moderately damaging broadband noise 2) three cycles of cisplatin treatment (IP, 3mg/kg) or 3) a single round window application of gentamicin or kanamycin (1 ml, 500 mg/ml). Post exposure ABRs and/ or c+VEMPs were recorded for 1-9 weeks (depending on exposure protocol) to track the progression and neuroprotection of hearing loss.
RESULTS: Animals that received the SOD gene therapy showed significant protection from noise and ototoxic induced hearing loss compared to the control group that developed moderate to severe hearing loss and lowered vestibular function.
CONCLUSIONS: These data show that increasing the bioavailability of natural cellular antioxidants in the inner and outer hair cells of the cochlea with AAV delivered SOD transgenes will protect auditory function.

Disclosures: S. Chandrasekar: None. **P. Wackym:** None. **T.M. Mowery:** None.

Poster

PSTR279: Hair Cells and the Periphery

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR279.10/D55

Topic: D.05. Auditory and Vestibular Systems

Support: Ministry of University and Research (MUR), National Recovery and Resilience Plan 40 (NRRP), project MNESYS (PE0000006)

Title: Early transtympanic administration of rhBDNF exerts a multifaced neuroprotective effect against cisplatin-induced hearing loss

Authors: *A. PISANI[1], F. PACIELLO[2], V. MOHAMED HIZAM[3], R. MONTUORO[3], C. GRASSI[2], A. R. FETONI[1];
[1]Univ. degli Studi di Napoli Federico II, Napoli, Italy; [2]Dept. of Neurosci., Catholic Univ. Sch. of Med., Rome, Italy; [3]Catholic Univ. Sch. of Med., Rome, Italy

Abstract: Early transtympanic administration of rhBDNF exerts a multifaceted neuroprotective effect against cisplatin-induced hearing loss
Anna Pisani[1], Fabiola Paciello[2],Veronica Mohamed Hizam[3], Raffaele Montuoro[3], Rolando Rolesi[3], Laura Brandolini[4], Cristina Giorgio[4], Andrea Aramini[4], Claudio Grassi[2], Anna Rita Fetoni[1]
[1]Department of Neuroscience, Unit of Audiology, Università degli Studi di Napoli Federico II, Naples, Italy;[2]Department of Neuroscience, Università Cattolica del Sacro Cuore, Rome, Italy;[3]Department of Head and Neck Surgery, Università Cattolica del Sacro Cuore, Rome 00168, Italy; [4]R&D, Dompé Farmaceutici S.p.A. - L'Aquila, Italy.
Cisplatin-induced sensorineural hearing loss is a clinical challenge and, currently, only one drug has been approved by Food and Drug Administration as an effective treatment. Thus, several efforts are needed to better understand cisplatin mechanism of damage and to explore new therapeutic strategies. Although the potential effects of brain-derived neurotrophic factor (BDNF) have previously been investigated in some ototoxicity models, its efficacy in cisplatin-induced hearing loss remains uncertain. This study aimed to investigate the therapeutic potential of recombinant human BDNF (rhBDNF) local delivery in counteracting cochlear damage in an in *in vivo* model of cisplatin-induced ototoxicity. Thus, adult Wistar rats were treated with cisplatin (12 mg/kg, intraperitoneally injected) and, after one hour, they received 5 mg/kg of rhBDNF suspended in a thermogel by a transtympanic injection. Auditory brainstem responses were recorded to evaluate hearing function at 3 and 7 days after treatment. At the end of treatment, we performed morphological, immunofluorescence and molecular analyses to investigate the molecular mechanisms underlying the beneficial effects of our rhBDNF formulation. Our data showed that rhBDNF mitigates hearing loss in cisplatin-exposed rats by preserving synaptic connections in the cochlear epithelium and reducing hair cell and spiral ganglion neuron death. rhBDNF maintains the balance of its receptor levels (pTrkB and p75), boosting TrkB-CREB pro-survival signalling and reducing caspase 3-dependent apoptosis in the cochlea. Additionally, it activates antioxidant mechanisms while inhibiting inflammation and promoting vascular repair. Overall, our study demonstrates that the early transtympanic treatment with rhBDNF plays a multifaceted protective role against cisplatin-induced ototoxicity, thus holding promise as a novel potential approach to preserve hearing in adult and pediatric patients undergoing cisplatin-based chemotherapy.

Disclosures: A. Pisani: None. **F. Paciello:** None. **V. Mohamed Hizam:** None. **R. montuoro:** None. **C. Grassi:** None. **A.R. Fetoni:** None.

Poster

PSTR279: Hair Cells and the Periphery

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR279.11/D56

Topic: D.05. Auditory and Vestibular Systems

Support: NIH Grant 2R15DC017866

Title: Exploring the Dynamics of Auditory Ribbon Synapse Development and Dysfunction using the mScarlet-I-RIBEYE Mouse Line

Authors: A. YEN[1], M. TRAN[1], G. LICWINKO[1], V. C. CHEN[2], C. NASIOS[1], C. GEBERT[1], *W.-M. YU[3,4];
[1]Biol., Loyola Univ. Chicago, Chicago, IL; [2]Engin. Sci., Loyola Univ. Chicago, Chicago, IL; [3]Loyola Univ. Chicago, Chicago, IL; [4]Biology, Loyola University Chicago, Chicago, IL

Abstract: Ribbon synapses, characterized by a presynaptic ribbon tethering a large number of vesicles in the active zone, play a crucial role in connecting auditory hair cells with bipolar spiral ganglion neurons in the cochlea. Despite our extensive knowledge of the physiological aspects of ribbon synapses, the mechanisms governing their development and degeneration in cochlear synaptopathy remain poorly understood. To address this gap, we created the mScarlet-I-RIBEYE mouse line by tagging the RIBEYE protein with a red fluorescent protein, mScarlet-I, in the synapse. To validate the utility of this mouse model, we confirmed red fluorescence in cochlear and retinal ribbon synapses. Moreover, these mice exhibit a normal number of auditory ribbon synapses and maintain normal hearing, affirming the structural and functional integrity of tagged synapses. Using the mScarlet-I-RIBEYE line, we plan to capture dynamic events during ribbon synapse development and investigate their degeneration after noise exposure. This research will advance our understanding of how ribbon synapses are compromised in cochlear synaptopathy.

Disclosures: A. Yen: None. **M. Tran:** None. **G. Licwinko:** None. **V.C. Chen:** None. **C. Nasios:** None. **C. Gebert:** None. **W. Yu:** None.

Poster

PSTR279: Hair Cells and the Periphery

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR279.12/D57

Topic: D.05. Auditory and Vestibular Systems

Support: NIH Grant 5R25NS080687-13

Title: Development of the hair cell synapse in the lateral line of the blind Mexican cavefish (Astyanax mexicanus)

Authors: *A. I. DUENO;
Biol., Univ. de Puerto Rico, Rio Piedras, San Juan, Puerto Rico

Abstract: Sensory system compensation is a process by which organisms that lose one or more sensory modalities activate morphological and functional expansions of other sensory systems for survival. A prime example of sensory compensation in nature is found in *Astyanax mexicanus*, a teleost fish species that exists as two morphotypes: sighted, river-dwelling surface fish, and blind, cave-dwelling populations of cavefish. While the mechanisms underlying eye regression in cavefish have been widely studied, whether these trigger compensatory mechanisms through constructive evolution of other non-visual sensory systems, like taste, olfaction, or mechanosensation, remains understudied. We propose to address how hair cell mediated mechanosensory compensation emerges in cavefish by examining development of the hair cell-containing lateral line system across different populations. Recent studies have shown increased neurotransmission in the cavefish lateral line that enhances mechanosensitivty, but it is not well understood whether these changes are a result of variations at the hair cell sensory synapse. To answer this, we performed immunohistochemistry on hair cells of the lateral line using antibodies against ribeye a/b and MAGUK that target pre- and post-synaptic densities at the hair cell. Our preliminary findings suggest that both morphotypes express the same synaptic components, and that these synaptic mechanisms are conserved in hyper-sensitive lateral line systems. Our future directions are to address whether there are changes in the amount, pattern, or distribution of synaptic proteins at the hair cell synapse in cavefish compared to sighted surface fish. This work will shed light onto the developmental and neural architecture underlying natural compensation between sensory systems in an emergent fish model for sensory neuroscience.

Disclosures: A.I. Dueno: None.

Poster

PSTR279: Hair Cells and the Periphery

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR279.13/D58

Topic: D.05. Auditory and Vestibular Systems

Title: Leveraging zebrafish to understand how Nrxn3 drives hair cell synapse assembly

Authors: *Y. TADESSE, K. PINTER, K. KINDT;
NIH/NIDCD, Bethesda, MD

Abstract: Ribbon synapses formed between hair cells and afferent neurons and are necessary for the transmission of sensory information to the central nervous system. The molecular mechanisms behind the ribbon synapse formation remain largely unknown. In recent research our group demonstrated that the long alpha form of the presynaptic adhesion molecule Neurexin 3 (Nrxn3) is required to form ~60% of the ribbon synapses in zebrafish hair cells. However, whether the shorter beta variant of Nrxn3 also impacts ribbon synapse formation is unclear. In addition, how Nrxn3 disrupts the dynamics of ribbon synapse formation is undefined.Using zebrafish genetics, we strive to characterize how different variants of Nrxn3 impact ribbon synapse formation. Currently, we are leveraging zebrafish genetics to create a pipeline to assess Nrxn3 variants in zebrafish by using the power of CRISPR-Cas-9 gene editing to generate F0 larvae with knockdown of Nrxn3 (Nrxn3 “crispants”). As proof of principle, we created F0 crispants that target the long alpha form of Nrxn3 to test whether the crispant phenotype was comparable to that of our germline Nrxn3 mutants. We then used immunohistochemistry to label both pre- and post- synapses to quantify the number of complete ribbon synapses in zebrafish hair cells. While our previous data has shown that germline Nrxn3 mutants have a 60% decrease in number ribbons synapses compared to wildtype animals, we found that Nrxn3 crispants have a robust, but milder 40% decrease in number ribbons synapses compared to wildtype animals. Our work demonstrates that CRISPR-Cas-9 can be used as an efficient tool to test Nrxn3 variants. We are currently assessing the shorter beta variant of Nrxn3 using this same approach. In the future we plan to also assess the role of more specific splice variants of alpha and beta Nrxn3.In addition, due to the access of hair cells in zebrafish and the transparency of larval zebrafish, we have begun to visualize the dynamics of ribbon synapse assembly through high-resolution live imaging. To image ribbon synapses we are using transgenic lines that label the presynapse (ribbon) and the postsynaptic density. Using these transgenic lines along with Airyscan imaging we are imaging ribbon synapses in developing hair cells in both wildtype and in our Nrxn3 crispants or germline mutants. Overall, understanding the molecular foundation of ribbon synapse formation is needed for the development of novel therapeutics to treat hearing loss caused by loss of hair cell synapse.

Disclosures: Y. Tadesse: None. **K. Pinter:** None. **K. Kindt:** None.

Poster

PSTR279: Hair Cells and the Periphery

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR279.14/D59

Topic: D.05. Auditory and Vestibular Systems

Title: Exploring sources of information in neural recordings using machine learning classifiers

Authors: A. MASHALKAR[1], D. ZHOU[1], N. G. SADEGHI[2], *S. SADEGHI[1];
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Abstract: The first step in the analysis of neural recordings involves tracking the timing and identity of firing neurons proximate to an electrode. Existing spike sorting algorithms, while effective, typically entail multi-step procedures and setting complex parameters by the user. Here, we introduce a novel approach for processing of unfiltered extracellular signals, enabling automatic detection of events and neuron identification without the need for user-defined parameters. Leveraging machine learning, we also demonstrate robust classification of inter-event intervals, conventionally regarded as 'noise', across various recordings within brief time frames of a few milliseconds. This suggests that temporal patterns in neural data contain information beyond mere spiking activity, potentially facilitating detection of fine modulations and categorization of neuronal recordings. Such 'noise' stems from diverse sources that could be neuronal (e.g., distinct neuron types, local/feedback inputs) or non-neuronal (e.g., electrodes, animal subjects, equipment noise). By contrasting recordings from analogous neuron groups under diverse experimental conditions, we explore potential contribution of these different sources. These methodologies offer a versatile approach for marking and classifying both events and inter-event 'noise'.

Disclosures: A. Mashalkar: None. **D. Zhou:** None. **N.G. Sadeghi:** None. **S. Sadeghi:** None.

Poster

PSTR279: Hair Cells and the Periphery

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR279.15/D60

Topic: D.05. Auditory and Vestibular Systems

Support: NIH R03 MH127401

Title: Gender specific dysfunction in the vestibular periphery in shank3 mouse model of autism

Authors: D. BALLINAS[1], Y. SHU[1], N. SHI[1], S. G. SADEGHI[2], *T. DEEMYAD[1];
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Abstract: Autism spectrum disorder (ASD) is associated with vestibular dysfunction, resulting in postural instability, gait dysfunction, and impaired gaze. As ASD is a neurodevelopmental condition, untreated vestibular dysfunction in children can lead to delayed milestones such as sitting and walking and poor motor coordination later in life. While abnormalities in central pathways, including the vestibular nuclei and the cerebellum have been linked to some of the pathologies of ASD, the function of the peripheral vestibular pathway has not been tested. However, there is evidence that peripheral dysfunction in other sensory systems following a

mutation in *shank3* could induce changes in central pathways and ASD-related phenotypes. Here, we investigated whether the peripheral vestibular pathway is affected in *shank3* KO mice by measuring vestibular sensory evoked potentials (VsEP) in response to rapid head movements. While VsEP responses did not differ between female *shank3* KO or heterozygote mice and WT mice, male *shank3* KO and heterozygote mice showed a 20% decrease in response compared to females. This gender-specific effect aligns with the higher prevalence of ASD and severity of phenotypes in males. VsEP relies on a unique non-quantal potassium-mediated transmission pathway, potentially linked to SHANK3 proteins' interaction with potassium channels like BK channels. Confirming this, we observed a similar decrease in VsEP amplitude in BK KO mice. Finally, we used 'contact righting reflex' to evaluate the effect of *shank3* mutation on quantal synaptic transmission between type II HCs and afferent terminals in these mice. This reflex relies on vestibular afferents responding to tonic stimuli, allowing a supine mouse to swiftly adjust to a prone position by detecting the gravity vector. We found that all WT and female *shank3* KO mice changed to the prone position in a few seconds (11 +/- 3 s, range: 2 – 20 s). In contrast, about half of male *shank3* KO mice remained in the prone position and walked between the two surfaces for more than 30 s (68 +/- 40 s, range: 30 – 370 s) before righting. This defect most likely results from a decrease in the amplitude of quantal synaptic events in *shank3* mice, as seen in other brain areas. These findings indicate a gender-specific dysfunction in the peripheral vestibular pathway in the *shank3* KO model of ASD, involving alterations in both quantal and non-quantal synaptic transmission. Given the significance of vestibular inputs, especially from the otolith organs, for cognitive development and spatial navigation, this dysfunction may contribute to balance, memory, and spatial navigation issues in individuals with ASD.

Disclosures: D. Ballinas: None. **Y. Shu:** None. **N. Shi:** None. **S.G. Sadeghi:** None. **T. Deemyad:** None.

Poster

PSTR279: Hair Cells and the Periphery

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Program #/Poster #: PSTR279.16/E1

Topic: D.05. Auditory and Vestibular Systems

Support: NIH Grant R01 DC019380 to SGS
NIH Grant R01 DC012957 to EG

Title: Functional contributions of quantal and non-quantal transmission in the vestibular periphery

Authors: *D. ZHOU[1], Z. YU[1], W. SCHOO[1], T. KODAMA[2], S. DU LAC[1], E. B. GLOWATZKI[1], S. G. SADEGHI[1];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Tokyo Women's Med. Univ., Tokyo, Japan

Abstract: Most vestibular afferents receive inputs from both types of vestibular hair cells (HC): type I HCs transmit signals through synaptic connections at the inner surface of specialized calyx terminals, while type II HCs form synapses either onto bouton terminals or directly onto the outer surface of calyces (Goldberg et al. 1990, Fernandez et al. 1995, Lysakowski and Goldberg 2008). Traditional quantal synaptic transmission via vesicular release of glutamate is well-documented, but an additional faster form of non-quantal (NQ) transmission has also been described between type I HC and calyx (Contini et al. 2022, Govindaraju et al. 2023). However, the precise role of the intricate network of connections between vestibular afferents and HCs along with the diverse forms of synaptic transmission remains a mystery. Recent studies have highlighted the role of type I HC – calyx synapses in eliciting normal vestibulo-ocular reflex (VOR) (Schenberg et al. 2023) and afferent responses to fast head movements (Pastras et al. 2023). Here, we further investigated the significance of quantal and NQ transmission in shaping afferent responses and encoding a broad spectrum of head movements. Using an optogenetic method, we selectively activated type I and II HCs in the cristae of the semicircular canals in young mice and found predominant quantal inputs from neighboring type II HCs onto the outer surface of most calyces, alongside NQ inputs from type I HCs onto inner surface of calyces. We used two mouse models deficient in quantal transmission either through the absence of vesicular glutamate transporter 3 (vglut3 KO) or by acute pharmacological block of glutamate receptors. As shown by a recent study in guinea pigs, in the absence of quantal transmission, vestibular sensory evoked potentials (VsEP) remained unaltered. Notably, VOR gain and phase also remained intact in response to horizontal rotational head movements up to 6 Hz. Single-unit extracellular recordings from the vestibular nerve showed normal proportion of regular afferents, which have been suggested to play a pivotal role in generating VOR responses. Finally, we investigated the importance of quantal and NQ transmission in encoding tonic stimuli. We utilized the contact righting reflex as a behavioral measure to detect changes in the gravity vector. Mice exhibited a longer time to turn from supine to prone positions following pharmacological inhibition of quantal transmission compared to the control. We propose that NQ transmission is necessary and sufficient for encoding fast and ultrafast head movements, while quantal transmission is necessary for encoding tonic changes in gravity and most likely, slow head movements.

Disclosures: D. Zhou: None. **Z. Yu:** None. **W. Schoo:** None. **T. Kodama:** None. **S. du Lac:** None. **E.B. Glowatzki:** None. **S.G. Sadeghi:** None.

Poster

PSTR279: Hair Cells and the Periphery

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Program #/Poster #: PSTR279.17/E2

Topic: D.05. Auditory and Vestibular Systems

Support: NIH Grant DC012347
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UIC Chancellors Undergraduate Research Award (CURA)

Title: Synaptic innervation of the mouse vestibular endorgans

Authors: M. HAMEED, F. IMRAN, T. MADAPPALLIL, J. ORUGANTI, B. SHAMSADDIN, *A. LYSAKOWSKI;
Anat. and Cell Biol., Univ. of Illinois at Chicago, Chicago, IL

Abstract: We decided to investigate the synaptic innervation of mouse vestibular hair cells (HCs) at the ultrastructural level using the disector method, as we have done before in the chinchilla and squirrel monkey (Lysakowski and Goldberg 1997, 2008). The reason for doing this is to have a comparable investigative method (TEM) and to confirm confocal data in a more recent study (Sadeghi et al. 2014). We need exact numbers for the mouse as we proceed to model quantal transmission after our recent non-quantal transmission model paper (Govindaraju et al. 2023). The number and type of synaptic ribbons were studied in 4 utricular macula and 9 crista ampullaris samples. Each sample was sectioned transversely and contained 30-32 serial ultrathin sections spanning the entire sensory epithelium. The disector method was used to estimate the total number of type I and type II HCs for each sensory organ (Sterio 1984), with a stringent criterion, in which the nucleus must be present for the HC to be counted. The utricular neuroepithelium was separated into 3 zones: medial extrastriola, striola, lateral extrastriola; while the crista sensory epithelium was separated into 2 zones: peripheral and central. Zones were determined using the density of complex calyces (calyx-shaped afferent endings enclosing more than one type I HC) to delineate the striolar/central zone and hair bundle polarization to locate the macular reversal line to delineate the striola (Desai et al. 2005; Li et al. 2008). Overall, we found regional and cell type variations in the synaptic ribbon distribution. The utricular extrastriola (90% of the sensory epithelial area) is much larger than the striolar region and the crista peripheral zone (56% of the cross-sectional area) is larger than the central zone. The ratio of ribbons/HC varies by zone and HC type, averaging 8.4 ± 1.1 vs 14.4 ± 2.3 (mean ± SEM) ribbons per type I and type II HC, respectively, in the crista. In the utricular macula, the numbers were 7.5 ± 2.0 and 12.1 ± 2.3 (mean ± SEM) for type I and type II, respectively. There were significant differences between type I and type II ribbons/HC. A distinction was made between the spherical “S” ribbon and the rod-like “R” ribbon; their distribution also varied according to region with “S” ribbons being far more prevalent than “R” ribbons throughout the neuroepithelium. Variations in synapse distribution between type I and type II HCs are consistent with the idea that the two different HC types serve different purposes, while variations in synapse shape can also be understood as ribbon types having different functions. These data should be useful in future electrophysiology and modeling studies of vestibular sensory epithelium.

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Poster

PSTR279: Hair Cells and the Periphery

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR279.18/E3

Topic: D.05. Auditory and Vestibular Systems

Support: R01DC018304
R01DC012347
F31DC021883
CBC-AG-002
TPCN 1R90DA060338

Title: Calcium Imaging of Mechanically Evoked Hair Cell and Afferent Population Activity in Mammalian Vestibular Inner Ear

Authors: *C. K. LUONG[1,2], M. KABIROVA[1], O. J. LUTZ[1,2], D. SILVIAN[1], R. EATOCK[1]; [1]Neurobio., [2]Committee on Computat. Neurosci., Univ. of Chicago, Chicago, IL

Abstract: We image Ca^{2+} activity in populations of utricular hair cells and afferents to capture moment-to-moment activity across the epithelium and correlate activity within and between distinct physiological zones. The utricle provides information about head tilt and linear head motions to the brainstem and cerebellum, facilitating rapid reflexes for maintaining stable visual fields, balance, and posture. Hair cell (HC) studies have shown major differences between HC and synaptic types, while *in vivo* afferent studies show distinct response properties that correlate with zone in the sensory epithelium and with spike regularity. Highly irregular afferents are more adapting and innervate the central zone (striola); highly regular afferents are more tonic and innervate the peripheral extrastriola. While single-unit recordings have shown that irregular and regular afferents differ strongly in average response properties, they do not establish moment-to-moment correlations of afferent activity. To address this information gap, we are using two-photon microscopy to correlate the Ca^{2+} activity of select populations of vestibular hair cells and afferents during a common fluid-jet stimulus.
Our preparation is the excised sensory epithelium and attached afferent nerve of the mouse utricle, for which exist extensive whole-cell recordings of hair cell and afferent responses to mechanical stimulation. We remove overlying structures to expose the mechanosensitive hair bundles, then apply a fluid jet driven with voltage waveforms for steps, sinusoids (0.5-20 Hz), or head motions as recorded from mice (courtesy KE Cullen). The evoked hair-bundle motions are viewed from above, captured in Dodt or DIC movies, and tracked with a custom algorithm (Silvian et al., this meeting). Ca^{2+} signals (dF/F) are correlated directly and/or deconvolved to generate “inferred activity”. Strategic choice of ROIs allows us to correlate activities within and across zones, between bundle polarities, and between hair cell types, in order to isolate the significance of each of these factors in shaping response activity. Preliminary results for ROIs

with ~50-125 HCs show activity with the expected zonal differences in stimulus orientation and frequency dependence. Complementary whole-cell recordings from hair cells and afferents (Kabirova et al., this meeting) reveal with great temporal precision the receptor and postsynaptic potentials and spikes driving the slower Ca^{2+} signals.
These data will provide input to a population model we are developing on how sensory information is represented across the mammalian vestibular epithelium (Lutz et al., this meeting).

Disclosures: C.K. Luong: None. **M. Kabirova:** None. **O.J. Lutz:** None. **D. Silvian:** None. **R. Eatock:** None.

Poster

PSTR279: Hair Cells and the Periphery

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Topic: D.05. Auditory and Vestibular Systems

Support: R01DC012347
CBC-AG-002
R01DC018304
F31DC021883
TPCN 1R90DA060338

Title: Comparing evoked calcium signals to whole-cell recordings from vestibular hair cells and afferents

Authors: M. KABIROVA[1], C. K. LUONG[2], O. J. LUTZ[2], D. SILVIAN[3], *R. EATOCK[1]; [1]Neurobio., Univ. of Chicago, Chicago, IL; [2]Committee on Computat. Neurosci., Univ. of Chicago, Chicago, IL; [3]Univ. of Chicago, Chicago, IL

Abstract: Within the first few milliseconds of a head motion, each mechanosensory hair cell in the vestibular inner ear generates a receptor potential that reflects directional and frequency component of the head motion. Many recordings exist from individual hair cells *ex vivo* and from individual primary afferents *in vivo*. There are also recordings of summated potentials that reflect output of the vestibular inner ear. Such recordings do not directly show how hair cell subpopulations in mammalian vestibular epithelia together represent head motions on a moment-by-moment basis. To address this question, we are recording Ca^{2+} signals from groups of hair cells and afferent terminals in the excised, intact epithelium of the mouse utricle, a linear accelerometer and widely used model vestibular epithelium. Accessory structures are removed to expose the mechanosensitive hair bundles to controlled fluid jets. Hair bundle deflections (tracked as in Silvian et al., this meeting) evoke Ca^{2+} signals detected by exogenous (e.g. fluo-

4ff) or genetically encoded (GCaMP) indicators (Luong et al., this meeting). Here we compare directly, for individual cells, evoked Ca^{2+} signals and concurrent whole-cell currents or voltage. Ca^{2+} indicators have a slower time course (seconds) than the trans-membrane Ca^{2+} currents (milliseconds) that give rise to the signals. During a hair bundle deflection, Ca^{2+} ions and other cations enter the bundle through transduction channels (TMCs), and the resulting receptor potential activates voltage-gated $Ca_V1.3$ channels in the basolateral membrane. Stimulus-evoked transmission to afferent synaptic terminals gives rise to postsynaptic Ca^{2+} signals likely due to voltage activation of $Ca_V1.2$ channels near the spike initiation zone. Direct comparisons of single-cell Ca^{2+} and electrical signals will guide our interpretation of population Ca^{2+} signals and modeling of how different populations within the vestibular epithelium and nerve represent head motions (Lutz et al., this meeting).

Disclosures: M. Kabirova: None. **C.K. Luong:** None. **O.J. Lutz:** None. **D. Silvian:** None. **R. Eatock:** None.

Poster

PSTR279: Hair Cells and the Periphery

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR279.20/E5

Topic: D.05. Auditory and Vestibular Systems

Support: TPCN 1R90DA060338 from NIDA
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R01DC018304
R01DC012347
CBC-AG-002

Title: Tracking stimulus-evoked hair-bundle deflections of sensory hair cell populations in an excised, intact vestibular organ

Authors: *D. SILVIAN[1], O. LUTZ[2], C. K. LUONG[2], M. KABIROVA[2], R. EATOCK[2];
[1]Univ. of Chicago, Chicago, IL; [2]Neurobio., Univ. of Chicago, Chicago, IL

Abstract: The vestibular system provides us with a sense of balance and coordination by detecting head motions through displacement of stereocilia bundles on hair cells, which are transduced into electrical signals relayed to the brain. The vestibular epithelium is organized into spatial zones corresponding to hair bundles' preferred direction, depolarizing maximally in response to head motions aligning with their orientation. While previous studies have characterized hair cell responses to bundle deflection at the single-cell level, our ongoing research endeavors to record (see Luong et al., Kabirova et al., this meeting) and model (see Lutz et al., this meeting) the collective responses of hair cell populations to stimuli delivered across

the epithelium. Stimuli across the epithelium may differentially deflect each hair bundle according to its preferred orientation and stiffness. A critical component of this research is to accurately track each hair bundle's motion for correlation with the activity of the corresponding hair cell and/or afferent.
We developed a custom motion-tracking pipeline to track individual bundle deflections evoked by a common fluid jet stimulus. We trained a machine learning algorithm (WEKA) to segment videos of moving hair bundles which we analyzed via custom algorithms and TrackMate (FIJI) to track the bundle positions with time. We established reliability with manual tracking. Our segmentation algorithm indicates motion relative to each bundle's preferred orientation. This tracking methodology allows us to quantitatively characterize the relationship between each hair bundle's motion and hair cell or afferent responses, as measured through calcium activity. For a particular head motion, bundle tracking will show how variation in bundle motions across the sensory epithelium affects the activity of the vestibular nerve.

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Poster

PSTR279: Hair Cells and the Periphery

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Topic: D.05. Auditory and Vestibular Systems

Support: F31DC021883
R01DC012347
R01DC018304
CBC-AG-002
1R90DA060338

Title: Spatiotemporal models to investigate population-level activity in the vestibular inner ear

Authors: *O. LUTZ[1], H. R. MARTIN[2], S. BAEZA LOYA[3], C. K. LUONG[1], M. KABIROVA[2], D. SILVIAN[2], B. D. DOIRON[4], R. EATOCK[2];
[1]Committee on Computat. Neurosci., Univ. of Chicago, Chicago, IL; [2]Neurobio., Univ. of Chicago, Chicago, IL; [3]The Univ. of Washington, Seattle, WA; [4]The Univ. of Chicago, Chicago, IL

Abstract: The vestibular inner ear detects head motion through displacement of mechanosensitive stereocilia bundles on hair cells (HCs). Primary vestibular afferents encode these bundle deflections with distinct firing patterns: irregular and regular. Both afferent classes encode head motion via spike rates, but irregular afferents provide more information based on

precise spike timing. Computational studies thus far have modeled HCs and neurons at the single-unit level to explain how various biophysical properties may influence neural coding but it is not clear which properties are the most salient. Furthermore, the vestibular epithelium is spatially organized into zones related to afferent subtype and to preferred stimulus direction: irregular and regular afferents emerge respectively from central (striola) and peripheral (extrastriola) zones; hair bundles reverse polarity (stimulus orientation preference) at the striola/lateral extrastriola boundary. Although we know key differences in the mean activity of HCs and afferents in the striola and extrastriola and across the line of polarity reversal, we lack information about how HC and afferent activities represent head motions moment-by-moment on the vestibular epithelium. Here we address this question with a computational model of population activity by hair cells and afferents of the mammalian utricle, the horizontal linear accelerometer of the inner ear. HCs and afferents are simulated with Hodgkin-Huxley style equations and incorporating physiological data from our work in excised utricles plus the in vivo literature. Parameters are fit using a Bayesian inference approach to estimate parameter combinations that match the physiological data. The vestibular epithelium is simulated as a three-layer feedforward model in which each layer represents a two-dimensional plane of, respectively, 1) HCs, 2) synaptic terminals and 3) spike initiation zones. To incorporate spatial features of the epithelium, the first layer contains HC orientation vectors. Feedforward integration between layers represents dendritic arbor size and types of synaptic inputs to afferents. We will systematically change parameters between the two zones and compare spiking statistics. Pairwise correlations will be calculated with respect to spatial distance between neuronal receptive fields and relative to the striola-extrastriola boundary. Ongoing work in our laboratory will allow us to compare our simulated responses to population activity in HCs and synaptic terminals evoked by bundle motions (Silvian et al., this meeting) and collected with two-photon calcium imaging (Luong et al., Kabirova et al. this meeting).

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Poster

PSTR279: Hair Cells and the Periphery

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Topic: D.05. Auditory and Vestibular Systems

Support: NIH R01 DC012347
NSF GRF
1U01-NS111695
NIH K12-GM123914

Title: Knockout of K_V1.8 subunits slows vestibular hair cell receptor potentials and impairs balance

Authors: *H. MARTIN[1], B. M. VERDONE[3], O. LOPEZ-RAMIREZ[1], D. SILVIAN[1], E. SCOTT[1], K. E. CULLEN[4], R. EATOCK[2];
[2]Neurobio., [1]Univ. of Chicago, Chicago, IL; [3]Johns Hopkins Univ., Baltimore, MD; [4]Dept. of Biomed. Engin., The Johns Hopkins Univ., Baltimore, MD

Abstract: In amniotes, head motions and gravity are detected by two types of vestibular hair cells (HCs) that express distinct basolateral voltage-gated potassium (K_V) conductances. Type I HCs, notable for their giant calyx synapse, express a large, low-voltage-activated K_V conductance, $g_{K,L}$, that is thought to augment non-quantal transmission. In contrast, type II HCs, contacted by more typical bouton synapses, express K_V conductances that activate positive to rest. Previously, we found that K_V channel subunit K_V1.8 is essential for the major K_V conductances in each HC (Martin et al. *eLife* 94342, 2024). Here, we present results on how absence of K_V1.8 affects different ends of the vestibulomotor pathway: HC receptor potentials and behavior. Any effects likely reflect K_V1.8 absence from the inner ear, given the low expression of K_V1.8 in other tissues (Lee et al. *Hear Res* 300:1, 2013).
We recorded from semi-intact, excised mouse utricles, an accessible sensory epithelium that detects horizontal translational acceleration. In K_V1.8-null, wildtype, and heterozygous littermates (postnatal days 10-60), we deflected hair bundles with step or sinusoidal waveforms delivered by stiff probes, and recorded HC currents and receptor potentials. In K_V1.8-null and heterozygous littermates (2-6 months), we tested activities that involve vestibular input: free swimming, open field activity, balance beam traversal, head stabilization, gait, and rotarod performance.
In K_V1.8-null HCs, greater input resistance increased the gain and rise time of the voltage response, and lowpass corner frequencies of receptor potentials fell from >100 Hz to ~20 Hz in type I HCs and from ~70 Hz to ~25 Hz in type II HCs. Absence of K_V1.8 increased phase (timing) lag of the receptor potential in both hair cell types above 10 Hz. These effects degrade temporal fidelity of receptor potentials, impairing accurate detection of head motions, particularly for fast head motions.
K_V1.8-null mice had normal motor abilities but struggled on challenging vestibulomotor tasks, such as crossing a narrow beam crossing and rearing on hindlegs. In the water, where proprioceptive cues are limited, K_V1.8-null mice did not maintain horizontal swim posture or stable head position. These results have implications for functional differentiation of type I and II vestibular HCs, synaptic transmission, and vestibular circuit function.
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Poster

PSTR279: Hair Cells and the Periphery

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Program #/Poster #: PSTR279.23/E8

Topic: D.05. Auditory and Vestibular Systems

Support: DC015135
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DC015252
AG051443
AG060504

Title: The Piezo channel is a mechano-sensitive complex component in the mammalian inner ear hair cell.

Authors: *M. C. PEREZ-FLORES[1], S. PARK[1], Y. CHEN[2], M. KANG[1], P. THAI[3], D. PEREZ FLORES[1], G. PERKINS[4], P. TRINH[3], X.-D. ZHANG[3], P. SIRISH[3], I. PESSAH[3], B. SOKOLOWSKI[5], B. FRITZSCH[6], N. CHIAMVIMONVAT[3], E. N. YAMOAH[1];
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Abstract: Background: The inner ear is the hub where hair cells (HCs) transduce sound, gravity, and head acceleration stimuli to the brain. Hearing and balance rely on mechanosensation, the fastest sensory signals transmitted to the brain. The mechanoelectrical transducer (MET) channel is the entryway for the sound-balance-brain interface, but the channel-complex composition is not entirely known.Methods: The prevailing data suggest TMC may be the pore-forming protein, but it only exists in a liposomal membrane as a reconstituted pore-forming protein. Moreover, whereas mutations of TMC1 in HCs may block conventional MET current, the anomalous current is spared. Experiments implicating Tmc1 as a pore-forming protein in HCs did not rule out; none did contemplate an allosteric role of Tmc on another channel. Previous evidence shows us that altering protein mutations or varying their concentrations may only provide evidence for an accessory protein rather than the sought-after pore-forming protein. Results: Here, we report that the mouse utilizes Piezo1 (Pz1) and Piezo2 (Pz2) isoforms as components of the MET complex. The Pz channel subunits are expressed in HC stereocilia, are co-localized and co-assembled, and are essential components of the MET complex in vitro and in situ, including integration with the transmembrane channel (Tmc1/2) protein. Mice expressing non-functional Pz1 and Pz2, but not wildtype Pz1 at the ROSA26 locus under the control of HC promoters, have impaired auditory and vestibular traits that suggest the Pz channel is integral to the MET complex. Conclusions: We propose that Pz protein subunits constitute part of the MET complex and that interactions with other MET components yield a functional hair-cell MET current.

Disclosures: M.C. Perez-Flores: None. **S. Park:** None. **Y. Chen:** None. **M. Kang:** None. **P. Thai:** None. **D. Perez Flores:** None. **G. Perkins:** None. **P. Trinh:** None. **X. Zhang:** None. **P.**

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Poster

PSTR280: Photoreceptors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR280.01/E9

Topic: D.06. Vision

Support: Foundation Fighting Blindness U.S. CD-RM-0821-0806-UHN

Title: Using gene therapy in human patient-derived retinal organoids to identify new therapeutic targets for USH2A-associated retinitis pigmentosa

Authors: *C. D'AMATA[1], K. ASHWORTH[2], B. G. BALLIOS[1];
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Abstract: Retinitis pigmentosa (RP) is a degenerative disease causing blindness through the progressive loss of photoreceptors. Pathological variants in the *USH2A* gene (e.g., c.2299delG) account for the majority of autosomal recessive RP cases. Animal knockout models have shown that USH2A is required for long-term photoreceptor maintenance, but clinically-pathogenic variants introduced into mice do not mimic the expected human disease progression. Our lab has created a human retinal organoid (RO) model of *USH2A* disease using patient-derived induced pluripotent stem cells (iPSCs). Our *USH2A*-ROs serve as a human preclinical model that enables us to study photoreceptor maturation, disease mechanism and facilitate new therapeutic discoveries. Patient-derived *USH2A*-ROs demonstrate photoreceptor maturation defects, including stunted photoreceptor inner/outer segment ("brush border") growth and reduced protein & gene expression of mature rod (Rhodopsin) and cone (Arrestin-3) markers from Week 24 to 34 of organoid development, compared with healthy control lines (N=3-5, min. 3 ROs). SEM and TEM of photoreceptor ultrastructure confirmed impaired outer structure development and markedly lower density of inner segments in *USH2A*-ROs compared to healthy ROs. To rescue this disease phenotype, we treated *USH2A*-ROs with an antisense oligonucleotide (ASO), which causes skipping of the c.2299delG mutation in exon-13, or a scrambled sequence control. While ASOs have been tested in Phase 2 clinical trials for patients with *USH2A*-associated RP, these preclinical studies only tested in 2D cultures of human cells. For the first time in a human RO model, we found that *USH2A*-ROs treated with 10uM of ASO from Week 18 to 20 show robust exon-13 skipping, faster rate of brush border growth, and improved Rhodopsin gene expression at Week 24, compared to scrambled and untreated controls (N=2, min. 3 ROs). Optimization of dosage and treatment window to find maximal rescue effects is ongoing. We have also generated an isogenic iPSC control line (iso*USH2A*corr), in which the c.2299delG was

corrected via CRISPR/Cas9 editing; culture and analysis of treatment in these ROs are underway. We hope that further gene expression analysis of diseased vs. healthy, treated, and corrected ROs will reveal differentially-regulated transcriptional pathways underlying a rescue effect, and potential new targets for neuroprotection and enhancement of photoreceptor maturation. We believe our work will provide new genotype-specific therapeutic targets against a common cause of inherited blinding eye disease.

Disclosures: C. D'Amata: None. **K. Ashworth:** None. **B.G. Ballios:** None.

Poster

PSTR280: Photoreceptors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR280.02/E10

Topic: D.06. Vision

Support: R01 EY027411
R01 EY026978
R01 EY034001

Title: A genome-wide in vivo CRISPR-screen identifies neuroprotective targets in retinal degeneration

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Abstract: A genome-wide in vivo CRISPR-screen identifies neuroprotective targets in retinal degeneration
Inherited retinal degenerations (IRDs) are a clinically and genetically heterogeneous group of blinding diseases characterized by progressive degeneration of photoreceptors. Most disease-causing mutations are rare, but many converge on common pathogenic pathways, raising hopes for mutation-agnostic neuroprotective strategies. Rhodopsin-P23H (RHO-P23H) mutation causes autosomal dominant retinitis pigmentosa (ADRP), accounting for ~10% cases in North America. In RHO-P23H mutation disease, misfolded Rhodopsin triggers ER stress, resulting in cell death through common pathways (e.g., unfolded protein response, UPR and apoptosis). In this study, we conducted the first in vivo genome-wide lentiviral CRISPR-screen in RHO-P23H knock-in mouse retina, and identified Ubiquitin fusion degradation 1 (Ufd1) and ubiquitously expressed prefoldin-like chaperone (Uxt) among the top neuroprotective candidate genes, whose removal accelerated photoreceptor death. We overexpressed Ufd1 and Uxt via adeno-associated viruses in

RHO-P23H knock-in mice, analyzed retina anatomical changes, electroretinograms (ERGs), visual cliff tests, and pupillary light responses to examine their ability to protect photoreceptors and rescue visual functions. Finally, we established RHO-P23H models in human retinal explant cultures to test the translational potential of the two candidate genes. We found that overexpression of Ufd1 or Uxt protects photoreceptors, rescues impairments in ERGs and visual behaviors in RHO-P23H knock-in mice, and supports photoreceptor survival in the RHO-P23H human retinal explant culture. Our study highlighted some promising candidate genes for neuroprotection in retinal degeneration and reveals the remarkable therapeutic potential of Ufd1 and Uxt gene augmentation.

Disclosures: N. Shen: None. **P. Ruzycki:** None. **E. Harding:** None. **F. Soto:** None. **D. Kerschensteiner:** None.

Poster

PSTR280: Photoreceptors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR280.03/E11

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant EY 020542
Foundation Fighting Blindness BR-CMM-06191763-UIA

Title: Mechanisms of Kv2.1/Kv8.2 associated vision loss

Authors: *S. BAKER[1], B. BERKOWITZ[2], J. G. LAIRD[1], S. M. INAMDAR[1];
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Abstract: Patients with Cone Dystrophy with Supernormal Rod Response (CDSRR) experience a childhood-onset decline in cone mediated vision followed by macular degeneration. About half of the patients also develop night blindness. CDSRR is caused by mutations in *KCNV2*, which encodes the obligatory Kv8.2 subunit of the rod and cone specific heteromeric Kv2.1/Kv8.2 voltage-gated potassium channel. Kv2.1/Kv8.2 participates in setting the resting dark current and filtering light responses. We developed two complimentary mouse models to study this disease. The Kv8.2 knockout (KO) retina is rod-dominant, like the human peripheral retina. These mice have the same electrical abnormalities as CDSRR, delayed or decreased rod responses in response to dim or brighter light respectively and decreased cone responses. In Kv8.2 KO there is slow rod degeneration and no loss of cones. The Conefull: Kv8.2 KO is a triple mutant mouse strain with only cone photoreceptors, similar to the cone-only fovea within the human macula. Conefull: Kv8.2 KO retina has the same decrease in cone-driven electrical responses as Kv8.2 KO and there is accelerated cone degeneration compared to the all-cone retina with intact Kv8.2. The deficits in electrical signaling are consistent with the predicted shift in the dark current.

Metabolomics was used to identify potential contributors to degeneration triggered by loss of Kv8.2. The two pathways most significantly altered in Kv8.2 KO retina were homocysteine degradation and taurine metabolism. High levels of homocysteine in neurons is associated with oxidative stress and inflammation. Taurine is an osmolyte and antioxidant abundant in photoreceptors that is essential for neuronal viability. In conclusion, together our Kv8.2 KO and Conefull: Kv8.2 KO mice allow us to model the different regions of the human retina affected by CDSRR. Rods appear to protect cones from degeneration, which could explain why the degeneration in CDSRR patients is limited to the macula. Further investigation of oxidative stress and inflammatory signaling in this disease is warranted to achieve the larger goal of identifying targets for drug development to slow retinal degeneration.

Disclosures: S. Baker: None. **B. Berkowitz:** None. **J.G. Laird:** None. **S.M. Inamdar:** None.

Poster

PSTR280: Photoreceptors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR280.04/E12

Topic: D.06. Vision

Support: Research to Prevent Blindness Career Development Award

Title: Zebrafish model for studying the metabolic activity in the retina and age-related macular degeneration

Authors: *T. TURER[1], T. YOSHIMATSU[2];
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Abstract: The macula is a central region in the retina that is densely saturated with cone photoreceptors and is responsible for high-acuity vision. It is prone to degenerative diseases such as age-related macular degeneration (AMD) which is the most common cause of blindness in the elderly. However, the pathogenesis and treatment of the macular degeneration are still unknown. In this study, we used zebrafish as an animal model since our previous studies showed that larval zebrafish have a macular-like cone-rich area in the retina that is physiologically, molecularly, and structurally specialized in a similar manner as seen in the human macula. This model also has the benefit of being easy to maintain and cost-efficient compared to other animals with known macular-like structures such as primates. We hypothesize that, in the macular-like area, the metabolic activity is higher; thus making this area metabolically more demanding, and more susceptible to environmental stress. We investigated metabolic activities in zebrafish by generating a series of transgenic lines that express biosensors for measuring the levels of various metabolic substrates such as ATP (biosensors: PercevalHR, iATPSnFR, GRABATP1), glucose (iGlucoSnFR), lactate (eLACCO 1.1) and hydrogen peroxide specific for different cell

compartments including mitochondrial matrix (MLS-HyPer7), mitochondrial intermembrane space (IMS-HyPer7) and cytosolic side of plasma membrane (HyPer7-MEM). In our experiments, we used the TrpR/tUAS system to drive the expression of these biosensors in a cell-type-specific manner. With these transgenic lines, we aim to reveal the retinal regions, cell compartments, and cell types that are metabolically more demanding, and how the variations in metabolic activities arise during development. In our future studies, we will investigate whether and how the metabolic activity is altered in AMD-associated gene mutants by knocking out these genes with F0 CRISPR screening in our biosensor lines.

Disclosures: T. Turer: None. **T. Yoshimatsu:** None.

Poster

PSTR280: Photoreceptors

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

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International Retinal Research Foundation
Purdue Institute Institute for Drug Discovery, Drug Investigational Screening and Chemigenomics Facility Project Grant

Title: A zebrafish functional screen identifies hits from FDA-approved drugs for treating retinitis pigmentosa

Authors: *B. WANG[1], L. GANZEN[2], M. TSUJIKAWA[3], Y. LEUNG[1,4];
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Abstract: Retinitis pigmentosa (RP) is a genetically inherited retinal degeneration that affects rods and may lead to visual impairment. RP can be caused by mutations in phototransduction genes, including *RHODOPSIN (RHO).* One *RHO* mutation, Q344X, can cause a severe form of RP in humans, which has no treatment. Hence, we aimed to discover new drugs for Q344X RP by repurposing FDA-approved drugs, which is faster and cheaper than *de novo* drug discovery. Thus, we screened an FDA-approved drug (FDA) library with 1430 drugs using a transgenic Q344X RP zebrafish model[1]. The screening utilized the visual-motor response (VMR), a

startle response displayed by zebrafish during drastic light changes. We previously detected a differential VMR between WT and Q344X larvae during light offset (light-off). This difference was the basis for our functional screening. We aimed to identify drugs that induced Q344X to display a light-off VMR similar to that of the WT controls, theorizing that these drugs may improve the vision of the Q334X mutant. To this end, we first exposed Q344X larvae to 10 µM drugs from 5 to 7 days post-fertilization (dpf). We began the treatment at 5 dpf because Q344X rods begin to degenerate substantially at this stage. We ran the VMR at 7 dpf because untreated Q344X larvae displayed a significantly reduced light-off VMR compared to WT. Using this treatment period would maximize our chance to identify drugs for Q344X RP. We eliminated 191 toxic drugs (13.4%) and then screened 1239 (86.6%) non-toxic drugs by VMR (n = 24 per drug). The positive and negative controls were drug-carrier-treated WT and Q344X, respectively. When analyzing the VMR data, we used an unbiased approach to detect similarities in VMR profiles by unsupervised machine learning. We combined the results of three clustering algorithms: hierarchical, K-means, and Gaussian Mixed Model with Expectation Maximization (EM-GMM). Since each algorithm has unique strengths in detecting similarities, combining their results will maximize our chance to discover drugs that induced a WT-like behavior in Q344X larvae. Out of the 1239 non-toxic drugs, 26 hits were identified by at least one algorithm (positive rate = 2.1%). One hit was identified by all three algorithms. The Q344X retina treated by this hit also contained more rods than the Q344X controls in the histology (n = 18 and 14; Wilcoxon two-sample test, W = 194, p-value = 0.028). In summary, we identified functional hits for Q344X RP from the FDA library. These hits can be further developed and repurposed for human Q344X RP treatment. **Reference:**1. Nakao T et al., PLOS ONE. 2012;7:e32472.

Disclosures: B. Wang: None. **L. Ganzen:** None. **M. Tsujikawa:** None. **Y. Leung:** None.

Poster

PSTR280: Photoreceptors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR280.06/E14

Topic: D.06. Vision

Title: Impact of ribeye deletion on ribbon synapse organization and function in the retinal photoreceptors

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Abstract: To encode a wide range of light information, retinal neurons including photoreceptors must be able to facilitate high rates of sustained neurotransmitter release over long periods. This is accomplished by a specialized structure anchored at the active zone of axon terminals of certain sensory neurons, known as ribbon. The ribbon tethers a large fraction of synaptic vesicles, enabling it to function as a conveyor belt for continuous vesicle exocytosis, thereby supporting tonic neurotransmitter release. Within the retina, ribbon synapses are located at the photoreceptor terminals in the outer plexiform layer (OPL) and at bipolar cell terminal in the inner plexiform layer (IPL). While the physiological properties and structural organization of ribbon synapses have been well studied, their role in synaptic assembly and circuit formation remains unclear. In this research, we utilized transgenic mice with a deletion of the ribbon synapse component RIBEYE to compare the differences in synaptic organization in OPL and retinal circuitry relative to wild-type mice, as well as how these structural changes affect the function of the retina. To assess these differences, serial block-face scanning electron microscopy was utilized to reconstruct neurons and their connectivity at the subcellular level in both RIBEYE knockout (KO) and wild-type (WT) mice. Additionally, we also conducted ex vivo electroretinograms (ERGs) to test the effect of synaptic ribbons on ERG responses to assess the impact of the loss of ribbon synapse on photoreceptor and bipolar cell output in response to light stimuli. Our results indicate that the specialized ribbon structure is completely lost from both rod and cone photoreceptor axon terminals in the KO mice. Rod photoreceptors in the KO mice form a lower percentage of conventional ribbon synapses which typically include four postsynaptic partners: two bipolar cells and two horizontal cells, compared to WT mice. The dendritic area of rod bipolar cells is nearly doubled, although the number of rod photoreceptors contacting the same rod bipolar cell remains unchanged. ERG responses indicate that scotopic bipolar cell responses were smaller in KO mice compared to WT. The synaptic organization of cone photoreceptors in KO mice is also significantly altered. However, ERG responses indicate that photopic bipolar cell responses in KO mice are unchanged compared to WT. In summary, absence of ribbon alters the rod terminal synaptic organization, which correlates to changes in rod bipolar cell response under scotopic light conditions. However, despite changes in the cone terminal synaptic organization, the cone bipolar response remains unchanged.

Disclosures: H. zhai: None. **R. Sinha:** None. **M. Hoon:** None.

Poster

PSTR280: Photoreceptors

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Topic: D.06. Vision

Support: NIH Grant EY022584
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NIH Grant EY034662

Title: Brn3b Shapes Intrinsically Photosensitive Retinal Ganglion Cell Electrophysiological Properties

Authors: *J. D. BHOI, M. ARANDA, O. PAYAN PARRA, T. YAMADA, Y. YANG, T. M. SCHMIDT;
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Abstract: Light is a fundamental feature of the environment, and it plays a critical role in entraining the circadian clock. Intrinsically photosensitive retinal ganglion cells (ipRGCs) are a highly conserved class of retinal photoreceptors that express the photopigment melanopsin, allowing them to detect light independent from rod and cone input. There are 6 types of ipRGCs, M1-M6, each with a distinct subset of physiological properties and transduction cascades. M1 ipRGCs, for example, relay ambient light information for circadian photoentrainment and pupil constriction, while M4 ipRGCs integrate contrast and environmental luminance to contribute to conscious visual perception. Despite extensive description of diverse ipRGC properties, little is known about the genetic programs shaping these differences. Recent work has shown that ipRGC subtypes express the transcription factor Brn3b in a gradient (M1<M2<M4). Interestingly, many subtype defining physiological features vary along this gradient including capacitance (M1<M2<M4), input resistance (M4<M2<M1), excitability (M1<M2<M4), and maximum firing rate (M1<M2<M4). Based on these relationships, we hypothesized that Brn3b may be required for development of these subtype-defining properties. If this is the case, then removal of Brn3b from the M4 ipRGCs, which have high Brn3b expression, should shift M4 physiological features toward those of M1 ipRGCs, which express the least Brn3b. To test this, we generated mice in which Brn3b is knocked out specifically in ipRGCs (Brn3b cKO) and used whole-cell patch clamp electrophysiology to measure the intrinsic properties of ipRGCs in Control and Brn3b cKO littermate mice. In agreement with our hypothesis, we find that Brn3b cKO cells show a shift toward M1-like properties in their intrinsic properties (capacitance, input resistance, excitability, and maximum firing rate), but little change in the photocurrent. Furthermore, Brn3b cKO M4s go into depolarization block following positive current injection, a characteristic of the lower Brn3b-expressing M2 and M1 ipRGCs. Finally, we also find that the action potential shape is altered in Control versus Brn3b cKO cells. Altogether, our results suggest that Brn3b plays a critical role in determining the electrophysiological properties of ipRGCs.

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Poster

PSTR280: Photoreceptors

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR280.08/E16

Topic: D.06. Vision

Support: NIH grant R01EY005121
EENT Foundation (NGB)

Title: Neuroinflammation and neurodegeneration in Adipor1$^{-/-}$ and Mfrp^{rd6} mice are associated with the downregulation of Fatty Acid Elongases 2 and 4

Authors: *M.-A. I. KAUTZMANN, N. G. BAZAN;
Neurosci. Ctr. of Excellence, New Orleans, LA

Abstract: Docosahexaenoic acid (DHA), an omega-3 fatty acid found abundantly in the central nervous system and retina, plays a crucial role in maintaining vision by preserving the morphology of photoreceptor discs. Its diverse benefits include antioxidative, anti-inflammatory, and anti-apoptotic properties through lipid mediator generation. Our study focused on mutant *Adipor1* and *Mfrp* mice, which showed reduced levels of DHA and very long-chain polyunsaturated fatty acids (VLC-PUFAs; ≥ 28 carbons), synthesized primarily by elongases Elovl2 and Elovl4. These mutant mice exhibited compromised retinal function and a progressive loss of photoreceptor cells. To understand the inflammation mechanism driving retinal degeneration in these models, we analyzed retinas and RPE-eyecups from *Adipor1*$^{-/-}$ and *Mfrp*^{rd6} and wild-type mice. We harvested retinas and RPE-eyecups from *Adipor1*$^{-/-}$, *Mfrp*^{rd6} and C57BL/6J (wild-type; WT) male and female mice. Utilizing capillary Western blot and qPCR we assessed the expressions of Elovl2 and Elovl4. Additionally, we conducted FAM-FLICA Caspase-1 Assay on mice eye sections to ascertain caspase-1 activation and employed immunohistochemistry with Iba-1 antibody to investigate microglia invasion in mutant mice. Elovl2 and Elovl4 proteins and RNA expressions were notably reduced in the mutant mice, suggesting a role in observed retinal abnormalities. Further analysis using FAM-FLICA demonstrated a significant increase in caspase 1 activity in *Adipor1*$^{-/-}$ and *Mfrp*^{rd6} eyes. Immunostaining revealed a higher number of Iba-1 positive cells in the retinas of both mutant groups, indicative of increased microglia invasion. These findings suggest that *Adipor1*$^{-/-}$ and *Mfrp*^{rd6} mice are prone to uncontrolled neuroinflammation, leading to microglia invasion and caspase 1-dependent retinal damage, potentially involving a pyroptotic immune response. Targeting the inflammasome pathway could be a promising therapeutic strategy for addressing retinal degeneration in these mutant models.

Disclosures: M.I. Kautzmann: None. **N.G. Bazan:** None.

Poster

PSTR280: Photoreceptors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR280.09/E17

Topic: D.06. Vision

Support: NIH EY032948-01
DoD #W81XWH-21-1-0884

Title: Improved response properties of single neurons in higher visual cortex of retinal degenerated rats following retinal sheet transplant

Authors: *A. ALIZADEH[1], B. LIN[2], R. SIMS[3], A. SUON[4], M. J. SEILER[5], D. C. LYON[6]; [1]Anat. & Neurobio., Univ. of California Irvine, Irvine, CA; [2]Stem Cell Res. Ctr., Univ. California Irvine, Irvine, CA; [3]UC Irvine, IRVINE, CA; [4]Stem Cell Res. Ctr., Univ. of California, Irvine, Irvine, CA; [5]PM&R, Univ. of California Irvine, Irvine, CA; [6]Anat. and Neurobio., UC Irvine, Irvine, CA

Abstract: Age-related retinal degeneration affects millions of people worldwide and often results in permanent loss of vision. In a previous study (Foik et al, 2018, J Neurosci) we showed in retinal degenerated rats (RD) that the number of visually responsive units and selectivity to stimulus size and orientation of primary visual cortex (V1) neurons were significantly improved after transplantation of a small fetal retinal sheets into one eye. The current study was designed to determine the effectiveness of retinal transplantation in rescuing neural responses of higher visual areas cortex of RD rats. We collected single unit responses from lateral medial area (LM, putative ventral visual stream in rodents) to drifting gratings and pattern motion. Briefly, the transgenic Rho-S334ter line-3 rat (both sexes), which loses photoreceptors at an early age and is effectively blind at postnatal day 30, received fetal retinal sheet transplants (E19) in one eye between 41 and 78 d of age. RD controls included sham surgeries and age-matched non-surgery rats (AMC). The quality of the transplant was then assessed using high-resolution OCT retinal imagining. Rats with qualitatively good transplants were used for recording. Responses from ~ 200 single-units from area LM of anesthetized rats we measured using tungsten sharp electrodes inserted perpendicular to the cortical surface. Cells were tested under optimal stimulus parameters for orientation, size, and spatial and temporal frequencies. Our primary results show that the proportion of visually responsive units increased from 4% in RD rats to 19% in transplanted animals. Also, firing rates to flashes of light were significantly increased in transplanted rats. Consistent with our previous findings, we observed improved orientation tuning and selectivity in transplanted animals compared to RD rats.Our results suggest that transplantation of fetal retinal sheets can improve responses of higher visual cortex and result in partial restoration of a functional connectivity in lower and higher visual cortex.

Disclosures: A. Alizadeh: None. **B. Lin:** None. **R. Sims:** None. **A. Suon:** None. **M.J. Seiler:** None. **D.C. Lyon:** None.

Poster

PSTR281: Visual System: Response Modulation and Adaptation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR281.01/E18

Topic: D.06. Vision

Support: NIH grant EY02874
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Moorfields Eye Charity GR001264

Title: Electrical stimulation alters behavioral state-dependent neuronal and population activities

Authors: M. W. FANG[1], M. P. STRYKER[2], M. C. DADARLAT[3], *Y. J. SUN[1];
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Abstract: Intracortical microstimulation is an important tool for directly altering neuronal responses and probing cortical functions, providing an essential foundation to the development of brain machine interface and neuroprosthesis. However, it remains unclear if and to what level the consequences of stimulation is dependent on the animal's behavioral states and pre-stimulus neural activities.

To answer this question, we applied two-photon calcium imaging in the awake mouse to track the single-cell activities of both excitatory and labeled inhibitory neurons in the primary visual cortex, before and after the stimulation. Electrical stimulation consisted of 25 biphasic cathode-leading train pulses was delivered through a tungsten electrode, once every 10 s at one of nine possible current amplitudes (3, 5, 10, 15, 20, 25, 30, 40, or 50 A) in pseudo-random order,for 10 repetitions at each amplitude (details in Dadarlat et al, 2023). We also differentiate behavioral states of the head-fixed mice based on their velocity on the floating ball.

We found that electric stimulation’s modulation depends on pre-stimulus activity, behavioral states, and cell types. Inhibitory neurons showed increased responses post-stimulation, while there is a decrease in post-stimulation activities for excitatory neurons. Interestingly, during quiescence, the excitatory neurons are also more dependent on the pre-stimulation activities. This may be due to changes of the local network connectivity during different states. While the mice were still, stimulation enhanced the overall population correlation, increasing the similarity among excitatory neurons. However, we did not find any similar effect on population correlation during locomotion. Pairwise noise correlation between excitatory neurons also has a similar pattern. While the average noise correlation increased for excitatory neurons during quiescence, we found an opposite trend during moving. For inhibitory neurons, the dependence on pre-stimulus activities was similar during both locomotion and quiescence. Both population correlation and noise correlation among inhibitory neurons increased after stimulation during both locomotion and quiescence state.

Overall, our study revealed key properties of the local neuronal network were significantly

altered after the stimulation, which are also contingent on the animal's behavioral states and cell types.

Disclosures: M.W. Fang: None. **M.P. Stryker:** None. **M.C. Dadarlat:** None. **Y.J. Sun:** None.

Poster

PSTR281: Visual System: Response Modulation and Adaptation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR281.02/E19

Topic: D.06. Vision

Support: 1R01EY027402-03
5T32 EY007135-24

Title: V1 population spiking does not show statistically significant differences between laminar compartments during binocular rivalry flash suppression.

Authors: *B. M. CARLSON[1], B. MITCHELL[2], P. G. POGGI[2], J. A. WESTERBERG[3], A. V. MAIER[2];
[1]Psychology, Vanderbilt Univ., Nashville, TN; [2]Dept. of Psychology, Vanderbilt Univ., Nashville, TN; [3]Dept. of Vision and Cognition, NIN-KNAW, Amsterdam, Netherlands

Abstract: Binocular rivalry flash suppression (BRFS) arises when one eye is adapted to a stimulus that persists after an incompatible stimulus is presented to the other eye. During rivalry, one eye's perception is suppressed while the other eye's perspective dominates. In the context of BRFS, the adapted eye is almost always briefly suppressed after the second stimulus is presented. Recent modeling work proposed that the upper layers of V1 might act as the primary site of rivalry-related activity (Evers et al., 2023). Several studies over the past decades scrutinized V1 activity during BRFS. However, most of these studies preceded the advent of laminar neurophysiology. Consequently, our understanding of how BRFS influences V1 responses at the laminar level remains limited. Here we test the computational model prediction of laminar anisotropy by detailing the laminar profile of BRFS responses in primate V1. We performed 43 acute laminar microelectrode penetrations in two awake macaques using linear array probes equipped with 32 contacts, each spaced 100um apart. Local field potentials (LFP) and spiking data were recorded during these penetrations. To localize the layer IV/V boundary, we employed power-spectral-density (PSD) analysis and validated these findings with current-source-density (CSD). Our analysis identified 28 penetrations that orthogonally spanned all V1 layers, yielding a total of 140 multi-units per laminar compartment. Population spiking did not follow the hypothesized laminar profile. In line with earlier work, BRFS-related spiking modulation was in the minority. The associated response profile of population averages was roughly equally distributed across V1 layers. We speculated that the effects of BRFS on V1

laminar responses might vary in ways that are challenging to detect by traditional rate-coding metrics such as averaged population spiking. As a first inroad to this question, we tested for laminar differentiation in V1 LFP coherence between regular and rivalrous visual stimulation. These analyses matched previous reports by Leopold & Logothetis in that V1 LFP exhibits greater coherence with dioptic stimulation than dichoptic stimulation. Even more interestingly, this effect was strongest within and between supragranular layers and infragranular layers, suggesting significant laminar differentiation. Taken together, these results suggest that more neuronal population-centered work is needed to fully determine the role of the columnar microcircuitry of primate visual cortex for binocular rivalry flash suppression.

Disclosures: B.M. Carlson: None. **B. Mitchell:** None. **P.G. Poggi:** None. **J.A. Westerberg:** None. **A.V. Maier:** None.

Poster

PSTR281: Visual System: Response Modulation and Adaptation

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Topic: D.06. Vision

Support: The Scientific and Technological Research Council of Turkiye (ARDEB Grant 123K833, BIDEB 2211 Program)

Title: Dynamic visual noise impact on contrast detection mimics transcranial random noise stimulation

Authors: *S. UNER[1], I. AKDOGAN[1], A. PAVAN[2], H. KAFALIGONUL[3];
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Abstract: Research on high-frequency transcranial random noise stimulation (hf-tRNS) revealed the benefits of noise to perceptual and cognitive processes, which have been modeled through stochastic resonance (SR, Simonotto et al., 1997). Using the SR framework, previous research identified enhanced stimulus processing when electrical (Pavan et al., 2019) or stimulus noise (van der Groen & Wenderoth, 2016) applied. Although these studies provide important insights into noisy processing, there is no systematic comparison of visual noise and tRNS. This requires a closer alignment of visual noise to the tRNS characteristics, such as continuous presentation of noise during the ongoing visual task and noisy stimulation of a wider area of the visual cortex. Here, we addressed whether dynamic visual noise could mimic the tRNS effects on visual processing when the noise characteristics were equated as possible. To achieve this, we generated several levels of dynamic white pixel noise continuously presented in the background

during a contrast detection task. To address SR effects of visual noise on stimulus processing, we presented Gabor stimuli at individualized contrast levels associated with either barely seen (subthreshold, n=25) or easily detectable (suprathreshold, n=25) stimuli. We calculated the performance improvement at each noise level with respect to the baseline accuracies to determine optimal noise levels leading to maximum performance. Our results demonstrated the robustness of visual noise in increasing the detection performance when presented concurrently but unrelated to the ongoing task. Importantly, we found a strong negative correlation between the baseline performance and maximum improvement ($p < .001$), indicating that participants with lower initial performance benefited most from the visual noise. We also confirmed that presenting an optimum level of visual noise is significantly more effective (Welch's t-test, $p < .001$) on the detection of subthreshold ($M = 19.79$, $SD = 19.96$) compared to suprathreshold ($M = 4.77$, $SD = 5.28$) stimuli, as the effect of tRNS shown in previous literature. Consistent with the SR phenomenon, we highlight that visual noise mimics the tRNS effect on visual processing. Specifically, we confirm the presence of individual optimum visual noise similar to the effectiveness of distinct tRNS levels, resulting in the maximum increase in perceptual performance. These findings suggest a potential use of dynamic visual noise to enhance visual perception and offer a safer procedure compared to electrical stimulation, especially for clinical populations that cannot tolerate electrical current application to the cortex.

Disclosures: S. Uner: None. **I. Akdogan:** None. **A. Pavan:** None. **H. Kafaligonul:** None.

Poster

PSTR281: Visual System: Response Modulation and Adaptation

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European Young Investigator Award

Title: Gamma in awake macaque V1 causes response-gain modulation

Authors: *M. PANDINELLI[1], T. NÄHER[1], E. PSAROU[1], I. GROTHE[2], P. FRIES[3]; [1]Ernst-Strungmann-Institut gGmbH, Frankfurt am Main, Germany; [2]Brain Res. Institute/Cognitive Neurophysiol. Group, ESI For Neurosci. In Cooperation With Max Planck Society, Bremen, Germany; [3]Max Planck Inst. for Biol. Cybernetics, Frankfurt, Germany

Abstract: A prominent feature of brain activity is oscillatory neuronal synchronization in the gamma range (40 – 90 Hz). Gamma rhythms have been hypothesized to implement the effective neuronal communication of selected stimuli through the alignment of excitability phases between lower and higher visual areas. Local gamma synchronization entails sequences of excitation and inhibition that have the potential to rhythmically modulate synaptic input gain. If synaptic inputs were consistently aligned to phases of high excitability, this would increase their gain. Indeed, we have previously shown that visually induced gamma in awake macaque area V4 and optogenetically induced gamma in anesthetized cat area 17 entails rhythmic gain modulation. Here, we tested whether visually induced gamma in awake macaque area V1 entails corresponding gain changes, and whether they constitute modulations of input or response gain.We recorded multi-unit activity (MUA) and local field potentials (LFP) from primary visual cortex (V1) of one awake macaque monkey. While the monkey maintained fixation, a uniform red background induced ongoing gamma-band activity, and a randomly timed probe stimulus evoked a MUA response. We investigated whether this MUA response was modulated by the phase of the ongoing gamma rhythm just prior to the probe presentation. Importantly, we quantified whether this putative gamma-rhythmic MUA-response modulation was multiplicative. That is, we tested, whether it exceeded an additive modulation that was expected from a simple superposition of an un-modulated probe-evoked response onto the background-induced ongoing gamma-rhythmic MUA modulation. Indeed, we found that gamma rhythmically modulated the MUA response, and that this modulation exceeded the additive component and therefore had a significant multiplicative component.We tested whether this effect constituted a gamma-rhythmic modulation of response gain or input gain. In the case of input gain, the contrast-response function is shifted to the left, with strongest response increases for mid-level stimulus contrasts. In the case of response gain, the contrast-response function is multiplied by an approximately constant factor for all stimulus contrasts. We therefore used probe stimuli with contrasts of 5%, 10%, 20%, 40%, 80% and 100%. The gamma-rhythmic gain modulation was approximately proportional to the probe-evoked response and thereby constituted a response-gain modulation.Based on these findings we propose that the gamma rhythm has causal consequences for neuronal communication, and that this effect is present in the primate visual system as early as primary visual cortex.

Disclosures: M. Pandinelli: A. Employment/Salary (full or part-time):; Ernst-Struengmann Institute for neuroscience. **T. Näher:** A. Employment/Salary (full or part-time):; Ernst-Struengmann Institute for neuroscience. **E. Psarou:** None. **I. Grothe:** None. **P. Fries:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent on thin-film electrodes. F. Consulting Fees (e.g., advisory boards); CoreTech GmbH (Freiburg, Germany).

Poster

PSTR281: Visual System: Response Modulation and Adaptation

Location: MCP Hall A

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Program #/Poster #: PSTR281.05/E22

Topic: D.06. Vision

Support: NIH Grant EY005253

Title: Optical blur affects differently ON and OFF visual pathways

Authors: C. PONS[1], R. MAZADE[2], ***J. JIN**[3], M. W. DUL[3], J.-M. ALONSO[3];
[1]Neurolog. Surgery, Univ. of Chicago Med., Chicago, IL; [2]Ophthalmology, Emory Univ., Atlanta, GA; [3]SUNY Col. of Optometry, New York, NY

Abstract: The human eye has a crystalline lens that focuses retinal images at the point of fixation. Outside this region, images are distorted by optical blur, which increases light scatter and reduces the spatial resolution and contrast processed by neuronal pathways. The negative and positive spectacle lenses that humans use for optical correction also minify or magnify the images, affecting neuronal surround suppression. Because light and dark stimuli are processed with ON and OFF pathways that have different spatial resolution, contrast sensitivity and surround suppression, lens distortions should affect differently the two pathways and the perception of lights and darks. Here, we provide support for this prediction by performing electrophysiological recordings from the visual cortex of cats (multiunit spiking activity) and humans (electroencephalography). All animals and human subjects wore contact lenses or spectacles while having their lens accommodation pharmacologically blocked. As expected from the effect of light scatter, optical blur expanded ON receptive fields measured with contact lenses (±10 vs. 0 diopters: $6.8^o \pm 0.1^o$ vs. $5.9^o \pm 0.1^o$, n=128, $p = 2.1 \times 10^{-18}$) or spectacles (±10 vs. 0 diopters: $6.7^o \pm 0.1^o$ vs. $6.2^o \pm 0.2^o$, n=70, $p = 9.2 \times 10^{-5}$, Wilcoxon tests and means ± standard errors in entire abstract). Opposite to ON receptive fields, optical blur shrunk OFF receptive fields measured with contact lenses (±10 vs. 0 diopters: $6.0^o \pm 0.1^o$ vs. $6.2^o \pm 0.1^o$, n=128, $p = 1.2 \times 10^{-5}$). However, when measured with spectacles, optical blur did not affect the average OFF receptive field size (±10 vs. 0 diopters: $5.7^o \pm 0.1^o$ vs. $5.8^o \pm 0.1^o$, n=70, p = 0.1186) because positive and negative lenses had opposite effects on image size (i.e. magnification and minimization), which affected differently surround suppression. Specifically, the image magnification of positive spectacles made OFF receptive fields smaller than ON receptive fields (OFF vs. ON at +10 diopters: $5.1^o \pm 0.1^o$ vs. $6.4^o \pm 0.1^o$, n=70, $p = 4.7 \times 10^{-11}$) and OFF receptive field size strongly correlated with spectacle blur ($R^2 = 0.98$, $p < 0.0001$, n = 70; slope: -0.1^o per diopter, blur range: -10 to +10 diopters). As expected from light scatter, optical blur also decreased the population response of OFF more than ON cortical pathways in humans (± 5 minus 0 diopters with contact lenses for OFF vs. ON: -4.8 ± 1.1 vs. 0.1 ± 0.7 microVolts, n=10, p = 0.002). Based on these results, we conclude that optical blur and image magnification reduce the receptive field sizes and cortical responses of OFF more than ON pathways, making OFF pathways better suited at optimizing the magnification/quality of retinal images during eye development.

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Poster

PSTR281: Visual System: Response Modulation and Adaptation

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR281.06/E23

Topic: D.06. Vision

Support: R01 NS121772

Title: Receptive field weight is robustly represented in divisive normalization.

Authors: *C. J. CHERIAN, J. H. MAUNSELL;
Neurobio., Univ. of Chicago, Chicago, IL

Abstract: When multiple stimuli lie within a cortical neuron's receptive field (RF), the combined response is typically less than the sum of the responses to those stimuli when presented individually. Divisive normalization models have been used to explain this non-linear relationship, a phenomenon that is widely observed in cortical responses. Normalization yields a response that approximates a contrast-weighted average of the responses to each stimulus when presented alone. Normalization thus preserves response selectivity by preventing multiple non-preferred stimuli from producing a response that is as strong as the response to a preferred stimulus. While normalization is widespread, neurons have been found to vary widely in how closely their response to multiple stimuli approximates a contrast-weighted average (Ni and Maunsell, 2017). An individual sensory neuron can even show differences in the strength of its normalization when the positions of a preferred and a non-preferred stimulus in its RF are swapped. The origins of this variance in normalization are not well understood, but differences in the strength of responses arising from different RF locations (i.e., RF weight) might be a factor. To test this, we recorded from neurons in the monkey middle temporal visual area (MT) while the animal held its gaze on a fixation spot and reported stimulus changes that occurred far from the RF. While the animal did the task, we flashed sequences of one or two, task-irrelevant Gabor stimuli in the RF to measure normalization. The positions of the stimuli were varied parametrically to measure how normalization depends on the RF weights at the stimulated locations. To date, we have recorded from 18 well-isolated MT neurons in one monkey. Presentation of individual Gabors at different RF sites revealed that the average MT RF weight profile is approximately Gaussian. Stimuli with preferred or non-preferred directions of motion produce Gaussian RF profiles that differ only in amplitude. When the Gabors were paired, one stimulus was always presented at the RF center while the other was presented at varying distance from the center. In all cases, as the second stimulus approached the RF limit, the paired response approached the response to the center stimulus by itself. Paired responses were well predicted by weighting each stimulus by the RF weight of its position. Failing to factor in the RF weight led to poor predictions of paired responses and variance in the apparent strength of normalization.

RF weighting, like contrast weighting, is a necessary factor for capturing a neuron's response to multiple stimuli.

Disclosures: C.J. Cherian: None. **J.H. Maunsell:** None.

Poster

PSTR281: Visual System: Response Modulation and Adaptation

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR281.07/E24

Topic: D.06. Vision

Support: NIH Grant R00 EY029323

Title: Thalamic stimulation modulates experience-dependent visual plasticity

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[1]Georgia Tech., Atlanta, GA; [2]Biomed. Engin., Emory Univ., Atlanta, GA; [3]Biomed. Engin., Georgia Tech. and Emory, Atlanta, GA

Abstract: Thalamocortical (TC) plasticity is a prominent feature of early sensory systems, enabling development of precise topographic maps in neocortical layer 4 (L4). As animals age, the capacity for thalamocortical long-term potentiation (LTP) and long-term depression (LTD) diminishes substantially, with numerous ex-vivo studies establishing the absence of TC-L4 LTP/LTD beyond early postnatal life. However, recent in-vivo studies in the adult rodent visual system have highlighted an important role for thalamic circuits in cortical plasticity beyond the canonical critical period. In this study, we sought to develop a system for directly monitoring and manipulating activity at TC-L4 synapses in vivo to assess their capacity for plasticity in adult mice. To enable high-frequency optical stimulation of the thalamus, we expressed the ultrafast depolarizing opsin, Chronos, into the dorsal lateral geniculate nucleus (dLGN). Meanwhile, we used chronically-implanted field electrodes in L4 to assess optically- and visually-evoked responses during different phases following stimulation. We first establish that the response in L4 of primary visual cortex (V1) increases monotonically with light power and that stimulus-response profile remains stable over the course of weeks. We then find that optogenetic stimulation of the thalamus alone has limited ability to produce thalamocortical LTP or visual response potentiation, but exerts significant influence over ongoing visually-driven cortical plasticity. We posit that although thalamic stimulation in the adult brain may not have the capacity to directly drive TC-L4 LTP in-vivo, it can act to augment or disrupt ongoing experience-dependent processes.

Disclosures: Y. Zhao: None. **M. Fong:** None.

Poster

PSTR281: Visual System: Response Modulation and Adaptation

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Program #/Poster #: PSTR281.08/E25

Topic: D.06. Vision

Support: NRF-2022R1I1A4063209
NRF-2022R1A2C2005062

Title: Direct and indirect response of retina ganglion cells to electrical stimulation in mice

Authors: *H. JEONG[1,2], S. HWANG[1], S. JUN[1,2,3];
[1]Dept. of Electronic and Electrical Engin., [2]Grad. Program in Smart Factory, [3]Dept. of Brain and Cognitive Sci., Ewha Womans Univ., Seoul, Korea, Republic of

Abstract: Retinal prostheses have been developed to restore vision in individuals who have lost sight due to retinal pigmentosa (RP) or age-related macular degeneration (AMD), which are diseases characterized by the degeneration of photoreceptors. Like other implantable prostheses such as cochlear implant system and deep brain stimulation systems, retinal prostheses also employ the principle of generating neural activity using electrical stimulation. By applying electrical stimulation to the retina, the surviving retinal ganglion cells (RGCs) are activated even in the absence of functional photoreceptor cells. A key aspect of developing retinal prostheses is optimizing electrical stimulation protocols by adjusting parameters to ensure successful vision restoration. In a previous study using the retinal tissue of C57BL/6 mouse (8 weeks, male) on microelectrode arrays, we have investigated the response of RGCs responding to the electrical pulse stimulations with various amplitudes (10~80 μA) and widths (100~1000 μs). It was observed that there exist short-latency responses and long-latency responses following subretinal stimulation. The short-latency responses were likely due to direct stimulation of RGCs, while the origin of the long-latency responses was less clear. In this study, the effects of the glutamatergic receptor antagonists (NBQX, AP5) were investigated. The synaptic blockers suppressed the long-latency responses of RGCs indicating the long-latency responses were mediated by glutamatergic synaptic transmission. Additionally, the RGC responses to light stimulation are also recorded in the absence and presence of the blockers.

Disclosures: H. Jeong: None. **S. Hwang:** None. **S. Jun:** None.

Poster

PSTR281: Visual System: Response Modulation and Adaptation

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Topic: D.06. Vision

Support: NIH Grant EY034503
IBACS 73

Title: Arousal-dependent modulation of membrane potential dynamics in the rabbit visual cortex

Authors: M. CHISTYAKOVA[1], N. L. RAFFONE[1], J.-M. ALONSO[2], H. A. SWADLOW[1], M. A. VOLGUSHEV[1], *Y. BERESHPOLOVA[1];
[1]Dept. of Psychological Sci., Univ. of Connecticut, Storrs, CT; [2]Biol. and Visual Sci., SUNY, New York, NY

Abstract: Studies of visual processing during different internal states of arousal revealed profound state-related changes in neuronal operations along the visual pathway. In the rabbit primary visual cortex a shift from alert to nonalert state is associated with a decrease of response gain and response reliability. Although there is a clear effect of arousal state on neuronal spiking activity, the subthreshold mechanisms underlying state-related changes of visual responses are poorly understood. We employed a novel technique which allows us to obtain stable intracellular recordings from primary visual cortex neurons in awake head-restrained rabbits. To minimize damage to the cortex, increase the stability of recording and allow multiple recording sessions from the same cortical area, we used a combination of coaligned guiding cannula that penetrates the dura and a “sharp” recording micropipette. Using this technique, we obtained intracellular recordings from visual cortical neurons across multiple episodes of alert and nonalert brain states. Brain states were defined by the characteristic signatures in hippocampal and cortical EEG. Recorded neurons were classified by the firing patterns evoked with depolarizing current steps and action potential waveforms. In regular spiking neurons we measured responses to visual stimuli and receptive field properties, using subthreshold membrane potential (Vm) dynamics and spiking. We found that upon transition from alert to nonalert state, regular spiking neurons showed a clear reduction in the Vm modulation and action potential firing in response to optimal visual stimulation. Changes in spiking were more pronounced than changes of the membrane potential responses, and relatively small changes in the Vm modulation amplitude could cause larger changes in spiking. In some cases, visually evoked spiking could be even completely shut off during nonalert state, but an underlying modulation in the Vm amplitude was still clear, though of a reduced amplitude. Interestingly, we found that the threshold for action potential generation and the transformation of membrane depolarization into spike responses did not change with state. These results point to the role of the threshold mechanism and involvement of the 'tip-of-the-iceberg' effect in shaping state-related transformation of neuronal responses in the visual cortex.

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Poster

PSTR281: Visual System: Response Modulation and Adaptation

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Program #/Poster #: PSTR281.10/E27

Topic: D.06. Vision

Support: 5T32DA018926-19
NIH-R01-EY028657

Title: In vivo measurements of rheobase in mouse primary visual cortex

Authors: *L. JENNINGS[1], N. J. PRIEBE[2];
[1]Neurosci., The Univ. of Texas at Austin, Austin, TX; [2]Neurosci., Univ. Texas, Austin, Austin, TX

Abstract: Neural spiking responses in the cerebral cortex are highly variable. One proposal to explain this variability is that it emerges from a balanced interaction between excitatory and inhibitory inputs. A critical prediction of this balanced hypothesis is that small changes in input lead to changes in spiking responses. This may occur if the underlying membrane potential lies near spike threshold. However, current injection thresholds for eliciting an action potential have not been measured in vivo. Using in vivo whole-cell electrophysiology, we have made measurements on the relationship between input current and spike rate using 20ms and 100ms current pulses in order to determine the rheobase of neurons in mouse primary visual cortex. We have found that the average rheobase for 100ms current injections is 80pA (SD = 53.0pA), while for 20ms current injections the rheobase is 150pA (SD = 64.3pA). These measurements are often complicated by the fact that membrane potential fluctuations occur in vivo on the order of 10mV. These fluctuations induce significant trial-to-trial variability in the relationship between injected current and spike rate. We compared responses to current injections when neurons were in a "down" state, receiving weak synaptic input, to current injections when neurons were in an "up" state, receiving strong synaptic input. Our results show that for many neurons, when a strong depolarizing event occurred prior to the 20ms current pulses, less current was required to initiate a spike (mean = 100pA, SD = 35.0pA). However, the rheobase measurement for many neurons remained invariant to membrane potential fluctuations. In tandem with these findings, we have made voltage-clamp recordings in a subset of these neurons to measure the currents that neurons receive during visual stimulation. Through optogenetic inactivation of cortex, we have measured the thalamic contribution to visual cortical neurons is small (mean = 20pA SD = 11.0pA), while the total drive to these neurons is on average 60pA (SD =20.4pA). Taken together, our results suggest that many neurons in cortex exist in a fluctuation-driven state, in which synaptic input during visual stimulation results in variable spike output. In addition, our rheobase measurements alone provide an estimate of cortical excitability under spontaneous conditions and suggest that there is diversity in sensitivity to small changes in input.

Disclosures: L. Jennings: None. **N.J. Priebe:** None.

Poster

PSTR281: Visual System: Response Modulation and Adaptation

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Topic: D.06. Vision

Support: NIH Grant R01 EY013588
NIH Grant P30 EY012576

Title: Quantifying the relationship between receptive field properties and response sparseness in V1

Authors: *H. J. ALITTO[1,2], M.-L. TRAN[3], W. USREY[3];
[1]Univ. of California Davis, Davis, CA; [2]Center for Neuroscience, University of California, Davis, CA; [3]Ctr. for Neurosci., Univ. of California, Davis, CA

Abstract: Primary visual cortex (V1) is the initial stage of cortical processing in the visual system, receiving retinal information via several parallel pathways within the lateral geniculate nucleus (LGN) of the dorsal thalamus. Based on decades of research, a strong model has emerged of V1 based on an array of divisive normalization modulated Gabor filters that distribute visual signals to a variety of higher-order, extrastriate cortical areas. These elegant linear-nonlinear (LN) models provide a satisfyingly intuitive understanding of V1 receptive fields; however, they fail at predicting the response of V1 neurons to arbitrary stimuli, including naturalistic stimuli, and have not been extended in a sophisticated fashion to account for network dynamics. More recent models that have employed convolutional neural networks (CNN) have been more successful at predicting V1 responses to novel stimuli; however, the "black box" nature of these models has allowed the underlying neural mechanisms and receptive field properties to remain enigmatic. The overarching goal of our research is to unify the concepts of single cell receptive fields and interneuronal network dynamics, with an eye toward bridging the gap between older Gabor models and newer CNN models.
As part of our research, we have explored the relationship between single cell receptive fields, and response sparseness in V1. Towards this aim we have recorded from two awake, behaving macaque monkeys using 128-channel laminar probes positioned in the operculum of V1 (parafoveal region, ~4°-6° eccentricity). By presenting a combination of synthetic and naturalistic stimuli we probed the interaction of receptive field properties and population dynamics, quantifying how certain population dynamics can be explained by stimulus dependent changes in classical and nonclassical receptive field properties. Our data establishes a link between response sparseness and receptive field properties including orientation bandwidth, contrast gain control, and extraclassical suppression. Further, we predict that contrast dependent changes in response sparseness can be modeled as a consequence of contrast dependent summation fields. Current efforts are focused on determining the benefits of "direct" model

parameter mapping vs “random estimation”. Finally, we are estimating the relative contributions of individual model parameters to residual error in order to determine the factors that limit the generalizability of Gabor models to naturalistic stimuli.

Disclosures: H.J. Alitto: None. **M. Tran:** None. **W. Usrey:** None.

Poster

PSTR281: Visual System: Response Modulation and Adaptation

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Topic: D.06. Vision

Support: NIH Grant R01GM143545
NIH Grant 1DP2EY022584

Title: Characterizing the expression patterns of sex steroid hormone receptors in ipRGCs and other retinal neurons

Authors: *E. CHAVEZ, K. C. MIGUEL, M. ARANDA, T. M. SCHMIDT;
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Abstract: Retinal function has been shown to differ across sexes and to fluctuate based on hormonal status. There is increasing evidence that visual behaviors are also sex dependent. Specifically, circadian photic responses, which are mediated by intrinsically photosensitive retinal ganglion cells (ipRGCs), are known to be sex- and estrus phase-dependent. However, it is unclear whether these responses are modulated at the level of photoreception in the retina. Sex steroid hormone (SSH) receptors are expressed in the retina, but the specific expression patterns of these receptors are unknown. Identifying the specific retinal cell types that express each SSH receptor would lend insight into which retinal circuits could be modulated by sex or the estrus phase. We aimed to characterize the cell type-specific expression patterns of SSH receptors and quantify expression across sexes and the estrus cycle in ipRGCs. We used RNAscope in retinal sections to visualize the expression pattern of 5 SSH receptor genes: Estrogen Receptor 1 (Esr1), Estrogen Receptor 2 (Esr2), G-protein Coupled Estrogen Receptor 1 (Gper1), Progesterone Receptor (Pgr), and Androgen Receptor (Ar). This was done in retinal slices of both male and female mice. Esr1 expression is predominantly localized to the inner nuclear layer, particularly in SEG amacrine cells. Esr2 does not show any detectable expression in the retina. Gper1 is expressed sparsely in both the ganglion cell layer and the inner nuclear layer. Pgr and Ar are widely expressed in the ganglion cell layer and are both expressed in ipRGCs. We observed a difference in Pgr and Ar expression in ipRGCs between males and females in sexually receptive and non-sexually receptive estrus phases. Overall, this data indicates that SSH receptors show sex-dependent and cell-type specific retinal expression patterns.

Disclosures: E. Chavez: None. **K.C. Miguel:** None. **M. Aranda:** None. **T.M. Schmidt:** None.

Poster

PSTR281: Visual System: Response Modulation and Adaptation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR281.13/E30

Topic: D.06. Vision

Title: Non-saturating contrast sensitivity curves for dark contrasts in primary visual cortex but not superior colliculus neurons

Authors: *C. TROTTENBERG[1,2], M. BAUMANN[3], Y. YU[4], T. MALEVICH[5], T. ZHANG[6], Z. M. HAFED[7];
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Abstract: The superior colliculus (SC) guides our eye movements to regions of interest in the form of saccades, and it is thus one of the more important brain regions involved in our day-to-day active vision. The large majority of the sensory information that the SC uses to accomplish this comes from the primary visual cortex (V1). Nonetheless, the neural signal transformation that happens from V1 to the SC is not thoroughly understood. Here, we aimed to further the understanding of this inter-regional communication by comparing SC and V1 visual burst activity with regard to contrast sensitivity and luminance polarity preferences. We analyzed neuronal recordings from three macaque monkeys that performed an active vision task in the form of reflexive visually-guided saccades. After initial fixation, a target disc of ~0.5 deg radius appeared within the response field of a neuron, and the fixation spot was extinguished simultaneously. Across trials, the target varied both in contrast and luminance polarity (dark versus bright) compared to the uniform task background. We found that both SC and V1 neurons generally prefer dark rather than bright contrasts, as evidenced by either earlier visual burst onsets or stronger visual bursts, but not necessarily both. Moreover, in both areas, we found neurons reacting faster for one stimulus polarity (e.g. bright) but stronger for the other (e.g. dark), suggesting a dissociation between neuronal response latency and response strength in both brain areas. V1 neurons also appeared to have stronger specialization than SC neurons: on average, the dynamic range in response latency or strength between the preferred and non-preferred contrast was larger for V1 neurons than for SC neurons. Next, we analyzed contrast sensitivity curves. While both areas generally showed increasing visual burst strenghts with higher contrasts, we were much more likely to encounter non-saturating contrast sensitivity

curves in V1 than in SC, and these non-saturating contrast sensitivity curves were most frequent for dark but not bright contrasts. Thus, shadows are represented in a more graded fashion than highlights in V1, and this asymmetry is not present in the SC population. These results suggest that somewhere along the visual processing pipeline from V1 to SC, some of the variability in stimulus representation is removed. We hypothesize that the graded representation of dark stimuli in V1 might help with jump-starting accurate image analysis in higher cortical areas, whereas the more step-like representation in the SC might help with detecting potential saccade targets of interest, with no need to much more accurately represent the exact stimulus shadings.

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Poster

PSTR281: Visual System: Response Modulation and Adaptation

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Title: Enhancing 3t retinotopic mapping precision: diffeomorphic registration and cross-validation

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Abstract: In the realm of visual cognitive neuroscience, retinotopic mapping plays a pivotal role in deciphering how the brain processes visual stimuli. However, despite its significance, this process heavily relies on BOLD functional magnetic resonance imaging (fMRI), which is hampered by low signal-noise ratio (SNR) and inadequate spatial resolution. These inherent limitations impede the creation of precise and reliable retinotopic maps. This research applies an innovative approach known as Diffeomorphic Registration for Retinotopic Maps (DRRM; Tu, et al, 2022) to improve the alignment of retinotopic maps. Utilizing the 3T NYU Retinotopy Dataset, which includes analyze-PRF and mrVista results, DRRM aims to overcome the challenges posed by traditional methods. By quantifying the diffeomorphic condition, it ensures accurate alignment of retinotopic maps while maintaining topological integrity through the

utilization of the Beltrami coefficient. Applying DRRM to the 3T NYU retinotopy dataset, we observed notable improvements in retinotopic map alignment and predictive accuracy compared to traditional methods. Visual inspection revealed refined alignment of retinotopic maps, evident in the absence of flipping triangles and enhanced topological integrity. Quantitative assessment confirmed DRRM's superior performance. Across 41 observers, DRRM achieved an average registration error (RMSE) of 1.129 for the left hemisphere and 1.181 for the right hemisphere, outperforming alternative mapping techniques. Pearson correlation coefficients were also notably enhanced, reaching 0.352 for the left hemisphere and 0.294 for the right hemisphere. Cross-validation revealed consistent predictive performance for the BOLD time series, with an average Pearson correlation of 0.168 and RMSE of 1.275 across 12 visual areas. Furthermore, a detailed analysis focusing on V1, V2, and V3 highlighted DRRM's consistent and superior performance across hemispheres. In summary, our study underscores the utility of DRRM as a valuable tool for enhancing the quality of retinotopic maps within the domain of 3T fMRI data. The successful integration of DRRM in our research showcases its potential to advance retinotopic map research and applications. By overcoming challenges associated with the limited signal-to-noise ratio and spatial resolution of BOLD fMRI, DRRM offers a promising avenue for improving the accuracy and interpretability of retinotopic maps.

Disclosures: N. Jalili Mallak: A. Employment/Salary (full or part-time):; Arizona State University. **Z. Lu:** A. Employment/Salary (full or part-time):; New York University, NYU Shanghai. **Y. Wang:** A. Employment/Salary (full or part-time):; Arizona State University.

Poster

PSTR281: Visual System: Response Modulation and Adaptation

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Topic: D.06. Vision

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China Scholarship Council

Title: Decision signals in the absence of spiking activity in macaque visual cortex

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Abstract: Choice probability (CP) offers a way to relate trial-to-trial fluctuation in neural responses to behavioral outcomes. However, whether CP indexes a causal feedforward influence of neural activity on decisions or if CP reflects a non-causal feedback representation of decisions is still debatable. To examine these possibilities, we inactivated spiking activity in a small patch

of the visual cortex, using the GABA agonist muscimol, while non-human primates performed various visual discrimination tasks. Because long-range communication in the brain relies on spikes, this manipulation precluded a causal influence of neural activity near the site of the inactivation. We then evaluated CP in the local field potentials (LFPs), which index nearby synaptic activity. Injection of muscimol into MT or V4 usually led to strong decrements in behavioral performance for motion discrimination (MT) and shape discrimination (V4) tasks. In these cases, we observed an inverse correlation between the power of beta oscillation (13-20 Hz) and the proportion of preferred stimulus decisions, so that CP values were on average below 0.5. This contrasted with the baseline conditions, in which beta oscillatory power positively correlated with these behavioral decisions, yielding CP values that were above chance. As reported previously (Liu & Pack, 2017) small changes in the stimulus or the training procedure often reduced or abolished the effect of muscimol injections on behavior. In these cases, the pattern of CP observed in the beta band was not observed, and instead, we found that muscimol injection actually increased CP values in the alpha LFP band (8-12 Hz), so that decisions were, on average, positively correlated with alpha power. These findings illustrate that CP generated by LFP, which reflects neural activity that is not propagating out of the local cortical circuit, can persist even in the absence of spiking activity within a cortical domain, relying on non-causal correlations with neural oscillations that vary with task engagement.

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Poster

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Title: Subdivisions of the midbrain reticular nucleus contain distinct connectivity, gene expression, and roles in context-dependent perceptual decision-making

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Abstract: A hallmark of mammalian behavior is the ability to rapidly remap actions in response to sensory stimuli depending on internal representations of the environment. Studies of the circuitry underlying context-dependent perceptual decision-making have classically focused on

frontal cortex, basal ganglia, superior colliculus (SC), and the recurrent connections between them. Here, we dissect a previously unidentified node within this circuitry: the midbrain reticular nucleus (MRN). Previous studies have implicated MRN in motor control, including locomotion, eye and limb movements, and releasing cue-triggered actions. It remains unclear how function localizes spatially within MRN and whether MRN plays a role in higher-order functions beyond motor control. Additionally, the connectivity and gene expression throughout MRN, and their potential alignment with function, is unknown. We first tested the hypothesis that MRN encodes abstract decision-related variables. Mice were trained on a novel reverse contingency task, wherein identical visual stimuli required opposing actions for reward depending on the uncued Block context. Mice successfully remapped sensorimotor contingencies between Blocks, which switched 8-15 times per session. Neuropixels 2.0 recordings were performed densely throughout MRN as well as SC, secondary motor cortex (MOs), caudoputamen (CP), and other structures (n=5 mice, 38 sessions). A single neuron encoding model, population decoding, and manifold trajectory analysis all revealed a significant representation of context in MRN, SC, MOs, and CP. The MRN also exhibited training-dependent plasticity: Representations of the specific task visual stimuli were found in trained, but not task-naive (n=6 mice, 22 sessions), mice. To assess MRN connectivity and cell types, we used open datasets, viral tracing, and whole-brain imaging. We found that MRN is densely interconnected with perceptual decision-making related circuitry, with inputs from substantia nigra pars reticulata, SC, and motor/somatosensory/retrosplenial cortex, and outputs to mediodorsal thalamus, SC, and brainstem motor centers. The input connectivity was topographically organized, and strikingly, aligned with context and motor representations within the space of MRN. Furthermore, spatial transcriptomic data from MRN revealed a clear spatial organization of cell types, which we integrate with the anatomical and functional data to provide a new understanding of the sub-regional organization of MRN.

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Poster

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Topic: D.06. Vision

Title: Selective effects of ongoing alpha-band activity on magno- and parvo-mediated detection

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Cruz, CA; [3]UC Santa Cruz, Camarillo, CA; [4]UC San Diego, San Diego, CA; [5]UC Santa Cruz, Santa Cruz, CA

Abstract: Spontaneous occipital alpha-band activity (8-12 Hz) has been shown to influence perceptual variability, leading one to report seeing a stimulus more often during states of weak alpha power, likely due to a shift in detection criterion. However, prior work has paid little attention to the specific stimulus properties mediating detection. In early vision, different stimulus properties are preferentially processed along the magnocellular (MC) and parvocellular (PC) pathways, which vary in their preference for spatial and temporal frequency and chromatic information. The goal of this study was to understand how spontaneous alpha power effects the detection of stimuli which are preferentially processed by either the MC or PC pathway. To achieve this, we used the "Steady/Pulsed Paradigm" which presented a brief, near-threshold stimulus in two conditions intended to bias processing to one or the other pathway. The pulsed condition presents the target stimulus atop a luminance pedestal, whose transient onset is believed to saturate MC firing and bias detection to the PC pathway. In the steady condition, which more closely resembles canonical detection paradigms, the luminance pedestals are present throughout the entire trial which is thought to evoke a sustained response from the PC pathway, biasing detection towards the MC pathway. Our results showed an interaction effect of alpha power on detection between the two conditions. While weak alpha power was predictive of seeing the stimulus in the steady condition (MC-biased), no significant effect was found in the pulsed condition (PC-biased). This interaction was driven by selective alpha-related criterion shifts between the two tasks, suggesting that alpha oscillations may deferentially regulate excitability in the MC and PC pathways.

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Poster

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Topic: D.06. Vision

Support: National Natural Science Foundation of China
Beijing Natural Science Foundation

Title: Cognitive control of instinctive decision-making in mice

Authors: *Z. LI[1,2], J. WANG[1], Y. SUN[1], J. LI[1], L. LI[3], Y.-T. LI[1];
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Abstract: When confronted by a predator, most animals make instinctive decision-making with a rapid reaction time, a trait shaped by natural selection to maximize survival chance. However, in complex and dynamic environments, a swift response is meaningful only when grounded on accurate judgments and correct decisions, which often involve cognitive control. Here we investigated how the decision to an overhead approaching threat during foraging is influenced by prior experience, reward, and social hierarchy in mice. Our results demonstrate that mice quickly learn from experience and make economic decisions by balancing risk and reward, and the decision is modulated by social status. Specifically, we observed both habituation and sensitization to the threat for the first five trials. When a reward is offered at the end of the linear track, mice show increased hesitancy to flee back to their nest, indicated by increased immobile duration. In addition, foraging mice are more vigilant than those exploring, evidenced by reduced detection latency and increased flight speed. This context-dependent decision-making is further shaped by social hierarchy, with dominant mice exhibiting heightened vigilance relative to subordinate peers.

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Poster

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Program #/Poster #: PSTR281.19/E36

Topic: D.06. Vision

Title: Communication between visual and executive areas is modulated post-locomotion in Rhesus macaques

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Abstract: Visual perception is a crucial sensory modality through which animals experience their surroundings and environment, and integration of incoming sensory information through parallel processing streams supports higher-order cognition and decision-making. While spatially navigating their environment via locomotion, nonhuman primates (NHPs) utilize perceptual information, such as distinct visual characteristics or cues to guide decision-making and executive function. Physical activity has been shown to benefit cognition by improving cerebrovascular perfusion and regulating hormone-mediated processes on a systemic level; however, the acute effects of physical activity such as locomotion on behavior and the neuronal circuits underlying communication between visual processing and executive function have yet to

be fully characterized. We performed high-yield electrophysiological recordings simultaneously from area V4, a mid-tier visual cortical region, and the dorsolateral prefrontal cortex (dlPFC) as the animal performed a visually-guided decision-making task following a period of sustained physical activity, finding that communication and feedback between V4 and dlPFC is modulated post-physical activity.

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Poster

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Topic: D.06. Vision

Support: NINDS RF1NS132910
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Title: Posterior Parietal Cortex Reflects Confidence-Dependent Changes in Decision Strategy

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Abstract: In our ever-changing world, we must learn from experiences for better future decisions. Decision confidence, the belief that a choice is correct, is thought to be crucial for adjusting decision strategies after feedback. The lateral intraparietal area (LIP) represents accumulated evidence in the form of a decision variable (DV) that also predicts decision confidence. Yet, whether and how LIP plays a role in utilizing previous confidence to influence the current decision process is unknown. We hypothesized that LIP maintains signals related to confidence on a previous trial, allowing it to mediate confidence-dependent choice bias and adjustments of reaction time (RT) in the subsequent trial. We trained two rhesus monkeys to perform a novel version of the random-dot motion task that measures choice, RT, and confidence on every trial ("peri-decision wagering"). The monkeys viewed a dynamic random-dot stimulus and reported both the perceived direction of motion and a wager on the decision outcome with a saccadic eye movement to one of four targets: left-high, left-low, right-high, or right-low. The wager served as a reliable indicator of their confidence in the direction decision on that trial, as validated by behavioral analyses. We found that both monkeys were more biased to repeat a previously rewarded choice when they reported lower confidence in the previous trial, as anticipated by previous work. Furthermore, larger changes in RT were observed after a less

expected outcome, i.e. a high-confidence error. LIP population activity before and after trial onset encoded the previous trial's choice and wager, and did so more strongly after a low-confidence rewarded trial. Moreover, stronger encoding of trial history predicted a larger choice bias. To characterize the population dynamics, we used a support vector machine to construct a neural DV from LIP activity on each trial. The monkeys' individual patterns of post-error RT were reflected in the slope of the neural DV (a correlate of the rate of evidence accumulation), and previous confidence further modulated this slope following an unexpected outcome (low-confidence reward or high-confidence error). In one monkey, the DV showed a higher starting point and lower ending point after a high-confidence error, suggesting choice bias and early stopping of the accumulation process counters the slowing-down effect from the reduced rate of evidence accumulation. In summary, this work suggests LIP could mediate confidence-guided learning and trial-by-trial strategy adjustments in perceptual decision-making.

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Poster

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Topic: D.06. Vision

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Title: Graded encoding of task context and its generalization in prefrontal cortex during face categorization

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Abstract: Our brains must use a variety of rules that associate sensory inputs and behavioral outputs in different behavioral contexts. The prefrontal cortex (PFC) encodes such task rules and shows distinct neural dynamics reflecting decision formation under different task rules. Previous studies have investigated these context-dependent dynamics using task paradigms that require subjects to switch between orthogonal rules, such as motion vs. color categorization. However, the rules we use to associate stimuli and responses in the real world are not always orthogonal but are often similar across contexts and even generalizable to new contexts. Here, we examined context-dependent signals in the ventrolateral PFC while a monkey switched between two similar face categorization tasks that had a common rule and generalized the rule to new tasks. In each task, we created face stimuli by morphing a human and a monkey face. Monkeys were

trained to categorize each face by choosing one of two targets corresponding to the two prototype faces. Thus, all the tasks were human vs. monkey face categorization, but because a specific facial identity was used as a prototype in each task, the monkey could improve its discrimination performance by relying on fine features diagnostic of the prototypes of each task. While the monkey performed the tasks, we recorded population neural activity from the ventrolateral PFC with a 96-channel Utah array. Analysis of the population response geometry reveals rotational dynamics and a curved manifold during decision formation, consistent with previous studies (Mante et al., 2013; Okazawa et al., 2021). In addition to these dynamics, we found distinct encoding of task context between the two tasks in the PFC population responses despite their common rule. This task-dependent signal cannot simply be explained as visual responses to different face stimuli, as it persisted after stimulus offset and differed from activity during passive viewing. We then introduced a third task with a new pair of prototypes to examine rule generalization. The monkey was able to generalize the rule to the new task with a small reduction in performance, but the PFC showed stronger context-dependent signals. As the monkey improved its performance, the signals gradually weakened and converged to the level we observed in the previously learned task. Altogether, we propose that the PFC encodes task contexts in a graded manner, showing differences in activity even under a common task rule depending on subtle differences in task conditions, allowing accurate learning, categorization, and generalization of perceptual tasks

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Poster

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National Natural Science Foundation of China 32171034
National Natural Science Foundation of China 32061143003
Israel Science Foundation 3318/20

Title: Dynamics of population neuron response to heading perception in different multisensory cortical areas

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Abstract: Neurons in multisensory cortices involved in heading perception, respond to multiple task-relevant variables, for example heading direction and choice. However, that makes it difficult to distinguish the functional properties of these neuronal populations. In our study, we recorded neuronal activity from the ventral intraparietal cortex (VIP), dorsal medial superior temporal cortex (MSTd), and parietoinsular vestibular cortex (PIVC) in 6 adult male rhesus macaques during a heading discrimination task. We applied a targeted dimensional reduction analysis by projecting the activity of each neuronal population onto a low-dimensional subspace, defined by dynamic motion parameters. We found VIP has a strong choice component, acceleration is not so clear in visual condition, but present in vestibular condition. MSTd has strong velocity component in visual condition, but also has the other two components in vestibular condition. PIVC has weak visual response, but a strong acceleration component for vestibular condition. Our results show that dynamic motion variables can be decoded from the different cortical populations, in accordance with the prevalence of the signals in each area.

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Poster

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Support: NSF NCS-2219876

Title: Sensory integration strategies exhibit distinct state-dependent dynamics

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Abstract: The perception of sensory stimuli is influenced by underlying cognitive or behavioral variables, known as internal states. Much like how a student's attention can fluctuate throughout the course of a class, internal states can fluctuate even during the course of a single experimental session. The inability to sustain engagement is a hallmark of many neuropsychiatric diseases and learning disorders such as Attention Deficit Hyperactivity Disorder (ADHD), leading to difficulties in learning, working memory, and sensory processing. Task-engagement directs attentional resources to relevant stimuli, which enhances the perception and encoding of sensory information. However, little is known about how engagement can impact the downstream computations necessary to integrate accumulated sensory evidence. To investigate this, we trained wild-type mice (WT) and mice with a heterozygous deletion of the dopamine transporter (DAT +/-) as a model of ADHD to perform a freely-moving evidence accumulation task. Mice are presented with a sequence of poisson-distributed stimuli and must determine whether the rate

of the stimulus is above or below an experimenter-defined category boundary. We then fit a hidden Markov Model coupled to a generalized linear model with Bernoulli emissions (GLM-HMM) to characterize latent states in WT and DAT +/- mice. We first verified that, as in previous studies, WT mice alternate between engaged and disengaged states. Interestingly, DAT +/- mice occupy a significantly greater proportion of disengaged states compared to WT mice and exhibit more frequent transitions between engaged and disengaged states. To probe the dynamics of sensory integration, we used psychophysical reverse correlation to calculate a time-varying kernel that captures the weight of sensory information on the animal's upcoming decision. WT mice exhibit distinct and consistent state-dependent differences in the strategy of evidence accumulation. Engaged decision-makers strongly and transiently prioritize early sensory evidence, while disengaged states exhibit decreased sensory weight across all time bins. Comparatively, DAT +/- mice show no transient prioritization of sensory information during engagement, and exhibit heterogeneity and idiosyncratic differences in sensory weight between states. Together, these findings reveal that sensory integration is altered in a state-dependent manner. These results highlight how increased fluctuations in engagement and unique temporal dynamics in sensory processing may underly behavioral and cognitive differences in ADHD.

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Poster

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Topic: D.06. Vision

Title: Decoupling choice from motor response reduces choice-history effects

Authors: *S. THOTHATHRI[1], B. TALLURI[2], S. SHUSHRUTH[3], M. N. SHADLEN[4], H. NIENBORG[5];
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Abstract: Perceptual decisions exhibit dependencies over time, a phenomenon known as serial dependencies or choice-history effects. Recent studies showed that choice-history effects depend on contributions from both previous perceptual choices and the motor responses used to report them. However, it is unclear whether the magnitude of modulation by previous choices depends on the coupling of perceptual choice to motor responses. To explore this, we analyzed datasets from non-human primates (macaca mulatta) performing two different perceptual decision-making tasks: disparity discrimination (DD) and random dot motion direction discrimination

(MD). Each task had a “coupled” variant where the mapping between perceptual choice and the motor response to report the choice was fixed, and an “uncoupled” variant where the mapping between perceptual choice and the motor response was varied randomly across trials. Critically, the choice-response mapping in the uncoupled variant was revealed to the animal only after stimulus presentation in each trial. Our dataset comprised eight animals (mean # of trials per animal = 40380), with two animals doing each task variant (2 tasks x 2 variants per task x 2 animals per task variant). Using regression models fit on choice behavior, we tested whether the strength of the choice-history bias depends on the coupling between perceptual choice and motor response. We found that, in both tasks, animals in the coupled variant had a larger magnitude of choice history bias (mean bias across animals = 8.34% signal coherence; bias quantified as the horizontal shift of the psychometric curve conditioned on previous choices, i.e. curves for negative minus positive previous choice) compared to animals in the uncoupled variant (mean bias across animals = -2.51% signal coherence). We quantified the choice prediction performance of previous choices in pure-noise (0% signal) trials as choice correlations (prediction performance was computed as the area under the receiver-operating characteristic curve on pure-noise trials and converted into choice correlations). The choice correlations were significantly higher in coupled tasks than in uncoupled tasks (mean choice correlations comparing coupled vs uncoupled tasks: in DD = 0.2 vs 0.05, p = 2.29e-09 & in MD = 0.22 vs 0.03, p = 2.37e-05; p-values computed using Wilcoxon Rank Sum test). This suggests that choice-history effects are only minimally driven by abstract categorical choices. Instead, they are driven by the action plans used to report them. Our findings support the idea that decisions represent intentions to pursue a course of action rather than abstract computations.

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Poster

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Title: Subcortical projecting neurons in mouse visual cortex encode sensory information during decision making

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Abstract: The mammalian cerebral cortex plays a central role in decision-making and different visual cortical regions have unique functional roles in processing visual information. Further, cortical excitatory pyramidal neurons (PyNs) have rich diversity, but the unique roles of different PyN types across primary and secondary visual areas in decision-making remains unclear. Recent work from our lab shows the particular importance of cortical output neurons, which reside in layer 5b (L5b), during perceptual decision-making—especially in the anterior visual areas (AM/A). Nonetheless, whether L5b PyNs in anterior visual areas serve a specific role during decision making is not known. Here, we address this gap in knowledge by characterizing the functional activity of AM L5b PyNs during visual decision-making and comparing with cells in AM L2/3 as well as V1 L5b and L2/3. We retinotopically mapped visual cortex and used 2-photon calcium imaging to record neural activity during passive visual stimulus viewing and while mice make perceptual decisions in a spatial and temporal accumulation of visual evidence task. In the task, visual patches appear in stochastically different screen locations. Head-fixed mice must judge the side of higher rate, hold their choice for a brief delay period, and report by licking. We recorded from over 6,000 cells in 7 mice (over 3000 in AM and V1), out of which approximately 60% were responsive during the task. We used full-field gratings to measure responses from L5b AM cells and found sharp orientation tuning and selectivity, similar to cells in V1 or other layers in AM. During the task, we found that L5b neurons in AM are stimulus selective: individual neurons respond to stimuli corresponding to one choice option but not the other. Stimulus selectivity during the task was not restricted to neurons in L5b of AM and selectivity was also observed in the response period. In future work we will determine whether this signal reflects cognitive or movement related aspects of choice reporting. Overall, we conclude that AM transmits information about the visual stimuli during evidence presentation in perceptual decision-making. Our results support the hypothesis that the interplay between cortical and subcortical areas is essential for the sensory evidence processing that enables perceptual decision-making.

Disclosures: L. Wilkins: None. **J. Couto:** None. **A. Khanal:** None. **A.K. Churchland:** None.

Poster

PSTR281: Visual System: Response Modulation and Adaptation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR281.26/F3

Topic: D.06. Vision

Title: Spatiotemporal properties governing the utilization of evidence for both choice and confidence in a perceptual decision

Authors: *M. VIVAR-LAZO[1], C. R. FETSCH[2];
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Abstract: Previous studies of confidence in perceptual decisions have converged on two predictions derived from accumulator-to-bound models. First, overlapping time periods of evidence accumulation should in parallel support both choice and confidence. Secondly, a balance of evidence (BOE) for and against a given option should support both choice and confidence. Yet, other studies have cast doubt on these fundamental characteristics, raising the possibility of a distinct mechanism for confidence. These studies have suggested that a BOE informs choice, but in contrast, confidence is informed by primarily the evidence that supports the selected option, known as a positive evidence bias (PEB). These contrasting mechanisms highlight the perplexing state of our understanding between sensory systems and their support of choice and confidence. To address this problem, we designed a peri-decision wagering (Peri-DW) task that simultaneously measures choice and confidence in visual motion discrimination. Monkeys made a saccadic eye movement to one of four targets arranged in a square: the left-right axis indicating the perceived motion direction and the up-down axis indicating a wager on the choice outcome. The decision was initiated at the monkey's discretion, thereby providing three measurements of the decision process: choice, reaction time, and confidence.We first show that deliberation about choice and confidence occurs concurrently in time. Reaffirmation of this theoretical prediction provides support for an accumulator-to-bound model. We found that lateral intraparietal area neurons (LIP; N=430 neurons) display temporal dynamics supporting a parallel updating of choice and confidence. To test the spatial contributions to choice and confidence we fit a generalized linear model (GLM) to a population of neurons recorded from visual areas MT & MST (N=406 neurons). Preliminary results suggest a contribution from strictly neurons whose preferred direction matches the choice outcome for confidence, reflecting a PEB. However, the initiation of the PEB phenomenon comes after the readout of choice, hinting at possible feedback contribution. Leveraging an extension to the GLM and simultaneous recordings from LIP and MT, we are able to infer functional feedback contributions from LIP to MT. This could reflect possible influences from the belief of the current choice onto the sensory representation, an idea previously postulated using hierarchical inference. Ultimately, we reconcile these differences by extending the accumulator-to-bound model in incorporating approximate hierarchical inference principles to explain the behavior and the neuronal results.

Disclosures: M. Vivar-Lazo: None. **C.R. Fetsch:** None.

Poster

PSTR281: Visual System: Response Modulation and Adaptation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

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Topic: D.06. Vision

Support: U19NS118246

Title: Eye movements as a window into the mind's strategy for tracking moving targets

Authors: *B. MUSANGU[1], J. VASTOLA[1], V. VENCATO[2], J.-P. NOEL[2], G. C. DEANGELIS[3], D. E. ANGELAKI[2], J. DRUGOWITSCH[1];
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Abstract: Selecting adaptive actions in noisy, dynamic environments requires continuously updating one's beliefs about the state of the environment. Understanding such action selection is challenging as it requires estimating beliefs, which are generally internal to the actor and so not directly observable. Past work has demonstrated that eye movements can be informative about the evolution of these beliefs (Lakshminarasimhan et al., 2020). In particular, eye movements of humans and monkeys navigating in a virtual reality environment to an invisible target have been shown to track the remembered location of this target. However, it was unclear whether eye movements indeed tracked the remembered target location through time or simply the actor's desired navigational end-point, which were in this case equivalent. To disambiguate these alternatives, we analyzed eye movement strategies of human participants in a task in which the target was either stationary - as before - or moving relative to the scene. In both cases, the target was visible only briefly at the beginning of each trial, and participants needed to either navigate to the target's location if the target was stationary, or intercept it if it was moving. Imposed self-motion during target presentation induced further ambiguity about the target's velocity in the world. As in previous work, we observed initial saccades toward the target's location while the target was visible, followed by smooth pursuit eye movements. Leveraging our task design, we asked whether eye movements either tracked the hidden target's location through time or instead focused on the expected location of target interception. Model comparison revealed the hidden target's location to be a significantly better predictor of the participants' eye movement throughout the trial than the target's interception point. The analysis furthermore revealed speed-dependent target tracking biases that might provide further insights into participants' beliefs while performing these tasks, but the origin of these biases requires further study. Overall, our work underlines the utility of using eye movements to gain insights into latent belief dynamics that would not be accessible otherwise.

Disclosures: B. Musangu: None. **J. Vastola:** None. **V. Vencato:** None. **J. Noel:** None. **G.C. DeAngelis:** None. **D.E. Angelaki:** None. **J. Drugowitsch:** None.

Poster

PSTR281: Visual System: Response Modulation and Adaptation

Location: MCP Hall A

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Topic: D.06. Vision

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Boston University (RND)

Title: Anticipatory dynamics of temporal expectation and voluntary temporal attention

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Abstract: Motivation: We can often anticipate the precise moment when a stimulus will be relevant for our behavioral goals. Voluntary temporal attention, the deliberate prioritization of sensory information at task-relevant time points, helps us see better at relevant times. How does the brain anticipate and select a relevant moment in a temporally precise manner? Anticipatory activity including fronto-parietal ramping and periodic modulations of sensory cortex in the delta band precede predictable and relevant moments. However, whether these anticipatory mechanisms are specific to voluntary temporal attention is unclear, because previous neural studies have not attempted to isolate its influence from that of temporal expectation, which reflects timing predictability rather than relevance. Voluntary temporal attention has been shown to improve behavioral performance and affect microsaccades over and above the effects of temporal expectation, suggesting dissociable neural mechanisms. Here we used time-resolved steady-state visual evoked responses (SSVER) to investigate how temporal attention dynamically modulates visual activity when temporal expectation is controlled. Methods: We recorded MEG while human observers (n = 10 x 2 sessions) performed a challenging perceptual discrimination task. Observers directed temporal attention to one of two sequential grating targets according to an auditory precue (75% validity) and reported the tilt of one grating according to an auditory response cue. On every trial, the targets were fully predictable in time following the precue, but the attended time point varied trial-to-trial. Meanwhile, we used a co-localized SSVER noise probe to continuously track visual cortical modulations leading up to the targets. Results: We found both ramping and a low-frequency (~2 Hz) periodic modulation of the SSVER that anticipated the arrival of the targets, tied to temporal expectation. Furthermore, the low-frequency modulation shifted in phase according to which of two time points was attended. Thus, temporal attention flexibly coordinates visual cortical excitability to proactively prioritize sensory information at precise moments.

Disclosures: K. Tian: None. **D.J. Heeger:** None. **M. Carrasco:** None. **R.N. Denison:** None. **K.J. Tian:** None.

Poster

PSTR281: Visual System: Response Modulation and Adaptation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR281.29/F6

Topic: D.06. Vision

Support: Boston University

Title: Decoding the dynamics of neural uncertainty using EEG

Authors: *J. NESTOR, R. N. DENISON;
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Abstract: Motivation: Observers are constantly faced with the challenge of uncertainty in their estimations of the state of the world. For example, when driving on a foggy day you might be more uncertain about how far the car in front of you is compared to when the day is clear. Even for the same physical stimulus, uncertainty may vary across presentations or across time due to internal noise. Functional magnetic resonance imaging (fMRI) studies support the approach of using model-based Bayesian classifiers to decode trial-by-trial probability distributions from population-level neural responses and show that uncertainty estimates derived from these distributions are related to human behavior. However, the poor temporal resolution of fMRI prevents these studies from investigating how probability distributions decoded from neural population activity evolve over time, which may illuminate the dynamics with which neural uncertainty unfolds during perception and decision making. Here we assess the feasibility of extending population-based uncertainty decoding to electroencephalography (EEG).
Methods: We recorded EEG data while participants fixated at the center of a screen and visual grating stimuli appeared at various locations around fixation at a fixed eccentricity. We implemented a decoding method based on TAFKAP (van Bergen & Jehee, 2021), developed for fMRI, training separate decoders at each timepoint. Stimulus-evoked activity at each EEG electrode was modeled as a linear combination of a set of hypothetical tuning curves which tiled the feature space. A generative model for the electrode response was estimated from training data to describe a conditional sampling distribution for the observed data given any possible spatial location. We then derived a probability distribution across possible locations whose center was the classifier's point estimate and whose width was the classifier's uncertainty.
Results: The trained classifiers decoded stimulus location above chance following stimulus presentation. The time course of decoded uncertainty mirrored the time course of decoder accuracy. In addition, decoded uncertainty correlated with decoder accuracy on a trial-by-trial level within time windows where accuracy was above chance.
Conclusion: Successful decoding supports the feasibility of probabilistic decoding methods for EEG, and trial-by-trial correlation between uncertainty and accuracy supports the validity of the uncertainty measure. These findings open up new possibilities for future studies which examine the temporal dynamics of probabilistic stimulus representations in the brain.

Disclosures: J. Nestor: A. Employment/Salary (full or part-time):; Boston University Psychological and Brain Sciences. **R.N. Denison:** A. Employment/Salary (full or part-time):; Boston University Psychological and Brain Sciences.

Poster

PSTR282: Cortical Pathways of Visual Perception

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR282.01/F7

Topic: D.06. Vision

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Title: Pareidolia is associated with changes in causal interactions among visual, salience, and sensory-motor networks

Authors: *T. KONDO[1], K. YOSHINAGA[2], T. HANAKAWA[2];
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Abstract: Pareidolia is the tendency to interpret a nebulous stimulus like wall stains as a meaningful object like a human face. Despite its frequent occurrence among individuals with neurodegenerative disorders, especially dementia with Lewy bodies (DLB), brain network alterations underlying pareidolia remain unclear. We aimed to unveil the neural mechanism of pareidolia using both functional connectivity (FC) and effective connectivity (EC) analyses of resting-state functional MRI.
The participants were healthy aged people (HA, n=53) and individuals diagnosed with Parkinson's disease (PD)/DLB (PD/DLB, n=56) and Alzheimer's disease (AD, n=43) recruited at four institutes in the Parkinson's and Alzheimer's disease Dimensional Neuroimaging Initiative (PADNI) cohort. First, we correlated FCs derived of 105 volumes of interest (VOIs) with log-transformed pareidolia test score (log-PS) through linear modeling (CONN toolbox). Second, focusing on the networks identified in the FC analysis, we conducted an EC analysis with dynamic causal modeling (DCM) in each participant. For group-level analysis, we estimated a Parametric Empirical Bayes model with the age, sex, log-PS, and group (HA, PD/DLB or AD) as covariates, and calculated the expected value and posterior probability of each connection.

The pareidolia scores were significantly higher in PD/DLB (mean ± standard deviation: 2.82±5.82) than in HA (1.42±3.89) (p=0.016, Wilcoxon rank-sum test), but not in AD (3.95±7.59). We found the log-PS were negatively correlated with FCs between the visual regions (i.e., the occipital pole and fusiform gyrus) and fronto-temporal regions (i.e., the central gyrus, insula, and operculum). EC analysis showed that EC from the right visual network to the right supplementary motor area positively correlated with log-PS, while EC from the right supplementary motor area to the left visual network showed negative correlation with log-PS. Several other ECs of inter- or left-hemispheric bottom-up pathway from the visual regions negatively correlated with log-PS.
Our findings suggest that pareidolia is related to reduced top-down regulation from the frontal regions to the visual network and strengthened bottom-up information flow from the visual network to the frontal regions. Together, we speculate the role of exaggerated bottom-up object recognition processing in the occurrence of pareidolia.

Disclosures: T. Kondo: None. **K. Yoshinaga:** None. **T. Hanakawa:** None.

Poster

PSTR282: Cortical Pathways of Visual Perception

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Topic: D.06. Vision

Support: SNSF 194957
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Title: Brain-wide functional ultrasound imaging reveals visual object sensitivity in the mouse spatial navigation system

Authors: *D. SIEGENTHALER[1,2,3], H. DENNY[4], J. L. MAYER[5], S. SKROMNE CARRASCO[6], A. PEYRACHE[6], S. TRENHOLM[7], E. MACÉ[8,3];
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Abstract: Visually recognizing objects is a fundamental step to guide a multitude of behaviors, including orientating oneself and navigating through an environment. Freely-moving experiments in rodents are well suited to study the spatial navigation system, but it is unclear if,

how and where visual object information is computed. To address these questions, we first took a comprehensive approach and performed a brain-wide screen for areas that preferred images of objects compared to images of scrambled objects in head-fixed mice. Interestingly, with this approach we do not find visual object sensitivity (VOS) in previously described higher order visual regions, but instead found that VOS was enriched in aspects of the hippocampal formation and the retrosplenial cortex - systems that are tightly linked to spatial navigation. Single-cell electrophysiology in both postsubiculum and restrosplenial cortex recapitulated this preference for objects over scrambled images. Furthermore, recordings in freely-moving mice revealed that object-preferring postsubicular neurons included both head direction and non-head direction cells. Overall, our data reveal that regions of the spatial navigation system support complex visual information processing in the mouse brain.

Disclosures: D. Siegenthaler: None. **H. Denny:** None. **J.L. Mayer:** None. **S. Skromne Carrasco:** None. **A. Peyrache:** None. **S. Trenholm:** None. **E. Macé:** None.

Poster

PSTR282: Cortical Pathways of Visual Perception

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR282.03/F9

Topic: D.06. Vision

Title: The part-whole effect in word recognition: the effect of language familiarity and handwriting

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Abstract: Visual words and faces have very different properties, but they are both visual stimuli for which humans have high expertise, raising the possibility that they might share common perceptual mechanisms and effects. One marker of this expertise in face recognition is the part-whole effect, which describes how recognition of a face's parts is superior when tested in the whole face than when tested alone. In the present study, to confirm the role of expertise in visual word processing, we measured the part-whole effect in word matching for familiar and unfamiliar languages. We also compared computerized font and handwriting to determine if stimulus regularity played a role. We recruited two groups of 20 human subjects, one fluent in Farsi (5 men, 15 women, aged 18-53, mean age=37.6 years, SD=10.9 years) and one in Punjabi (9 men, 11 women, aged 20-65, mean age=37.3 years, SD=13.4 years) with neither group familiar with the other language. Stimuli were comprised of single letters, 4-letter words, and 4-letter pseudowords (a string of letters lacking semantic meaning), in one of 4 handwriting or 4 font styles, shown in either upright or inverted orientations. The subjects engaged in an

alternative forced-choice task, in which subjects were required to discern whether the letter displayed on the screen was included in the real word or pseudoword presented on the preceding screen. Repeated-measures ANOVA revealed that subjects displayed greater accuracy and quicker reaction times when engaging with the familiar language in the upright orientation. There was no difference between computerized fonts and handwriting, and age and sex did not affect the results. Importantly, one sample t-test against chance level confirmed that there was a whole-word effect - i.e., accuracy was higher for words than pseudowords and single letters - but only in the familiar language in the upright orientation (all ps < .05). We conclude that experience generates an orientation-dependent whole-word effect similar to the face part-whole effect, irrespective of the script's regularity.

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Poster

PSTR282: Cortical Pathways of Visual Perception

Location: MCP Hall A

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Program #/Poster #: PSTR282.04/Web Only

Topic: D.06. Vision

Title: A meta-analysis of color sensation in the congenital blind after gaining vision

Authors: *C. Q. WU;
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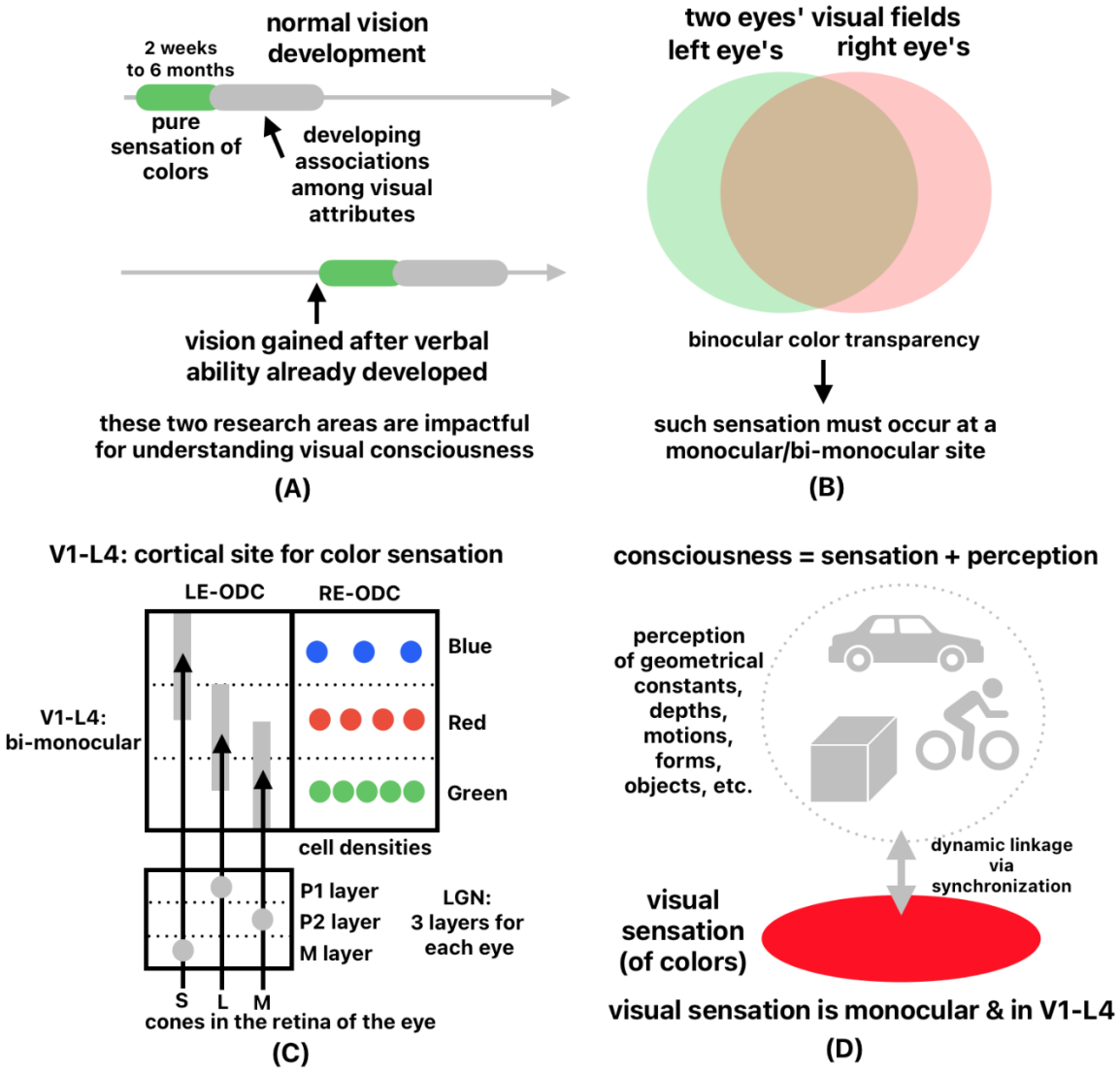
Abstract: “All vision is color vision”: This is Maxwell's (1871) opening statement in a paper on human color vision. Expressed in another way, this view states that color is the basis of all conscious vision: It is a culmination of the British empiricist’s view regarding color sensation, beginning from Thomas Hobbes and continuing up to Maxwell’s philosophical mentor Sir William Hamilton when Maxwell was attending University of Edinburgh. As noted by Maxwell, in this view, color includes the sensation along the black-white dimension. In contrast, the currently dominant view concerning color’s role in visual perception is that color is just one visual attribute in parallel with others such as form, depth, and motion. Which view is closer to the truth with respect to the neuroanatomical organization of the human visual system? In an attempt to answer this question, a meta-analysis has been conducted concerning over 100 cases of congenital cataracts who had gained vision later in life when the patients were already able to verbally express their visual experience. The sources of such cases include Cheselden (1728), Latte (1904), Miner (1905), von Senden (1960), Gregory and Wallace (1963), Fine et al. (2002), Sinha and Held (2012), and Barry (2021). Overall, this meta-analysis yields the following conclusions: 1. As illustrated in Fig 1A, newly gained vision is at first purely phenomenal and consists only of color sensation, followed by a learning stage when color is used for developing associations among various visual attributes; 2. Newly sighted may experience binocular color

transparency—as illustrated in Fig 1B, this implies that color sensation is monocular or bi-monocular (i.e., a neural substrate where monocular neurons for both eyes co-exist); 3. As illustrated in Fig 1C, mapping onto the known neuroanatomical organization of the human visual system, we can infer that color sensation occurs in V1-L4 (i.e., layer 4 in the primary visual cortex / visual cortical area V1); 4. As illustrated in Fig 1D, color is not just one of visual attributes—instead, it is the basis (carrier) of visual consciousness.



Disclosures: C.Q. Wu: None.

Poster

PSTR282: Cortical Pathways of Visual Perception

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

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Topic: D.06. Vision

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Title: Chromatic information in single-cell and population activity in the mouse early visual system

Authors: *J. SANTIAGO MORENO[1], D. J. DENMAN[2];
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Abstract: The shape and texture of our environment is enriched and informed by our perception of color. From the retina, information about the intensity, spectral content, and spatial organization of light are progressively integrated through the visual hierarchy to generate visual images. Light intensity signals relative differences in luminance while contrasts in spectral content are the basis of colors. The joint coding of color and luminance in space is at least partially established by networks connecting the retina, lateral geniculate nucleus of the thalamus (LGN) and primary visual cortex (V1). By extension, these networks are necessary for image segmentation, object recognition, and visual memory. Despite fundamental implications for understanding visual processing, it is still unclear how and where color and form are integrated in the early visual system.
The evidence for strong color opponency in single mouse visual neurons has been mixed, but nonetheless, mice are able to behaviorally discriminate colored stimuli. However, it is not currently understood how chromatic signals are relayed and integrated through the LGN and into V1. Here, we used multi-Neuropixels recordings in awake mice to broadly capture the network dynamics and visuospatial distribution of neurons that could convey color information in mice. We hypothesize that while spatial tuning relies on strong synaptic relationships between neurons with overlapping retinotopy, chromatic tuning emerges as a byproduct of pseudorandom convergence of opposing opsin signals. We found diverse single-cell chromatic tuning across regions with only rare instances of strong color opponency. Despite this, we found chromatic information within the aggregated population responses of these non-opponent neurons to chromatic stimuli. To understand potential mechanisms integrating this chromatic information, we compare chromatic populations across areas and retinotopy using a series of supervised and unsupervised machine learning methods. Finally, to elucidate the relationship between color and luminance coding, we compare the structure and dynamics of population responses to chromatic and luminance stimuli.

Disclosures: J. Santiago Moreno: None. **D.J. Denman:** None.

Poster

PSTR282: Cortical Pathways of Visual Perception

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR282.06/F11

Topic: D.06. Vision

Title: The multistage model of color thresholds and color perception

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Abstract: The two aspects of color vision - perception and thresholds - correspond to different stages of neural processing of color. While perception depends on the magnitude of neural signals at the last stage of color processing, thresholds are set by noise, which originates at all stages of neural processing. The simplest model of color thresholds (Vorobyev and Osorio, 1998, *Proc.R. Soc. B,* 265: 351-358) - the receptor noise limited model (RNL) - takes into account only the noise originating at the first stage of processing (photoreceptors) and, hence, does not have free parameters. Surprisingly, the RNL model describes the increment threshold spectral sensitivity in humans and in a number of animals, such as bees and birds. The RNL model postulates that the second stage mechanisms are not sensitive to changes in intensity of the light stimuli. This assumption is valid only for large stationary stimuli, which limits the applicability of the RNL model. Here I present a multistage generalization of the RNL model, which is applicable to a wide range of viewing conditions. This multistage model assumes that noise can be added at each stage of neural processing and does not restrict the nature of color processing. Therefore, unlike the RNL model, the multistage model has many free parameters that can be adjusted to fit experimental results. The multistage model is aimed to describe both color thresholds and perceptual judgements of color difference. While thresholds are inferred from the signal-to-noise ratio of neural mechanisms, the perceptual difference is given by the magnitude of the last stage neural signals. Therefore, by comparing color thresholds to perceptual judgements of color it is possible to make inferences about the higher order mechanisms. Analysis of results of a number of experiments suggests that thresholds can be explained by the noise of the first two stages of neural processing - the photoreceptors and the retinal ganglion cell together with the lateral genicular nucleus, i.e. the noise added at higher order mechanisms can be ignored. We observe that thresholds are generally similar among observers, which indicates the similarity of the early stages of color processing among observers. In contrast, perceptual judgements vary among observers significantly. This indicates that the higher order mechanisms revealed using a psychophysical method differ between people. It is unclear what are the neural corelates of he higher order mechanisms and whether the differences in the psychophysically revealed higher order mechanism indicate significant variations in the neural wiring of color pathways in human brain.

Disclosures: M. Vorobyev: None.

Poster

PSTR282: Cortical Pathways of Visual Perception

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Title: Spatial localization of visual stimulus processing in the occipitotemporal cortex using SEEG broadband gamma activity

Authors: *Z. LI[1,2,3,4,5], G. TAN[6,3,4,5], T. XIE[7,4,5], H. CHO[7,4,5], J. R. SWIFT[7,4,5], J. T. WILLIE[6,3,4,5], P. BRUNNER[6,3,4,5];
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Abstract: Perception of faces is understood to be processed by specialized brain mechanisms that occur in distinct brain regions. Recent evidence suggests that there are neurophysiological differences between areas involved in face and object recognition. For example, damage in the occipitotemporal cortex may cause an inability to recognize faces, but little or no deficit in recognizing objects. In another study, a small region in the right lateral fusiform gyrus was found to respond more to faces than to objects. Most previous studies used fMRI to study processing of faces and bodies within fusiform gyrus and adjacent ventral occipitotemporal cortex. However, stereoelectroencephalography (SEEG) can provide better spatial and temporal resolution and provide insight into local neural activity. Broadband gamma activity, which is known to reflect local neuronal firing, makes SEEG well-suited for localizing task-related cortical activity. In this

study, we investigated the spatiotemporal neural dynamics within the occipitotemporal cortex during visual perception. Thirteen patients implanted with SEEG electrodes participated in this study. Subjects attended to rapid serial visual presentation of images (in color or black and white), randomly selected from ten categories (faces, bodies, scenes, objects, scrambled objects, digits, lines, words, shuffled words, and character strings). Each image was presented for 200 ms with an 800 ms interstimulus interval. Visual attention was assured through a 1-back task. BBG was extracted from the SEEG signal recorded throughout the experiment. Electrode locations with statistically significant responses were determined by comparing broadband gamma activity during baseline and task phases. Electrode locations were transformed into MNI coordinates, and the distance to the AC-PC line was used to classify responsive electrode locations into clusters. Our results show that electrode locations responsive to all ten image categories were clustered in the posterior portion of fusiform gyrus for both hemispheres. Further, electrode locations responsive to specific image categories were more likely to be located in the anterior than in the posterior aspect of the fusiform gyrus. Electrode locations that responded to scenes were generally also responsive to bodies, objects, and scrambled objects, while electrode locations that were responsive to words were generally also responsive to perturbed words, digits, and colored strings. These findings are preliminary and require a larger sample size and broader electrode coverage for conclusive interpretation.

Disclosures: Z. Li: None. **G. Tan:** None. **T. Xie:** None. **H. Cho:** None. **J.R. Swift:** None. **J.T. Willie:** None. **P. Brunner:** None.

Poster

PSTR282: Cortical Pathways of Visual Perception

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR282.08/F13

Topic: D.06. Vision

Support: NIH 1DP2-EY022584
Christina Enroth-Cugell and David Cugell Fellowship for Visual Neuroscience and Biomedical Engineering
NIH T32-EY025202

Title: Evaluating Circadian Rhythmicity of Visual Function Using Deep Learning Based Analysis

Authors: *M. FLORES-NARVAEZ[1], K. C. MIGUEL[2], T. M. SCHMIDT[2];
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Abstract: Animals adapt their visual behaviors to the changing environmental light levels throughout day and night, with these adaptations being crucial for survival and maintaining

normal visual function. Contrast sensitivity, or the ability to sense spatial differences in luminance, is a key element of proper visual function. Prior work has shown that contrast sensitivity displays circadian rhythmicity in mice. This rhythmicity is characterized by heightened sensitivity during the day and reduced sensitivity at night, highlighting the cyclic nature of this visual adaptation. Traditionally, contrast sensitivity has been gauged through the Optomotor Response (OMR), relying on tracking of head movements in response to moving stimuli as a proxy for visual function. The neural circuit driving the OMR is complex, where each region involved possesses its own circadian clock. However, it remains unknown where in the OMR circuitry the circadian rhythmicity observed arises. For a more precise assessment of visual function, we sought to measure the Optokinetic Reflex (OKR), reflexive eye movements induced by motion in the visual field. The OKR retinal and brain circuitry is well characterized and less complex, providing us with the ideal system to study where circadian rhythmicity in visual function arises from. To achieve this, we developed a method to record OKR responses in head-fixed mice exposed to drifting sinusoidal gratings at various contrast levels. To test whether the circadian rhythmicity observed in OMR contrast sensitivity extends to the OKR, we recorded OKR responses (~ZT6) and Midnight (~ZT18). Using DeepLabCut, an open-source pose estimation toolbox, we tracked the pupil's location in space and automated the measurement of eye-tracking movements (ETMs) characteristic of the OKR. Our deep-learning-based analysis successfully detected ETMs both during Midday and Midnight. Importantly, we observed differences in contrast sensitivity thresholds, with animals exhibiting lower thresholds and enhanced contrast sensitivity during the day (~ZT6), confirming that the circadian rhythmicity observed in OMR holds true for the OKR. These results highlight the robustness and reliability of our methods across various time points. This automated behavioral analysis pipeline will allow us to delineate the effect of circadian rhythms on the OKR, deepening our understanding of this fundamental visual behavior. Ultimately, this will aid to investigate how light and circadian rhythms influence visual function.

Disclosures: M. Flores-Narvaez: None. **K.C. Miguel:** None. **T.M. Schmidt:** None.

Poster

PSTR282: Cortical Pathways of Visual Perception

Location: MCP Hall A

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Program #/Poster #: PSTR282.09/F14

Topic: D.06. Vision

Title: Perception of visual noise patterns depends upon correlations produced in V1: a simulation study

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Abstract: How is an external noise stimulus encoded and then decoded by the visual system? One might assume that when presented with spatially independent noise, e.g., white noise, responses of V1 neurons will be independent. However, recent electrophysiological work has shown that white noise triggers correlated responses in V1, theoretically increasing information represented (Meytlis et al., 2012). But the perceptual importance of such correlations remains unknown. Here we use decoding of simulated V1 neural responses to show that the correlations are necessary to produce a spatially independent noise-like percept. Our simulation was based on a CNN-like model of V1 consisting of simple and complex neurons. The model used a convolutional layer of 512 channels: 256 were Gabor-shaped linear filters of various frequencies and orientations followed by rectification, and the other 256 were similar filters in quadrature pairs that were squared and summed (Dapello et al., 2020). Internal noise was then added independently to each neuron at different locations. External visual noise was then fed to the V1 network. The neural activity produced appeared independent, but reducing internal noise revealed strong correlations between neurons of the same type at nearby locations and weaker correlations across different neuron types. To test whether these correlations were necessary for perception, a separate CNN was trained to reconstruct a large set of natural images from the model V1 activity, and then tested on activity from external noise images. Reconstructing the image from V1 model output with correlated channel activity produced a noise-like image, resembling the input. However, removing the correlations from the V1 output produced a reconstructed image that did not resemble noise. Specifically, it produced a more spatially dependent "tiger stripe" pattern (similar to Perlin noise). These results suggest that despite external noise being spatially independent, the neural "noise" it generates internally is not, and conversely that adding independent internal noise to neurons will not result in a percept similar to external noise. People with visual snow syndrome, a recently isolated and surprisingly common condition, perceive noise continuously in their vision. Our results suggest that snow is unlikely to arise from noise originating (independently) in V1 neurons, and instead points to an increase in retinal noise to produce the needed correlations in V1 activity.

Disclosures: S. Nie: None. **S.A. Engel:** None.

Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

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Japan Society for the Promotion of Science (JSPS) KAKENHI (Grant-in-Aid for Scientific Research (B), JP24K03240)

Title: Evaluating the spatial distribution of negative BOLD induced by an auditory task in the human visual cortex

Authors: *T. MIYATA[1], M. FUKUNAGA[1,2], J. LUO[1], I. YOKOI[1], T. YAMAMOTO[1], A. YOSHIOKA[1,3], J. YANG[4], T. MORITA[5,6], H. TAKEMURA[1,2,5];
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Abstract: Functional magnetic resonance imaging (fMRI) is a non-invasive measurement of human brain activity with relatively higher spatial resolution based on blood oxygenation-level dependent (BOLD) signals (Ogawa et al., 1992). While most studies have focused on analyzing positive BOLD responses, the negative BOLD response (NBR) is observed under particular conditions. Previous fMRI studies reported that the NBR is induced in the visual cortex during tasks for auditory stimuli (Laurenti et al., 2002; Mozolic et al., 2018) at relatively coarse spatial resolution. We performed a block-design fMRI experiment to evaluate spatial distribution of the NBR in the visual cortex occuring during auditory task using 7T MRI and individual subject-based analysis. 15 healthy adult volunteers participated in the experiment. Experiment consisted of two conditions (visual and auditory); in visual condition, the wedge-like gratings were presented in either left or right visual hemifield whereas in auditory condition, the beep sounds were presented in one of the ears. Participants were instructed to detect the changes occuring in the grating or beep sound while maintaining a fixation. In each hemisphere, we identified regions of interest (ROIs; V1, V2, and V3) by using an anatomical template (Benson et al., 2014) on structural data and divided each ROI based on eccentricity representation. FMRI data were analyzed after distortion correction, motion correction, and co-registration with structural image acquired from the same participant (Yamamoto et al., 2021). We evaluated the percent signal change of the BOLD in each ROI. All ROIs exhibited positive BOLD responses at the eccentricity corresponding to stimulus position when visual stimulus was presented in the contralateral visual field (10 deg). Importantly, NBRs were observed during auditory tasks in V2 and V3 ROIs representing peripheral visual fields (40-80 deg), suggesting a possibility that these responses are spatially localized in the peripheral area of the visual cortex. These results have potential implications for elucidating neural mechanisms involved in processing of multi-sensory inputs in the human visual cortex.

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Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR283.02/F16

Topic: D.06. Vision

Title: Using natural images to compute receptive fields in primary visual cortex

Authors: M. SOMARATNA, S. NG, *A. FREEMAN;
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Abstract: Introduction. The receptive field of a visual neuron conveys valuable information about the cell's signalling properties - visual field location, spatiotemporal characteristics, and more. A variety of stimuli have been used to compute the receptive field, including spots, bars, gratings, and white noise. We instead aimed to use stimuli, natural images, which neurons have evolved and developed to process. **Methods.** Stimuli were photographs of macaque monkeys (Deng et al., 2009, *ImageNet*), and were presented to a signal-processing model of the macaque visual system (Somaratna et al., 2023, *SfN*). The model included cones, horizontal cells, on- and off-centre bipolar and ganglion cells, geniculate cells, and both excitatory and inhibitory cells in layer 4Cβ of primary visual cortex. Each cell was implemented as a first-order differential equation, and all equations were solved simultaneously to obtain time-varying responses. Receptive fields were calculated by presenting each image for 50 ms, weighting the image by the cell's peak response, adding all weighted images, and normalising the sum by the number of images (about 1600). As a control we also used gratings which varied across the full range of orientation, spatial frequency, and spatial phase. Receptive fields were calculated from responses to flashed gratings using the same stimulus/response correlation method as for natural stimuli. **Results.** Receptive fields were calculated for excitatory cells in the input layer of primary cortex. The fields obtained with images approached those for gratings as the number of images increased. The correlation coefficient between the two receptive field types for the cell shown in the figure was 0.74 ($p < 0.001$). This is quite representative of the coefficient, 0.69, for all 529 neurons in our sample. **Discussion.** While neurons in primary visual cortex respond well to gratings, the preferred stimulus for downstream cells is typically unknown. Finding optimal stimuli for these downstream cells may be assisted by calculating their receptive fields from responses to natural images.

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Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

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Topic: D.06. Vision

Support: R01EY028905
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Title: Fast-spike interneurons in visual cortical layer 5: Heterogeneous response properties are related to thalamocortical connectivity

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Abstract: Layer 4 of rabbit V1 contains fast-spiking GABAergic interneurons (suspected inhibitory interneurons, SINs) that receive potent synaptic input from the LGN and provide fast feed-forward inhibition to local spiny neurons. These cells display overlapping ON/OFF subfields, very broad orientation tuning, non-linear responses to drifting visual gratings, and high spontaneous and visually driven firing rates. Such fast-spike interneurons are also found in layer 5 (L5), which receives much less LGN input, but their response properties and thalamocortical connectivity are relatively unstudied. Here, we examine L5 SINs in awake rabbits and compare their response properties with previously studied SINs of layer 4. We also assess thalamocortical connectivity of L5 SINs using both cross-correlation of retinotopically aligned LGN-SIN pairs and electrical stimulation of the LGN. We found that the response properties of many L5 SINs are similar to L4 SINs, but show considerably more heterogeneity. Moreover, some of this heterogeneity was related to the L5 SINs thalamocortical connectivity. Thus, L5 SINs with longer latencies to LGN stimulation display (1) better tuning to stimulus orientation, (2) lower spontaneous activity, (3) lower visually driven firing rates, and (4) longer latencies to visual stimulation. Importantly, cross-correlation analyses confirmed that a population of L5 SINs does receive monosynaptic LGN input. Moreover, all these first-order L5 SINs respond with short synaptic latencies to LGN stimulation and are found in the upper ½ of L5. Thus, we find that many L5 SINs, like those of L4, receive a strong and fast synaptic drive from the LGN, and this results in similar visual response properties to L4. However, L5 SINs that respond synaptically at

a longer-latency to LGN stimulation are better tuned to stimulus orientation and have more heterogeneous response properties. We suggest that the long-latency responses to thalamic stimulation in L5 SINs reflects a multi-synaptic intracortical pathway that generates lower spontaneous and visually driven firing rates, longer latencies to visual stimulation, and more selective orientation tuning.

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Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

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Title: Increasing robustness of ventral visual cortex revealed by neurally-guided deep neural networks

Authors: *Z. SHAO[1], L. MA[2], B. LI[2], D. M. BECK[3];
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Abstract: Humans excel at visual processing, effortlessly recognizing objects even in dynamic and cluttered environments. In contrast, deep neural networks (DNNs), despite being the only artificial systems with human-level performance in visual tasks, show surprising vulnerability to image perturbations that remain imperceptible or innocuous to humans. This disparity raises the question of what underlies the robustness of the human visual system. Some theories suggest that the ventral visual stream plays a key role in achieving stable object representations. In particular, all identity-preserving changes to objects form continuous representation manifolds. These manifolds are highly tangled upon entry but become progressively disentangled across successive stages of the ventral visual stream. If such representational space disentangling is indeed the key and unique to human brains, then training DNNs to produce more human-like representations should also improve their robustness. We trained DNNs to emulate neural representations while performing visual classification and captioning tasks, through a method we term “neural guidance”. More importantly, different DNNs were trained to each emulate successive stages of the ventral visual stream. Thus, we should expect to see progressively

increased robustness if these evolving representations are indeed essential for human visual robustness. We extracted neural activity patterns from seven hierarchical regions of interest (ROIs) in a 7T fMRI dataset (Allen et al., 2022) obtained while human participants viewed a large number of natural images. Five of these ROIs were obtained from the ventral visual stream: V1, V2, V4, VO, and PHC, and two lateral ROIs, LO and TO were included to capture the shape-sensitive lateral occipital cortex (LOC). DNN models were simultaneously trained to perform the visual task and emulate neural activity from each ROI. Our findings show not only a significant improvement in DNN robustness but also a hierarchical effect: greater robustness gains were observed in DNNs guided by later stages of the visual hierarchy. More importantly, through several analyses, we found that neurally-guided DNNs developed not only smoother decision spaces, but also qualitatively different representational spaces compared to conventional models. These unique representational spaces likely contribute to the desirable property of neurally-guided DNNs showing robustness to image perturbations. Our results, thus, support the hypothesis that human robustness gradually emerges along the ventral visual stream as a result of smoother and more disentangled object manifolds.

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Poster

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Topic: D.06. Vision

Support: National Center for Artificial Intelligence CENIA FB210017, Basal ANID

Title: Unveiling the visual cortex microcircuit: Modeling the extra-classical receptive field effect

Authors: *S. MADARIAGA[1,2], C. JARA[3], C. MURUA[3], P. E. MALDONADO[1,2], C. DEVIA[1,2];
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Abstract: Understanding visual information processing in the cortex is crucial for neuroscience and visual AI. Studying how different stimuli modify the receptive field characteristics of neurons in the cortex has been a focus for decades. Despite the accepted organization of the visual cortex in columns, understanding how microcircuits process information from various sources remains unclear. Here, we explore the mechanisms and dynamics between microcircuits underlying classical and extra-classical receptive fields effect (ECRF). To do this, we developed a model representing a small portion of the visual cortex consisting of 5 interconnected microcircuit models (4 in V1 and 1 in V2) based on the Potjans model, which describes

interactions among excitatory and inhibitory neuron groups across 4 layers of a cortical column (layer 2/3, 4, 5 and 6), and all model parameters were chosen for bio plausibility. We study how long-range lateral connections and top-down interactions from nonstriated cortices modulate V1 neuron activity. To simulate the ECRF effect, we present a preferred stimulus to a microcircuit, followed by the appearance of a new stimulus outside its receptive field. Each stimulus and its characteristics are simulated as an increase in the firing rate of a group of simulated thalamic neurons connected directly to V1. All simulations in our model maintain neuronal activity within reported ranges, indicating stable network parameters. Results show expected neuronal activity during the first stimulus presentation in both complete and modified (without V2) networks. As expected, simulating a stimulus outside the receptive field shows minimal changes in the first microcircuit. Unlike classical effects, simulating the preferred stimulus first and then the outside stimulus shows no significant change in the V2-absent model but suppresses activity in the complete model. Our findings suggest that lateral and vertical connections jointly contribute to the generation of receptive field effects. Furthermore, we noted that achieving the observed dynamics required very fine tuning of the top-down and bottom-up connections between modules, the sensitivity of which could be the origin of all observed effects in the cortex.This work was supported in part by The National Center for Artificial Intelligence CENIA, Chile FB210017.

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Poster

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Title: Comparison of visual responses on electrocorticography and intrinsic signal optical imaging in macaque visual cortex

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[1]Col. of Biomed. Engin. & Instrument Sci., [2]Sch. of Med., Zhejiang Univ., Hangzhou, China

Abstract: Electrocorticography (ECoG) offers high spatial resolution and signal-to-noise ratio by placing electrodes directly on the cortical surface. ECoG allows for the decoding of complex cognitive information through multivariate pattern analysis of oscillatory signals. This capability

suggests that ECoG captures oscillatory patterns based on the cortical functional organization. However, it remains unclear how well ECoG signals represent the functional organization. In this study, intrinsic signal optical imaging (ISOI) was used to visualize the functional organization of the macaque visual cortex. ECoG recordings were then performed in the same region to compare responses to visual stimuli between ISOI and ECoG signals. One macaque underwent surgical exposure of V1, V2, and V4 under isoflurane anesthesia, followed by ISOI and ECoG under propofol anesthesia. Visual stimuli consisting of horizontal or vertical drifting gratings made up of achromatic black and white or isoluminant chromatic red and green were presented to each eye. After the ISOI, a 64-channel ECoG electrode array (500 μm spacing) was implanted on the imaged cortical surface. The dura mater over the ECoG electrodes was replaced with a transparent artificial dura, and a chamber with a glass window was fixed to the skull with dental cement for observation of the electrodes. Visual inspection revealed that the ECoG electrodes were gradually covered by a neomembrane containing vessels within two weeks after implantation and were obscured by cloudy cerebrospinal fluid after four weeks. The mean displacement of electrode positions after two weeks was 138 μm (SD = 78 μm, n = 64). ISOI signals showed site-specific response preferences to visual stimulus attributes. Similarly, ECoG signals showed electrode-specific response preferences to visual attributes, particularly in gamma power. The extent to which electrodes showing preferences for visual attributes correlated with ISOI signals was investigated. In V1, gamma power responses of ECoG electrodes showing eye dominance correlated significantly with ISOI responses only in conditions where the dominant eye was stimulated. In addition, the gamma power of electrodes with preferences for achromatic or chromatic stimuli showed a significant correlation with ISOI responses when the preferred stimuli were presented. These results suggest that the spatial patterns of ECoG signals can indeed reflect the cortical functional organization. Furthermore, our observation reveals how the cortex responds to implanted ECoG electrodes.

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Poster

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Topic: D.06. Vision

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Title: The role of inhibition in divisive normalization in the mouse primary visual cortex

Authors: *H. ROCKWELL[1], C. CLULOW[1], J. N. MACLEAN[2];
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Abstract: Understanding the circuit-level mechanisms of neural computation is a key goal of neuroscience. Divisive normalization is a “canonical computation” that is common to sensory processing across multiple visual and auditory cortical areas. Phenomenological models of normalization help to explain various individual neuronal responses in the visual cortex. Recent studies have identified differences in pairwise correlations during responses to normalizing stimuli, suggesting the involvement of a network mechanism. However, the specific neural circuits in the neocortex that are responsible for carrying out normalization remain unclear. Recurrent inhibition is a putative mechanism for providing the divisive signal needed for this computation, but its function has not been extensively studied in mammals. In this work, we characterize the responses of two types of inhibitory neurons, parvalbumin-expressing (PV) and somatostatin-expressing (SST) cells, to normalizing stimuli in mouse primary visual cortex. Specifically, we induce cross-orientation suppression, a form of normalization, in V1 neurons by presenting sine-wave gratings and their corresponding plaids, which consist of two superimposed perpendicular gratings. We label specific inhibitory neuron subtypes with the constitutively expressed fluorescent protein tdTomato. We monitor neuronal activity in superficial layers of V1 in several male and female mice using two-photon microscopy of neurons expressing the fluorescent calcium indicator GCaMP6f, imaging a 750x750um field of view at 30Hz. Population imaging enables us to analyze the responses of numerous inhibitory cells following the presentation of normalizing stimuli. We also assess their functional connectivity with the local population of excitatory cells. We investigate whether the responses of PV and SST cells show activity changes consistent with implementing normalization, specifically examining if these cells are particularly active during the presentation of plaids. Additionally, we explore how the strength of normalization in individual excitatory cells is related to their functional connectivity with neighboring interneurons and the network organization of normalization among the local cell population.

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Poster

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Support: NIH Grant U24NS120053

Title: A mouse connectome for the community

Authors: *B. P. DANSKIN[1], C. ZHANG[1], E. NEACE[1], R. SWANSTROM[1], S.-C. YU[2], H. S. SEUNG[2], F. C. COLLMAN[1], N. M. DA COSTA[1], R. C. REID[1];
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Abstract: What scientific questions could you answer with a cubic millimeter of cortex, reconstructed at nanometer resolution? The Machine Intelligence from Cortical Networks (MICrONS) dataset is a volumetric electron microscopy (vEM) reconstruction of the mouse visual cortex containing more than 200,000 cells and half a billion synapses. Dense calcium imaging recorded the activity of 75,000 neurons while the mouse viewed natural movies and synthetic stimuli. This massive dataset was collected, processed, automatically segmented, manually proofread, and is now public and open access.
The Virtual Observatory of the Cortex (VORTEX) is here to assist scientists who want to analyze the data to further their own research interests: across scales from organelles to cells, circuits, or functional dynamics. Investigate the structure of synapses, and map their partner cells to find connectivity motifs. Or find the principals underlying visual computation across space, within and across primary visual cortex (VISp) and three higher visual areas (VISrl, VISal and VISlm). Inspect the soma and nucleus features of cells, such as chromatin patterning or membrane invagination, to identify cell types and render their structural details. Quantify the myelination of axons across layers of cortex, and locate nodes of Ranvier. Dissect and quantify the elements of the neurovascular unit, astrocytes and pericytes, smooth muscle and endothelium, across complete networks of arterioles, capillaries, and venules. Observe microglia and their phagolysosomes, and oligodendrocyte precursor cells at stages of division and differentiation. All these examples reflect current use of the MICrONS dataset, some by scientists with the support of VORTEX resources.
In VORTEX, we provide analysis consultation and tutorials to work with the data. Our team of proofreaders support further refinement and annotation of the dataset, directed by the needs of the scientific community. We are committed to contributing to the advancement of brain science, and are actively reviewing and accepting requests. Access the data portal and submit a research request at microns-explorer.org/vortex today.

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Poster

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Title: Somatostatin dendritic inhibition onto L2/3 pyramidal neurons during adult ocular dominance plasticity.

Authors: *K. BURNELL[1], J. BOIVIN[2], E. NEDIVI[2];
[1]Brain & Cognitive Sci., MIT, Cambridge, MA; [2]MIT, Cambridge, MA

Abstract: Somatostatin (SST) interneurons comprise approximately 30% of inhibitory interneurons in the cortex and are thought to preferentially innervate the dendritic arbor of pyramidal neurons. Inhibition on a broader scale has been shown to alter plasticity in the primary visual cortex (V1) of rodents. More specifically, silencing somatostatin cells in mice during adult monocular deprivation (MD) increases ocular dominance plasticity (ODP), the ability for cells to shift their response from one eye to the other. It has been shown that during adult MD there is a net loss of dendritic inhibitory synapses onto L2/3 pyramidal neurons, consistent with data suggesting that disinhibition is permissive to plasticity. While dendritic inhibitory synapses are thought to be innervated by SST afferents, the identity of dendritic inhibitory synapses removed during ODP has not been determined. Further, how SST innervation may differ across pyramidal neurons in V1, and how their response to ODP may depend on an individual cells' ocular dominance properties has not been studied. To ask if the removal of SST synapses onto individual pyramidal neurons in response to MD relates to their initial OD preference and the extent of their OD shift, we used a combined genetic and molecular strategy which sparsely labels L2/3 pyramidal neurons with a cell fill and a functional calcium sensor, as well as all inhibitory post-synaptic sites, and SST presynaptic boutons. By crossing SST-cre mice with a cre-dependent synaptophysin-TdTomato mouse, we generated pups expressing TdTomato in all their SST presynaptic boutons. These pups were then *in-utero* electroporated to express a cell fill (YFP), inhibitory post-synaptic markers (teal-gephyrin), and a calcium indicator (jRGECO) in a sparsely labeled population of pyramidal neurons in L2/3 of V1. By combining 3-color structural and functional imaging in the same cells we will be able to use MD to induce ODP and measure the functional ocular dominance shift of individual cells, as well as the inhibitory synapses lost, their position along the arbor, and their afferent source (SST or non-SST), allowing us to examine the relationship of SST synapses removed along the dendritic arbor with the functional OD shift in individual neurons.

Disclosures: K. Burnell: None. **J. Boivin:** None. **E. Nedivi:** None.

Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR283.10/F24

Topic: D.06. Vision

Support: Howard Hughes Medical Institute

Title: Towards a simplified model of primary visual cortex

Authors: *F. DU, M. A. NUÑEZ, M. PACHITARIU, C. STRINGER;
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Abstract: Deep convolutional neural networks have been shown to predict neural responses in primary visual cortex (V1) substantially better than classical models. However, this performance comes at the expense of simplicity because these models have at least four hidden layers with many feature maps in each layer. Here we show that V1 encoding models can be substantially simplified while retaining high predictive power for both monkey and mouse neurons. To show this in mouse V1, we recorded over 3,000 neurons simultaneously using two-photon calcium imaging, while presenting 30,000 natural images. We fit encoding models to the V1 neural activity, and found that the models only required two convolutional layers for good performance, with a relatively small first layer. We further found that we could also make the second layer small without loss of performance, by fitting models separately for each neuron. Similar simplifications applied for a public dataset of monkey V1 neurons (Cadena et al 2019). We show that these relatively simple models can be used for tasks such as object and visual texture recognition and we use the models we fit to gain insights into how the texture invariance properties arise in real neurons.

Disclosures: F. Du: None. **M.A. Nuñez:** None. **M. Pachitariu:** None. **C. Stringer:** None.

Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

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Topic: D.06. Vision

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Title: Sub-millimeter functional domains for different visual features in IT cortex

Authors: *C. LIANG, K. YAN, X. CAI, H. D. LU;
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Abstract: Objective Inferotemporal cortex (IT) is the final stage of the ventral visual pathway. It occupies a large portion of the temporal cortex and receives main visual inputs from area V4. Neurons in IT cortex usually exhibit selectivity to complex object features, and are organized into functional modules that can be detected at different spatial scales. Although there have been

many studies on functional architectures in IT cortex, fine-scale functional modules in this area remain unclear. For example, it is unknown whether IT cortex contains orientation or curvature maps similar to those found in earlier visual areas.**Method** With intrinsic signal optical imaging, we imaged posterior part of IT cortex and adjacent V4 in 5 anesthetized macaques (6 hemispheres). Visual stimuli included gratings at different orientations, motion directions, SFs and colors, and more complex patterns such as curvatures, shapes, faces and nature pictures. We obtained maps by comparing cortical responses to different visual stimuli. A cortical coordinate system for imaged IT surface was established to compare maps across different cases.**Result** We observed clear sub-millimeter curvature and orientation domains in posterior IT (PIT). They were similar to the same types of domains in the adjacent V4 regions, and no clear V4-PIT border can be discerned based on these maps. In the imaging region, however, map strength became weaker from posterior to anterior, and single domains became smaller. In addition, evidence of motion direction domains was also observed in PIT, although direction domains were much weaker and fewer than the orientation and curvature ones.Overall, functional domains in IT are smaller and sparser than those in earlier visual areas.**Conclusion** This is the first evidence of sub-millimeter structure for simple visual features in area IT. Additionally, our evidence suggests the penitential existence of other types of functional maps, which exhibit structures consistent with the previously observed mosaic-like organization in the IT cortex. Thus, although IT processes higher-level object features, it still contains functional structures for simple visual features. This means that different ventral visual areas possess some common types of functional modules, despite their clear differences in hierarchical order. These findings are important for understanding the visual information processing in the ventral pathway and the functional role of functional modules.

Disclosures: C. Liang: None. **K. Yan:** None. **X. Cai:** None. **H.D. Lu:** None.

Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: PSTR283.12/F26

Topic: D.06. Vision

Support: NIH R01 NS109978
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Title: Representation of visual space in the mouse frontal cortex

Authors: *A. D. LIEN, B. HAIDER;
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Abstract: Frontal cortex is implicated in cognitive control of visual spatial behavior, as classically observed in studies of spatial attention in primates. This control is presumed to rely on "top down" inputs from frontal cortical areas to visual cortical areas. Recent studies in mice have revealed similar control functions of frontal cortex - particularly anterior cingulate (ACA) and secondary motor cortex (MOs) - on visual spatial behaviors. However, the representation of visual space by neurons in these areas, and their underlying circuit organization, remains poorly understood. Here we addressed this with Neuropixels recordings across both ACA and MOs in awake, non-locomoting, head-fixed mice that passively viewed visual stimuli (no task). We presented black or white vertical and horizontal bars across the binocular and monocular visual fields to map receptive fields (RFs). Surprisingly, a subset of neurons (10-15%) responded robustly to the bars and showed clear spatially-localized receptive fields (RFs), tiling all of visual space but particularly in the binocular visual field. Recordings across a 2mm rostral/caudal extent showed no retinotopic organization. The size of the RFs (32 ± 14° full width at half max; mean ± s.d; n=411) was comparable to those recorded in higher visual cortical areas AM and PM (32 ± 18°; n=189) but larger than V1 (19 ± 12° degrees; n=263). Their peak response latency (107 ± 34 ms) lagged both areas AM and PM (80 ± 31 ms) and V1 (76 ± 37 ms). Antidromic responses (driven by ChR2-evoked axonal spikes) revealed functional projections of ACA and MOs neurons to multiple visual cortical areas. Finally, optogenetic silencing of visual cortex largely abolished frontal cortical receptive fields. In conclusion, we show that spatially selective visual receptive fields in the mouse frontal cortex prioritize binocular visual space, and depend on input from visual cortex. We are currently investigating how visuospatial properties of these frontal cortex neurons relate to the functional organization of their feedback projections to multiple areas of visual cortex.

Disclosures: A.D. Lien: None. **B. Haider:** None.

Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR283.13/F27

Topic: D.06. Vision

Title: Decoding Visual Features from Ultra-High-Density EEG Recordings

Authors: *L. SCHREINER, K. MAYR, C. GUGER;
g.tec Med. Engin. GmbH, Schiedlberg, Austria

Abstract: Exploring the sensitivity of the early visual cortex to a range of fundamental visual attributes, including orientation, contrast, spatial frequency, and colors, constitutes a significant area of inquiry within neural engineering. Building upon prior research demonstrating improved outcomes with higher EEG electrode density, this study seeks to deepen our understanding of

visual information processing in the brain. To achieve this, ultra-high-density EEG (uHD EEG) surface scalp recordings were employed to capture richer spatial and temporal data. Focusing on the occipital region, known for its role in visual processing, the study involved densely covering this area with 512 channels using flexible surface electrode grids across three healthy subjects. The analysis centered on decoding responses to colored and black-and-white stimuli. Visual evoked potentials (VEPs) were scrutinized to elucidate the temporal dynamics of neural activity, while topographical maps, rendered on 3D reconstructed head models, provided enhanced spatial insights. Furthermore, classification models were deployed to gauge the discriminatory capacity of extracted VEPs, distinguishing between features such as colors, spatial frequencies, orientations, and contrasts of colored and black-and-white stimuli. The findings highlighted consistent VEP timing and amplitudes across subjects, with notable variations observed among colors and black-and-white stimuli. Moreover, distinct focal areas of activation corresponding to each stimulus type within the occipital region were identified. The classification results showed that, on average, accuracies were 82.1% for distinguishing between low and high spatial frequencies, 71.1% for low and high contrast images, and 79.43% for the four-color classes (red, green, blue, and black and white) across all subjects. These findings highlight the effectiveness of the approach in decoding various visual features concurrently.

Disclosures: L. Schreiner: A. Employment/Salary (full or part-time):; g.tec medical engineering GmbH. **K. Mayr:** A. Employment/Salary (full or part-time):; g.tec medical engineering GmbH. **C. Guger:** A. Employment/Salary (full or part-time):; g.tec medical engineering GmbH.

Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

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NIH BRAIN initiative (9R01DA056404-04)
U19 NIH-NINDS BRAIN Initiative Award (5U19NS104648)

Title: Continuous partitioning of neuronal variability

Authors: *A. RUPASINGHE[1], A. S. CHARLES[2], J. W. PILLOW[1];
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Abstract: Sensory neurons exhibit substantial variability in response to repeated presentations of a sensory stimulus. To account for this variability, recent studies have proposed a Modulated Poisson model [Goris et al 2014; Charles et al 2018], in which a Poisson firing rate is multiplied

by a stochastic gain variable representing stimulus-independent fluctuations. The resulting model is effective at capturing greater-than-Poisson variability in spike counts across a wide range of visual areas. However, the model typically assumes that the stochastic gain variable is constant within each trial, and drawn independently across trials. This imposes strong assumptions about how spike count variability changes with time bin size, which limits its ability to capture the statistics of real neural spike trains. To address this shortcoming, we introduce the Continuously partitioned Modulated Poisson model. In this model, the firing rate is given by the product of a time-varying stimulus drive (signal) and a stimulus-independent time-varying stochastic gain process (noise), which we model with an exponentiated Gaussian process. We fit this model to spike train data from a variety of brain regions and show that the modulatory noise process exhibits temporal correlations that decay according to an exponentiated power law, thus offering new insights into the temporal dynamics of signal-independent modulatory signals within and across trials. We apply our model to spike responses from four visual areas (LGN, V1, V2, and MT), revealing novel insights into trial-to-trial variability across the visual hierarchy. We found that rapid temporal fluctuations in trial-to-trial variability decrease as information propagates along the pathway, while the strength of the trial-to-trial variations increases along the visual hierarchy. Furthermore, a comparison of the strength of the inferred signal and noise processes revealed that they tend to be negatively correlated in all visual areas. Our method thus supports existing hypotheses, outperforming several existing methods in accurately modeling spiking observations and their over-dispersion across multiple time scales.

Disclosures: A. Rupasinghe: None. **A.S. Charles:** None. **J.W. Pillow:** None.

Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: PSTR283.15/F29

Topic: D.06. Vision

Support: SPP2205 Evolutionary optimization of neuronal processing

Title: Developmental speed advantages may have favoured an evolutionarily conserved ordered organisation of cortical stimulus preference in primates and carnivores

Authors: *Z. R. STAWYSKYJ[1,2,3], F. WOLF[1,2,3,4,5];
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Abstract: In mammalian primary visual cortex (V1), form vision is supported by networks of orientation selective neurons. In carnivores, primates, and some marsupials orientation domains comprised of local clusters of similarly tuned neurons are organized in quasi-periodic patterns. This architecture is quantitatively invariant both among deeply separated lineages and across substantial evolutionarily timescales (Ho et al. 2021, Kaschube et al. 2010, Schmidt & Wolf 2021). Orientation columns may provide visual encoding or other benefits. Irrespective, they require more remodelling for convergence than random architectures. If such remodelling is necessary, it is should ideally be slight and fast. Here we present a mathematical theory uncovering that the experimentally observed universal architecture of V1 orientation domains and pinwheels uniquely enables development with minimal representational turnover of neuronal orientation encoding. We argue that this fundamental advantage may explain the surprising evolutionary stasis and potential convergent evolution observed for this system.
We model orientation selectivity by order parameters governed by some symmetry constrained variational dynamics. In the regime of biologically realistic architectures attractors are composed of an exponential number of toroidal state manifolds. Considering a large class of dynamical rules for the formation of orientation domains, we, using gradient descent optimisation, find that dynamics with realistic attractor states are singled out by the requirement of minimal representational turnover.
We formalize the principle of minimal turnover by requiring that for an ensemble of random initial conditions, attractors can be reached with the shortest path length in circuit space. Analytically this is achieved by minimising a gradient of the dynamical system. In gradient descent space the solutions closest to the initial condition should have the flattest gradient. We validate equivalence of this approach with the principle of shortest average path length by numerical calculation in a subset of analytically tractable systems. The characteristics of minimal turnover may be advantages for the animal lineages particularly when evolving into visual specialists with deep cortical processing hierarchies during mammalian evolution.

Disclosures: Z.R. Stawyskyj: None. **F. Wolf:** None.

Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR283.16/F30

Topic: D.06. Vision

Support: Wellcome Trust (223144)

Title: Apical dendrites drive surround responses in the visual cortex

Authors: *A. LIU[1], **K. D. HARRIS**[2], L. F. ROSSI[1,3], M. CARANDINI[1];
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United Kingdom; [3]Ctr. for Neurosci. and Cognitive Systems, Italian Inst. of Technol., Rovereto, Italy

Abstract: Apical dendrites drivesurroundresponses inthevisual cortex*Anyi Liu[1],Kenneth D.Harris[3],L.FedericoRossi[1,2]& MatteoCarandini[1] [1]UCL Instituteof Ophthalmology,University CollegeLondon,London,UK [2]Center for NeuroscienceandCognitiveSystems,ItalianInstituteof Technology,Rovereto,Italy[3]UCL QueenSquareInstituteof Neurology,University CollegeLondon,London,UK*Introduction. Pyramidal neurons haveprominent apical dendrites that arehypothesizedtomodulatesomatic output. Inthevisual cortex, apical dendrites may receivecontextual signalsviatop-downinputs andlocal inhibition. It is not known, however, whether they mainly driveorsuppress somatic activity, andhow they shapeaneuron's visual tuning.Methods. Werecordedtheactivity of pyramidal neurons sparsely expressing GCaMP7s inlayer5of thevisual cortex inawakemicebeforeandafter pruning their apical dendrites withtwo-photondendrotomy. Together with theneuron's receptivefieldandorientation/directionselectivity, wemeasuredproperties that dependonsensory andbehavioral context:sizetuningandcorrelationwith facial movements. Thenwerepeatedthesemeasurements after pruning theapical dendriteof 51% randomly selectedneurons (n=118, N =6), sparing theothers ascontrols.Results. Pruning theapical dendritesignificantly reducedaneuron's visual response(onaverage, by 65%, $p<0.05$), but didnot affect its selectivity for orientationor direction, or itscorrelationwith facial movements. Inneurons that exhibitedsurroundsuppression, it left thissuppressionintact. However, inneurons that preferredlargestimuli, it reducedresponses tothosestimuli ($p<0.05$).Conclusions.Inconclusion, theapical dendriteprovides amultiplicativegaineffect onall visual responses;moreover, inneurons that integrateresponses over alargeregionof visual space, it deliverssignals originating inthedistal parts of thereceptivefield. Perhaps surprisingly, theapicaldendritedoes not appear necessary toprovidebehavioral signals such as thosereflectedinfacial movements.(Characters:1725

Disclosures: A. Liu: None. **K.D. Harris:** None. **L.F. Rossi:** None. **M. Carandini:** None.

Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

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Title: Acute and chronic windows for macaque multi-photon Ca^{2+} imaging in areas V1, V2 and V4 of the visual cortex

Authors: *G. HATANAKA[1], S. CHATTERJEE[2], K. TAKASAKI[2], C. J. M. DYLLA[1], N. WARREN[1], T. KIM[1], A. PASUPATHY[1], J. WATERS[2], R. C. REID[2], W. BAIR[1];
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Abstract: We describe the design, implantation and use of several types of cranial windows for in vivo multi-photon Ca^{2+} imaging in the macaque cerebral cortex. Our designs fall into two broad categories, those that are implanted at the time of a terminal imaging session (acute) and those that are implanted chronically. Both designs address several key challenges to achieving successful imaging. First, the window needs to be as small as possible while allowing water-immersion microscope objectives with working distances of only a few millimeters to be moved laterally, close to the cortical surface. Second, we designed the windows to be completely sealed within the craniotomy at the time of implant so that there is no maintenance and to limit tissue growth on the cortex below the window. Third, the window must be placed at an appropriate elevation to achieve enough pressure to prevent z-axis (vertical) motion. Our chronic windows have allowed us to record for up to five consecutive days in terminal, anesthetized, paralyzed sessions while visual stimuli are presented to characterize the physiology of ROIs in areas V1, V2 and V4 at many depths down to 500 um with 2-photon and 800 um with 3-photon imaging. Our chronic windows are implanted right after injection of AAV-GCaMP6s. They consist of small titanium rings that hold a coverglass (7-10 mm diam) in the center and have a surrounding annular silicone artificial dura (AD) that extends several millimeters. The AD is tucked below the native dura, and dental acrylic seals the ring to the skull within an 18-20 mm craniotomy. We tested three designs for attaching the AD to the rings: a one-piece ring attached using silicone sealant, and two 2-piece designs where the AD is either squeezed between two press-fit titanium rings (no sealant) or held between rings with sealant. For imaging with acutely implanted windows, we first attach an oval headplate to the cranium with a 23 mm diameter aperture overlying a matching craniotomy. A window plate (18 mm diam coverglass) of appropriate depth to prevent z-motion is sealed to the headplate. We will describe our surgical implant technique, including for dual V1 and V4 windows in the same hemisphere, and will provide scale diagrams to allow others to copy and fabricate our designs.

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Poster

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Topic: D.06. Vision

Support: European Research Council (ERC) under the European Union's Horizon Europe research and innovation programme (Grant agreement No. 101041669)

Title: Morphology explains part of neurons' stimulus-response function in the mouse visual cortex

Authors: *P. TURISHCHEVA[1], M. WEIS[2], S. PAPADOPOULOS[4], A. S. TOLIAS[5], A. S. ECKER[3];
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Abstract: Understanding the relationship between a neuron's function and its morphology or genetic profile is crucial for achieving a unified taxonomy of cell types in the brain. Previous work linking different modalities has focused on summarizing a neuron's "function" by relatively simple features such as its preferred orientation for neurons in primary visual cortex. However, recent work has shown that the stimulus selectivity in the mouse primary visual cortex is much more complex and not captured well by a single dimension such as orientation tuning. Here we took a data-driven approach and used recent deep learning models to obtain a vector space representation that captures the full stimulus-response function of a population of neurons to arbitrary visual stimuli and represents each neuron's response function with a fixed-length vector embedding. Using a recent combined structure-function dataset from the MICrONS Consortium, we investigated to what extent a neuron's stimulus-response function could be explained by its morphology, and vice versa. To do so, we used redundancy analysis (Stewart and Love, 1968) to project functional and morphological embedding vectors into a common latent space and extracted the percentage of variance explained in one modality (function) by the other (morphology). We found that, across visual areas V1, AL and RL and after accounting for the cortical location, 18.6% of the functional variance was explained by morphology. This number was higher (26.6%) when restricting to V1 neurons. The morphological features most predictive of function were the number of dendrite branches, the dendrite area, the skeletal length, the total number of apical branches, and the number of synaptic shafts. Counting the number of synaptic shafts provided insight into the connectivity as well as branching structures of dendrites are the projections that receive signals from other neurons. These results suggest that the morphological features that are most predictive of function are the ones that are involved in the communication between neurons.

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Poster

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Topic: D.06. Vision

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EPSRC grant EP/Y020316/1

Title: Mesoscopic multiphoton calcium imaging reveals a confluence of overlapping avalanches with varying distance to criticality and distinct roles

Authors: *C. STEFENS[1], H. RAJPAL[2], J. CANZANO[3], M. SAEEDIAN[2], M. YANG[1], L. DE ARCANGELIS[4], H. JENSEN[2], M. BARAHONA[2], S. L. SMITH[3], S. R. SCHULTZ[1];
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Abstract: Criticality in a complex system is defined by a phase transition point between order and disorder. The hypothesis that information processing properties emerge at this critical point has been of interest in neuroscience, inspiring a phenomenological framework for analysis of "neural avalanches" of activity that propagate with power-law distributions of size and duration. There is growing experimental support for neural systems across species (and observed via a range of recording modalities) operating at or near criticality. However, the way in and extent to which this criticality might contribute to cognitive information processing remains unclear. One important limitation of previous studies is the assumption of at most a single avalanche at any time. Yet, in a system as large as the brain, it is highly likely that multiple avalanches co-exist, intersecting in space (cell membership) or time. In this work, we investigate neural avalanches with mesoscale, single-cell resolution optical imaging, finding that as neural recording technology scales up, this assumption is no longer viable.
We recorded the activity of ~7000 layer 2/3 neurons from a 3x3 mm2 field of view (FOV) including striate and extrastriate visual cortical areas in awake, head-fixed mice, using a Diesel2p mesoscope (Yu et al, Nat Comms 12:6639, 2021). The mice were presented with randomised visual stimuli, and an infrared camera recorded pupil diameter. We used the seqNMF algorithm to decompose spatiotemporal patterns of neural activity into components which we found to contain individual propagating neural avalanches, for which we calculated criticality and information metrics. Avalanches detected using entire FOVs revealed a proximity to criticality (2 animals, 3 recordings). seqNMF demixed the 3 FOVs neural activity into 23 sets of co-occurring avalanches that displayed a range of distances to criticality, and differing information content (with some containing mutual information about pupil diameter, and others stimulus-related information). Pupil-related avalanches appeared to behave closer to criticality than the stimulus-related. The former tended to propagate along the antero-posterior axis, while the latter typically localised in V1 then diffused into higher visual areas. Consistently, pupil-avalanches appeared close to the critical point on the phase transition diagram. Lastly, information and criticality metrics corroborated that information processing is enhanced closed

to criticality. Overall, our study suggests that the interplay of co-occurring, near-critical avalanches may play an important role in information processing across cortical circuits.

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Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: PSTR283.20/F34

Topic: D.06. Vision

Title: Intravenous injection of AAV-CAP-B10 and AAV-PHP.eB to deliver cytoplasmic and soma-targeted GCaMP8 for robust visual responses in mouse V1

Authors: *A. LEIKVOLL, Z. CRABTREE, S. SWIGGUM, N. SIMCO, R. PAVAN, P. KARA;
Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Advancements in instrumentation have increased the maximum achievable size of the two-photon imaging field of view (FOV), from less than 500 × 500 µm to 5,000 × 5,000 µm, allowing for the near simultaneous measurement of activity from enormous populations of neurons. However, large FOV imaging requires that genetically encoded calcium indicators (GECIs) be expressed over equally large areas of the brain. Transgenic mouse lines with brain-wide GECI expression are regularly created, but this technique is not feasible in non-rodent species, e.g., marmosets. We previously demonstrated that an AAV capsid variant which crosses the blood brain barrier, i.e., AAV-PHP.eB, can be intravenously injected to provide brain wide GECI expression that produces high fidelity sensory-evoked responses in the mouse visual cortex (Leikvoll and Kara 2023 Front Neurosci). A weakness of the PHP.eB capsid is that it produces good expression only in mice. Moreover, cytoplasmic expression of GECIs can generate large sensory-evoked calcium transients from the neuropil (axons and dendrites), contaminating soma responses.
Here, we present our extensive testing of an alternative AAV GCaMP8 GECI which is also capable of crossing the blood brain barrier, i.e., AAV.CAP-B10-GCaMP8. Critically, the CAP-B10 capsid has been tested and showed strong expression of non-GECI proteins in several rodent and non-rodent species, including non-human primates like marmosets (Goersten et al 2021 Nat Neurosci). We found that AAV-CAP-B10-GCaMP8 provides brain-wide expression in neurons with similar efficacy as AAV-PHP.eB in male and female C57BL6/J mice. We compared these AAV capsids in terms of expression patterns and fidelity of two-photon calcium imaging for two versions of GCaMP, traditional cytoplasmic Syn-GCaMP8s and ribosome tethered Syn-RiboL1-

GCaMP8s (Grodem et al 2023 Nat Commun). Ribosome tethering limits GECI expression to the neuronal soma and thus eliminates neuropil contamination (Chen et al 2020 Neuron). Regardless, all GCaMP8 variants we tested produced robust trial-by-trial visually evoked responses that matched expected retinotopic mapping and orientation tuning properties. We are currently performing histological assays to determine the ratio of excitatory to inhibitory neurons that express these GECI variants. Based on our findings, we anticipate that cytoplasmic and ribosome tethered GCaMP expressed with the AAV-CAP-B10 vector will become invaluable for population calcium imaging of neurons in non-rodent species such as marmosets.

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Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR283.21/F35

Topic: D.06. Vision

Support: DFG grant (ZA990/1-)
ZIAMH002838
ZIAMH002898
ZIAMH002899

Title: Single unit fMRI mapping reveals intermixed neural populations with distinct functional connectivity during rest

Authors: *L. V. IVES[1], D. ZALDIVAR[2], K. W. KOYANO[3], R. BHIK-GHANIE[4], F. Q. YE[5], D. A. LEOPOLD[6];
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Abstract: The brain exhibits spatial patterns of fMRI correlations at rest, a phenomenon commonly known as functional connectivity. While it is well established that such fMRI correlations capture important aspects of network structure and function, their relationship to spontaneous electrophysiological signals is only partly understood. A recent study employing concurrent electrophysiological and fMRI measurements determined that neurons in a local cortical population are functionally heterogeneous at rest. Specifically, two major cohorts of intermixed neurons (S+ and S-) exhibited opposite polarity correlation with cortical fMRI signals. In the present study we conducted a detailed investigation of these two functional

subpopulations. In five macaque subjects, we simultaneously measured spontaneous fMRI activity from the entire brain and single unit activity from local neural populations in the inferior temporal cortex. In each subject, MRI compatible electrode arrays were implanted to sample single units within a volume of 1 mm^3, smaller than a single fMRI voxel. A total of seventy-six simultaneous fMRI/electrophysiology scanning sessions were carried out while monkeys sat quietly in the dark scanner in the absence of any visual input or a task. We found that S+ cells, which were positively correlated with regional cortical signals, had a much higher spiking frequency than S- cells, which were negatively correlated with the same regions. Beyond their polarity and rate differences, the S+ and S- cells also exhibited significant differences in their spatial pattern of functional connectivity. Namely, the positive correlation of S+ cells was largely restricted to other STS face patches and a few areas of the visual and frontal cortex, whereas the negative correlations of S- cells were more widespread and included regions such as the hippocampus and retrosplenial cortex. Together, these results demonstrate that two physiologically distinct populations of neurons, which coinhabit the same cortical voxel, differ in both their resting firing rates as well as their functional connectivity with voxels locally and across the brain.

Disclosures: L.V. Ives: None. **D. Zaldivar:** None. **K.W. Koyano:** None. **R. Bhik-Ghanie:** None. **F.Q. Ye:** None. **D.A. Leopold:** None.

Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: PSTR283.22/F36

Topic: D.06. Vision

Support: CIHR Grant

Title: Topographic variation of visual feature preference in thalamic inputs to mouse V1

Authors: *K. CHA[1], A. RANGEL OLGUIN[2], E. P. COOK[2], A. KRISHNASWAMY[2];
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Abstract: Recent work suggests that the visual cortex enhances or attenuates its representation of visual features (such as orientation) based on retinotopic position. However, the circuits of the retina encode many of these features uniformly. The non-uniform cortical representation of visual features could reflect variations in both intracortical circuitry and corticothalamic projections. To learn more, we investigated visual feature representation across the dorsal lateral geniculate nucleus of the thalamus (dLGN). We injected viruses containing axon-localized calcium indicators (aGCaMP8s) into the entire mouse dLGN and then implanted a cranial window over the visual cortex. Next, we imaged dLGN terminals across the visual cortex in

lightly sedated mice to full-field static gratings, drifting gratings, and luminance modulations and compared these dLGN feature maps to those obtained following chemogenetic ablation of Layer 6 visual cortical (L6V1) neurons and to those obtained from the retina. We observed topographic variations in spatial frequency, preferred orientation, direction, and temporal frequency in dLGN terminals distributed across the cortical surface. dLGN terminals representing the optical axis of the mouse eye showed a preference for higher spatial frequencies with a gradual change along eccentricity. Terminals had a bias for horizontal orientations in the anterior visual field which transitioned to a bias for vertical orientations in the posterior visual field. Preferred direction also changed along the anterior-posterior field axis from posterior to upward direction. Lastly, high temporal frequencies were preferred by dLGN terminals encoding stimuli at the top and posterior parts of the visual field with gradual tapering towards the lower, anterior part of the visual field. Ablation of L6V1 neurons significantly altered the maps of preferred motion direction and temporal frequency while those of spatial frequency and orientation were largely preserved. Our results indicate that the mouse dLGN conveys a location-variant feature representation to cortex that is partly dependent on cortical feedback. These results provide new circuit-level insights into geniculocortical feature organization and functional architecture of the visual pathway.

Disclosures: K. Cha: None. **A. Rangel Olguin:** None. **E.P. Cook:** None. **A. Krishnaswamy:** None.

Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: PSTR283.23/F37

Topic: D.06. Vision

Support: NIH R01EY006821

Title: The contribution of spontaneous linear wave-fronts to the emergence of functional modular networks in developing visual cortex

Authors: *A. GRIBIZIS, J. M. GUEST, B. GRANITTO, R. SATTERFIELD, D. FITZPATRICK;
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Abstract: In the primary visual cortex (V1) of many mammals, orientation preference is organized in a periodic pattern across cortical space known as orientation maps. The iterated clusters of similarly responding neurons, or modules, resemble spontaneous patterned cortical activity observed before eye opening, consistent with an experience-independent origin of a fundamental modular structure. The sequence of early events that leads to the initial emergence of modular network structure remains unknown. Interestingly during this period of early visual

system development, linear wavefronts of spontaneous activity also sweep across the retina and downstream visual pathways before eye opening in many species. Here we examine the contribution of patterned spontaneous feed-forward activity for setting up mature cortical networks.
To address these questions, we have developed a chronic imaging preparation to record changes in patterns of spontaneous V1 activity over several days in early developing tree shrews. The mature tree shrew has long served as a model for studying the functional organization of circuits in V1, due to its well-defined modular representation of visual properties and resemblance to primate V1. In this study, we use multi-color imaging to measure calcium signals in genetically targeted cell types. We find that in the tree shrew, patterned spontaneous waves are broad and unspecific early in the developing visual cortex and then rapidly transform within a day into a modular coactivation of multiple patches extending millimeters across the cortical surface. Surprisingly, early on, we find a correlation between wavefront trajectory and modular pattern identity, with orthogonal wavefronts activating complimentary modular patterns. As the neonate approaches eye opening, wave complexity and modular pattern dimensionality increases. Ongoing studies continue to evaluate laminar and structural changes that could be developing concurrently during this time period by using multiphoton functional imaging, laminar electrophysiological probes, and structural analyses of cell morphology. Preliminary reconstructions in layer 2/3 of V1 show that axon length, dendritic length, and spine density remain similarly immature until eye opening. Thus, the developmental emergence of functional modules appears to precede the development of modular L2/3 recurrent connectivity. Further investigation into the dynamics of these processes promises to deepen our understanding of developing networks in the visual cortex.

Disclosures: A. Gribizis: None. **J.M. Guest:** None. **B. Granitto:** None. **R. Satterfield:** None. **D. Fitzpatrick:** None.

Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: PSTR283.24/G1

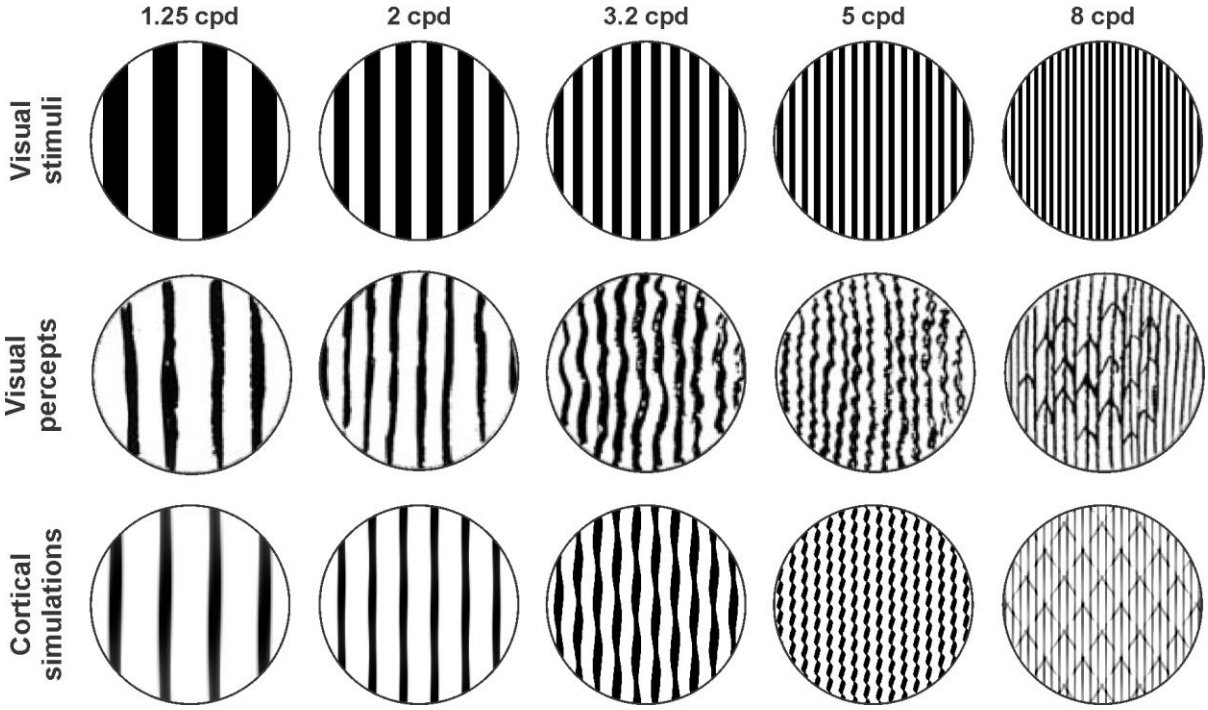
Topic: D.06. Vision

Support: NIH grant EY005253
NIH grant EY035085

Title: A computation model of visual spatial distortions in human amblyopia

Authors: *F. OLIANEZHAD, J. JIN, A. MARUYA, Q. ZAIDI, J.-M. ALONSO;
State Univ. of New York Col. of Optometry, New York, NY

Abstract: Amblyopia (lazy eye) is a developmental visual disorder of the cerebral cortex that compromises contrast sensitivity, spatial resolution, and shape perception. Based on our recent work (Pons et al., 2019; Najafian et al. 2022), we hypothesize that amblyopia affects differently ON and OFF visual pathways and their sampling of visual space in the cortex. We test this hypothesis with a computational model that simulates visual distortions perceived by humans with amblyopia, reported as drawings of grating patterns (Barrett et al., 2003). The grating distortions are simulated by sampling visual space with multiple receptive fields from area V1 (V1 filters) that, through neuronal convergence, generate large grating-like receptive fields in extra-striate cortex (Ve filters). Each visual percept is simulated as a weighted sum of Ve filters with different preferred orientations, spatial frequencies, and phases (e.g., 2-5 Ve filters for Figure 1). The Ve filter that best matches the stimulus has a weight of one whereas the mismatched Ve filters have lower weights calculated as convolutions between filter and stimuli. Before integrating the Ve filters, we apply an offset for background adaptation followed by response saturation to stimulate ON-OFF asymmetries. We then normalize and saturate the integrated response to simulate sharp edges. As amblyopia severity increases, the model decreases the response of the matched Ve filter, causing a reduction in the ratio of matched/mismatched filters that distorts the visual percepts. Preliminary simulations reproduce the visual distortions (Figure 1, cpd: cycles per degree), and the correlations between the 2D-Fourier spectrums of simulations and drawings are strong (mean ± standard deviation for R = 0.92 ± 0.03 and Rc = 0.68 ± 0.15 in 2 subjects and 10 visual percepts, Rc = (R-Rmin)/(1-Rmin), where Rmin is the minimum correlation between the lowest-spatial-frequency drawing and its copy rotated by 90^{o}). We are currently optimizing the model to quantify the entire parameter space for all amblyopia drawings available in the scientific literature.



Disclosures: F. Olianezhad: None. **J. Jin:** None. **A. Maruya:** None. **Q. Zaidi:** None. **J. Alonso:** None.

Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: PSTR283.25/G2

Topic: D.06. Vision

Support: NIH Grant EY011488

Title: Functional architecture of source-identified synaptic inputs in ferret visual cortex

Authors: *J. JAEPEL[1], R. SATTERFIELD[1], N. C. SHULTZ[1], S. MODY[1], A. FERGUSON[1], N. T. URBAN[2], D. FITZPATRICK[1];
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Abstract: Cortical neurons integrate information from a large number of inputs originating from various sources, including local connections and long-range projections to generate well-tuned somatic responses. Functional imaging of dendritic spines has revealed diversity rather than specificity and organization of inputs on multiple spatial scales. However, it remains unclear how different input sources contribute to the observed functional synaptic architecture. By combining in vivo two-photon Calcium imaging of spines and posthoc source identification with multi-colored 3D Stimulated Emission Depletion (STED) Microscopy, we are able to visualize inputs from one source onto an entire cell. This new tool enables us to successfully link functional properties of spines and their location within the dendritic field to defined input sources.
Here, we present preliminary results disentangling how two prominent input sources are contributing to the observed functional diversity, spatial organization and somatic drive of spines in layer 2/3 of ferret visual cortex. Specifically, we explore the anatomical and functional properties of spines identified as receiving input from either recurrent axons from within V1 or feedback axons from visual area 19. Preliminary results suggest a remarkable difference in the tuning of these source-identified synaptic inputs relative to somatic output. Recurrent inputs are strikingly co-tuned with the somatic orientation preference of their target neurons, while A19 identified inputs are less likely to exhibit somatic co-tuning. Recurrent inputs tend to terminate within a field of similarly tuned neighboring spines. These functional clusters do not show an anatomical preference in terms of their location within the dendritic tree. In contrast, A19 inputs lack functional clustering, appearing in functionally heterogeneous neighborhoods that are randomly distributed within the dendritic tree.

Together, these results highlight the critical importance of source identification in understanding the functional synaptic architecture of a cell.

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Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: PSTR283.26/G3

Topic: D.06. Vision

Support: NIH
NSF
Research to Prevent Blindness

Title: Optogenetic inactivation of top-down feedback modulates rhythmic activity in primate primary visual cortex

Authors: *A. M. CLARK[1], A. ANGELUCCI[2];
[1]Univ. of Utah, Salt Lake City, UT; [2]Ophthalmol, Moran Eye Inst., Univ. of Utah, Salt Lake Cty, UT

Abstract: Visual stimuli evoke rhythmic activity throughout visual cortex. Theoretical studies have suggested these oscillations could be important for effective inter-areal communication, with different channels operating within distinct frequency bands. This hypothesis has been supported by reports that oscillations within feedforward (FF) pathways are synchronized within the gamma band, while feedback (FB) circuits exhibit directed interactions in a lower (beta - 16-29Hz) frequency range. However, studies have also suggested that FB also plays a role in V1 gamma oscillations. Specifically, the effect of stimulus size on V1 gamma power is greater in FB-recipient layers, and cooling higher-order visual areas decreases evoked gamma power. To investigate the specific role of FB from the second visual area (V2) on rhythmic activity in V1, we employed selective optogenetic inhibition of V2-to-V1 FB in sufentanil-anesthetized marmosets. We expressed the inhibitory opsin ArchT in V2 pyramidal cells via injections of AAV9-CaMKII.Cre and AAV9.CAG.Flex.Arch.eGFP viruses into V2 (N=3 cases). We measured V1 activity using linear electrode arrays (LEAs) (N = 9 penetrations) while optogenetically inactivating nearby V2 FB terminals in V1. We measured size tuning to full contrast drifting sinusoidal gratings of optimal orientation and spatiotemporal frequency with and without FB inactivation. Consistent with prior work, V1 gamma power was size tuned, with some suppression at the large stimulus diameters in the supra- (SG) and infragranular (IG) layers. Also consistent with prior results, we observed size tuning of beta power. However,

unlike for gamma oscillations, beta power increased with stimulus size across all layers. FB-inactivation effects were size and layer dependent. Stimuli matched to the RF size generated a modest but significant decrease within the high-gamma band. Further increases in stimulus size yielded significant decreases in both high-gamma and gamma band power (stimuli ~ 1.5 x RF size), gamma and beta power (stimuli > 3.3 x RF size) and only beta power at the largest stimulus sizes (stimuli > 6.67 x RF size). Thus, the dominant effects were a reduction of beta power for the largest stimuli and of gamma power for smaller stimuli. These effects varied across layers, with FB-recipient SG and IG layer contacts exhibiting all of the aforementioned changes, while signals in layer 4C only showed significant decreases in beta power in response to large stimuli. These results suggest FB signals can shape rhythmic activity within lower order areas, suggesting one role of FB could be to adjust the coordinated activity of neuronal assemblies providing FF input.

Disclosures: A.M. Clark: None. **A. Angelucci:** None.

Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: PSTR283.27/G4

Topic: D.06. Vision

Support: NIH
NSF
Research Prevent Blindness Inc

Title: Computational function of parvalbumin-expressing inhibitory neurons in the primate primary visual cortex (V1)

Authors: *A. VAFAEI[1], A. M. CLARK[2], F. C. FEDERER[3], A. ANGELUCCI[4];
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Abstract: Parvalbumin-expressing (PV) inhibitory neurons (INs) are the largest IN class in many species (Federer et al. 2024). Studies of PV IN function in mouse V1 have been inconclusive. Some studies found PV INs modulate gain but not orientation tuning (Atallah et al. 2012; Wilson et al. 2012), while others found subtractive inhibition by PV INs alters tuning (Lee et al. 2012). Primates offer a better model to understand the role of cortical PV INs in generating and sharpening orientation selectivity, because, unlike in mouse, in primates this property first emerges in V1. Thus, we studied how PV INs affect visual responses in sufentanil-anesthetized marmoset V1. We injected a C1V1- or stGtACR2-expressing AAV-PhP.eB-S5E2 vector into V1

to optogenetically activate or inhibit PV INs, respectively. We optogenetically manipulated PV INs while measuring orientation tuning across V1 layers, using linear electrode arrays (n=171 single units in 3 animals). PV-IN manipulation affected both response gain and orientation selectivity, with the magnitude of the effects depending on the magnitude of PV perturbation. PV perturbation led to both subtractive/additive and divisive/multiplicative changes in tuning. The former dominated when moderate levels of optogenetic manipulation changed firing rates between -50% (PV excitation) to +100% (PV inhibition), whereas the latter increased for high manipulations that drove larger changes in spike rate. PV-IN manipulation did not affect orientation preference, but significantly changed orientation selectivity (measured as OS Index or as circular variance, CV, a measure of the global shape of the tuning curve which varies from 0, highly tuned, to 1, untuned). The magnitude of these changes scaled with the magnitude of the change in firing rate induced by PV-IN manipulation. At moderate and high PV manipulations, averaging across all layers, ΔOSI=+0.09±0.01 and +0.28±0.04 (PV activation), -0.05±0.01 and -0.15±0.01 (PV inhibition), respectively, and , ΔCV=-0.05±0.01 and -0.21±0.05 (PV activation), +0.03±0.01 and +0.07±0.01 (PV inhibition), respectively. The half-bandwidth (HBW) of the tuning curve was less altered by PV-IN manipulation (ΔHBW at moderate and high manipulation=-0.8°±0.6 and -8.5°±3.5, PV activation, -2°±3.5 and 1.5°±0.7, PV inhibition). Moreover, as predicted by some models (Shapley et al. 2003), the effects of PV-IN inhibition were strongest in the most selective cells (CV<0.8; ΔOSI=-0.23±0.01, $p<10^{-4}$, ΔCV=+0.13±0.01, $p<10^{-5}$, ΔHWHH=+4.27°±0.5, $p<10^{-5}$;Wilcoxon rank-sum test). A simple “iceberg effect” could explain changes in tuning at the highest PV activation but not changes caused by PV-IN inhibition.

Disclosures: A. Vafaei: None. **A.M. Clark:** None. **F.C. Federer:** None. **A. Angelucci:** None.

Poster

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Program #/Poster #: PSTR283.28/G5

Topic: D.06. Vision

Support: NIH
NSF
Research to Prevent Blindness Inc.

Title: Brain-wide long-range inputs to parvalbumin-expressing inhibitory neurons in marmoset primary visual cortex

Authors: *M. R. GIELOW[1], F. C. FEDERER[1], S. JAYAKUMAR[1], C. WINEBRENNER[1], Q. XU[2], J. DIMIDSCHSTEIN[3], G. J. FISHELL[4], G. POUCHELON[5], A. ANGELUCCI[1];
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Univiersity, Abu Dhabi, United Arab Emirates; [3]Regel Therapeut., Boston, MA; [4]Harvard Med. Sch., Jamaica Plain, MA; [5]CSHL, Cold Spring Harbor, NY

Abstract: In marmoset primary visual cortex (V1) parvalbumin-expressing (PV) inhibitory neurons (INs) are about 60% (80% in layer 4C) of all GABAergic cells (Federer et al 2024). An important step towards understanding PV-IN function in primate V1 is to elucidate their connectivity. We developed a viral TRIO strategy to map brain-wide monosynaptic inputs to PV cells. In marmoset V1, we injected helper virus AAV1/2-S5E2-TVA-V5-P2A-N2cG to express the avian TVA receptor, the glycoprotein for rabies variant N2c (N2cG), and a V5 tag, selectively in PV INs. EnvA-nuclear-N2c(ΔG)-NLS-tdTomato rabies was injected at the same location 7 weeks after helper injection, and perfusion occurred 1 week later. Following immunolabeling of V5 tag, we mapped (by hand) labeled starter cells in V1 and input cells throughout the brain (excluding local input cells within 0.8 mm from the injection center). We quantified the percentage of long-range inputs labeled in all cortical and subcortical regions. Starter cells were found across all layers of V1. About 30% of long-range inputs to V1 PV-INs arise from within V1, with more V1 label found in supragranular (SG) than in infragranular (IG) layers (SG/IG=~1.5). Of the extra-V1 inputs, 85% (~60% of total inputs), arise from other visual cortical areas, specifically from areas previously demonstrated to send feedback projections to V1. Projections were more numerous from areas V2 and MT, moderate from VLP, VLA, DM, MTc, MST and DI, and sparse from areas DA, parietal and temporal cortices. Differing from reports in mouse V1, we found almost no inputs from prefrontal areas, and inputs from non-visual areas (including auditory, somatosensory, entorhinal, perirhinal, and motor) were very sparse (~0.1% of total label, or 0.2% of extra-V1 label). Cortical feedback to V1 PV INs arises predominantly from the IG layers, specifically at the border between L5 and L6A, with some contributions from SG layers (mainly L2/3A) in areas V2 (18% SLN=SG / (SG+IG), MT and VLP (7% SLN), DM and MTc (3 and 2% SLN, respectively). Thus, as previously described for feedback projections in primate visual cortex, the proportion of SG input to PV IN also decreases with hierarchical distance from V1 (Markov et al 2013). About 15% percent of extra-V1 input to V1 PV INs arises from subcortical nuclei (LGN, pulvinar, midline thalamus, endopiriform/claustrum and basal forebrain). Among these subcortical inputs, those from the LGN and endopiriform nuclei were most numerous. This study expands our knowledge of the circuitry involving PV INs and demonstrates important differences between rodent and primate regarding the global connectivity of PV cells situated in primary sensory areas.

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Poster

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Program #/Poster #: PSTR283.29/G6

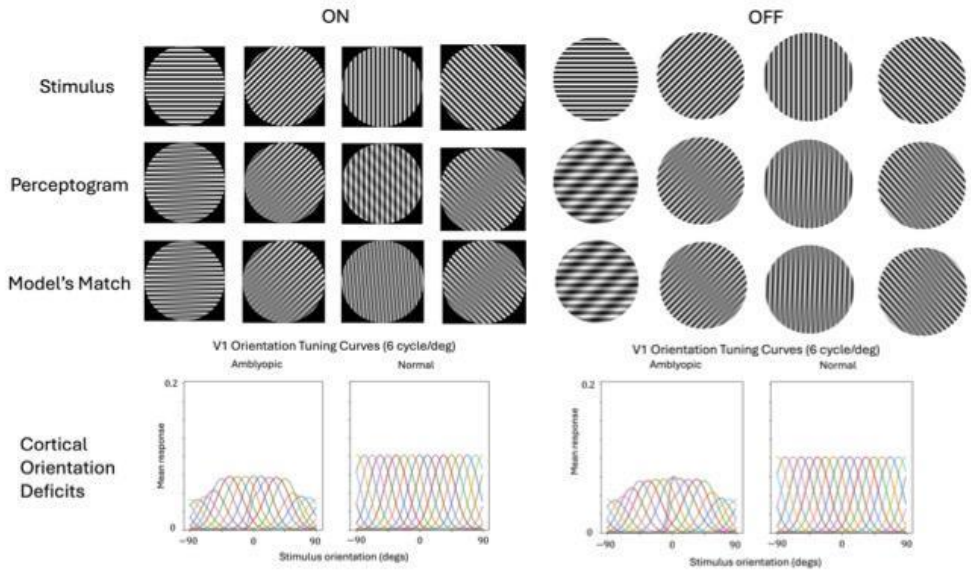
Topic: D.06. Vision

Support: NIH grant EY035085
NIH grant EY027361

Title: Perceptograms of form distortions seen by Amblyopes and models of underlying cortical deficits

Authors: *A. MARUYA, F. OLIANEZHAD, J. WANG, J.-M. ALONSO, Q. ZAIDI;
Grad. Ctr. for Vision Res., SUNY Optometry, New York, NY

Abstract: Striking form distortions seen by amblyopes have been documented by showing high contrast sinusoidal gratings to the amblyopic eye (AE) and drawing the percept through the fellow eye (FE). The drawings fall into 7 types, each resembling sums-of-gratings plaids (Barrett et al 2003), suggesting aliasing in orientation (OR). Drawings could conceal distortions of contrast, shading, spatial frequency (SF) and linearity, so we have measured Perceptograms for 4 amblyopes ages 22-45. In a dichoptic display, AE sees a test grating in the center (6, 9, 12 cyc/deg, 4 orientations, ON or OFF). FE sees 8 surrounding patterns: the test grating and 7 plaids matching every distortion type adjusted to the SF and OR of the test. The observer picks the pattern most like the central image, ignoring possible differences in contrast, SF, OR and phase. Then the AE sees the test grating and the FE sees just the chosen match while 8 parameters of the 2 plaid gratings (contrast, SF, OR and phase) are adjusted until the two percepts match perfectly (3 repeats). Fig 1 shows 8 ON & OFF gratings and the Perceptograms for one observer. The linking hypothesis for our model: In visual extra-striate cortex (Ve) the signals generated by the test grating through AE match the signals generated by the matched plaid through FE. We separately pass the image of the grating and the matched plaid through Steerable Filters representing a normal V1 cortex and use the Portilla-Simoncelli algorithm to calculate marginal statistics and correlations which reflect Ve responses. The multi-dimensional difference between the responses drives the model. Our algorithm selectively modifies the response magnitude and orientation tuning width of the filters for just the grating input until the two Ve responses match. We test the model by finding its best match to the Perceptogram from 1,000 plaids (Fig 1). The modified set of orientation filters with selective broader tuning and lower magnitude compared to normal V1 (Fig 1) reveals the deficit that generates distortions through AE. The modified V1 provides the target for neural development models of amblyopia.



Disclosures: A. Maruya: None. **F. Olianezhad:** None. **J. Wang:** None. **J. Alonso:** None. **Q. Zaidi:** None.

Poster

PSTR283: Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: PSTR283.30/G7

Topic: D.06. Vision

Support: NEI F32EY034024

Title: Dynamic cortical representations of rewarded stimuli in tree shrew primary visual cortex (V1) during learning of a visual discrimination task

Authors: *G. RODRIGUEZ[1], C. MORALES[1], R. SATTERFIELD[2], D. FITZPATRICK[3]; [1]Max Planck Florida Inst. for Neurosci., Jupiter, FL; [2]Fitzpatrick Lab., Max Planck Florida Inst., Jupiter, FL; [3]Max Planck Florida Inst., Jupiter, FL

Abstract: Visual discrimination learning modifies cortical neural representations according to changes in the behavioral relevance of sensory stimuli. Previous studies in primary visual cortex (V1) of the tree shrew (*tupaia belangeri*) show that learning a fine visual discrimination task leads to long lasting enhancement in responses of a subset of excitatory neurons whose orientation preferences allow optimal encoding of differences between task-relevant stimuli (Schumacher et al 2022). However, exactly how his process unfolds throughout stages of learning and the role of different types of neurons remains elusive. We aim to understand the progression of discrimination learning related changes in the responses of cortical neural populations. To address this question, we employ chronic 2-photon calcium imaging of excitatory and inhibitory neurons simultaneously, within V1 layer 2/3 of head-fixed tree shrews, throughout learning of a fine orientation discrimination task. The task is based on a Go/No-Go paradigm where animals learn to distinguish between a rewarded orientation (target) and a distractor orientation (22.5 degrees difference). In the task structure, successful responses (lick) after viewing the target provides juice as reward while failing to respond or licking for the distractor results in time out. Comparison of tracked neural activity at different learning stages uncovered a period of feature specific changes in both excitatory and inhibitory populations during the learning process. Our data suggest that a selective decrease in the inhibitory population response to the rewarded stimulus, but not the distractor, precedes the long-term enhanced activity described in the excitatory network. This decrease in inhibitory responses is transient, as it recovers once proficiency is achieved in task performance. Coincidentally, we observe neuronal population responses to the rewarded stimulus, but not the distractor, transiently become variable during learning and later regain stability in expert stages. Our data suggest that during learning, when the target stimulus is presented, V1 L2/3 neural populations sample alternative network configurations some of which enhance discriminability between task relevant stimuli. Moreover, the selective nature of the observed changes to the rewarded, but not the distractor stimuli, suggest reward association may underlie the altered neural population responses during learning. Our current efforts are focused on dissecting the role reward history may play in opening a period of dynamic network configurations and in instructing more efficient stimulus representations across learning.

Disclosures: G. Rodriguez: None. **C. Morales:** None. **R. Satterfield:** None. **D. Fitzpatrick:** None.

Poster

PSTR284: Visual System: Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR284.01/G8

Topic: D.06. Vision

Support: VR220084

Title: Neurogenesis in the adult mammalian retina induced by an alpha 7 nicotinic acetylcholine receptor agonist eye drop treatment following blast exposure

Authors: *G. V. NOLASCO DE CARVALHO[1], J. B. SPITSBERGEN[1], D. M. LINN, Jr.[2], C. L. LINN[1];
[1]Biol. Sci., Western Michigan Univ., Kalamazoo, MI; [2]Biomed. Sci., Grand Valley State Univ., Allendale, MI

Abstract: Previous studies from this lab have explored the neurogenic capability of an alpha7 nicotinic acetylcholine receptor agonist, PNU-282987, in the retina. The goal of this study is to investigate the neurogenic effects of PNU-282987 in adult mouse retinas following blast-induced ocular trauma. Functional recovery and neurogenesis was evaluated using electroretinogram (ERG) recordings in dark adapted animals and IHC. Blast injuries were induced in 3-8 month old adult mice with a 35-psi blast delivered to the left eye using a modified paintball gun. One month post blast, mice were treated bilaterally daily for two weeks with PBS eye drops containing either 1% DMSO/1 mg/mL BrdU or 1 mM PNU-282987/BrdU. ERG recordings were conducted before blast exposure and every week following blast exposure for two months. At the end of two months, retinas were processed immunohistochemically for BrdU incorporation and for retinal cell markers. ERG analysis revealed that the a-wave amplitude significantly decreased by an average of 24% +/- 5% the b-wave amplitude decreased by an average of 32% +/- 18%, and oscillatory potentials (1, 2, and 3,) decreased by an average of 41% +/- 1%, 55.4% +/- 5%, and 55.8% +/- 5% (N=7; P less than 0.05) respectively following blast injury. However, amplitudes recovered to control-like levels (136% +/-34%; a-wave, 121% +/- 25%; b-wave, 120% +/- 5%, 111% +/- 2%, and 129% +/- 6% ; OP1, 2, and 3) when treated with PNU-282987 after blast. Morphological analysis demonstrated significantly decreased cell counts in all layers after blast but recovered with agonist treatment. Using DAPI stained cells, ONL cell counts under control conditions averaged 208.15 cells +/- 10.2, the INL cell counts averaged 90.1 +/- 5.2, and the GCL counts averaged 15.1 +/- 2.1 from 200-μm² confocal images. After blast, ONL cell counts significantly decreased to an average of 180.1 +/- 3.4, INL cell counts decreased to an average of 70.4 +/- 4.1, and GCL cell counts decreased to an average of 6.8 +/- 2.8. After PNU-282987 treatment following blast, the ONL cell counts significantly increased to an average of 225.22 +/- 12 cells, INL cell counts increased to an average of 94.4 +/- 2.1, and GCL cell counts increased to an average of 16.2 +/- 1.8 (N=7; P less than 0.05). In IHC studies, BrdU incorporation was seen in retinal ganglion cells and photoreceptors after PNU-282987 treatment, with or without blast exposure. These are the first experiments to demonstrate neurogenesis in the adult mammalian retina after treatment with an alpha 7 nicotinic acetylcholine receptor agonist. Results of this research may have implications for neurodegenerative diseases, trauma, and aging in adult mammals.

Disclosures: G.V. Nolasco de Carvalho: None. **J.B. Spitsbergen:** None. **D.M. Linn:** None. **C.L. Linn:** None.

Poster

PSTR284: Visual System: Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR284.02/G9

Topic: D.06. Vision

Title: Development of Perceptual Grouping Ability in Visually Impaired Humans following Late Sight Onset

Authors: *S. GUPTA[1], T. K. GANDHI[2];
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Abstract: The adaptability of the human visual system to comprehend complex environmental inputs is indeed remarkable. This skill of the brain to organize visual information into meaningful patterns or structures is referred to as perceptual grouping. However, it is uncertain whether this ability is innate or develops over time with experience. A compelling scenario that provides an exceptional opportunity for conducting such investigations arises in cases involving the restoration of a deprived sensory modality. Individuals born with bilaterally dense cataracts, which block patterned light from reaching the retina due to opaque lenses, present a distinctive opportunity. We are presented with a remarkable opportunity to collaborate with special subjects from Project Prakash (referred to as PP subjects). Project Prakash operates as part of humanitarian and scientific endeavors aimed at studying and treating congenitally blind children. The PP subjects have experienced prolonged early-onset blindness, which commenced before the age of one and persisted for 8-17 years before the removal of bilateral cataracts. The present study investigates the development of perceptual grouping abilities following long-term visual deprivation (age group 7-20 years, mean age: 15.63 years, 4 females) after the visual restoration surgery. The electroencephalography (EEG) data was collected for these subjects while the display was with or without a `structure' (a line segment formed by dots) amidst random dots (referred to as `non-structure'). The EEG data were also collected from age-matched healthy controls with the same age and socio-economic background. The ERP was evaluated in the occipital region in the left and right hemispheres for structure and non-structure separately before sight onset and after a long period of sight-restoring surgery. This study focuses on analyzing the brain responses of PP long-term subjects (LTS) and comparing them with brain responses from age-matched control subjects. The LTS subjects were examined for the development of the skill of differentiation between structures and non-structures through EEG and behavioral experiments. The results demonstrate the development of the visual ability to discriminate between structure and non-structure following long-term visual impairment. Furthermore, our findings suggest that newly sighted children exhibit comparable neural responses to control subjects, indicating the acquisition of perceptual grouping skills over time. The findings

elucidate that perceptual grouping skills may evolve with experience and not necessarily depend on early visual input.

Disclosures: S. Gupta: None. **T.K. Gandhi:** None.

Poster

PSTR284: Visual System: Plasticity

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Program #/Poster #: PSTR284.03/G10

Topic: D.06. Vision

Support: R15EY035803

Title: Differentially expressed genes and pathways identified in primary murine Müller glia cells associated with adult neurogenesis as a result of exposure to supernatant from PNU-282987-treated retinal pigment epithelium cells

Authors: *H. K. VANZO-SPARKS[1], D. M. LINN, Jr.[2], A. R. NEWCASTLE[1], C. L. LINN[1]; [1]Biol. Sci., Western Michigan Univ., Kalamazoo, MI; [2]Biomed. Sci., Grand Valley State Univ., Allendale, MI

Abstract: Previous studies from this lab have suggested Müller glia are the source of new cells generated by the selective α7 nicotinic acetylcholine receptor (nAChR) agonist, PNU-282987. The goal of this study was to identify transcriptome changes in primary cultures of Müller glia after indirect exposure to PNU-282987 to further our understanding of the pathways being activated and genes being expressed. Primary Müller glia cultures sourced from adult (3-6 months) murine retinae were exposed to either 1% DMSO (control) or 100 nm PNU-282987-treated retinal pigment epithelium cells for 8, 12, 24, and 48 hours and total RNA was extracted. Novogene conducted mRNA-seq and mapped reads to the *Mus musculus* GRCm39 reference genome. Novogene provided Deseq2 bioinformatics and gene ontology (GO). Additional functional enrichment was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and PANTHER pathway analysis. RT-qPCR was performed to validate the mRNA-seq using the comparative Ct method ($2^{-\Delta\Delta Ct}$) for several genes of interest. Principle Component Analysis revealed transcriptome differences between DMSO control samples and samples exposed to PNU-282987 for various time points, suggesting a time-dependent effect on Müller glia gene expression. Pathway analysis revealed that differentially expressed genes (DEGs) in earlier time points were associated with MAPK, Hippo, TNF signaling, and PI3K-Akt pathways that are initiated in regenerative responses in lower vertebrates. Later time points were significantly enriched for the cell cycle. Furthermore, significant (p less than 0.05 and $\log_2$ fold change greater than 1 or less than -1) GO terms at earlier time points included those involved in neural retinal development, cell differentiation, and pathways that are upregulated in retina

regeneration. GO terms at later time points were associated with the cell cycle. mRNA-sequencing results were verified with RT-qPCR and were found to follow similar patterns of up and down-regulation at their respective time points. These data suggest PNU-282987 allows the expression of genes involved in differentiation and retina regeneration in Müller glia. This furthers the notion that PNU-282987 can induce neurogenesis in the adult murine retina. The results of this study have significant implications for retinal degenerative diseases.

Disclosures: H.K. Vanzo-Sparks: None. **D.M. Linn:** None. **A.R. Newcastle:** None. **C.L. Linn:** None.

Poster

PSTR284: Visual System: Plasticity

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR284.04/G11

Topic: D.06. Vision

Support: 5F31EY033996

Title: Elucidating the role of dorsal lateral geniculate nucleus burst-mode firing in retinal inactivation induced recovery from amblyopic rearing

Authors: *M. ECHAVARRI-LEET[1], T. CHAUHAN[1], A. THOMAZEAU[2], M.-F. FONG[3], M. F. BEAR[1];
[1]Picower Inst. for Learning and Memory, MIT, Cambridge, MA; [2]IPMC, CNRS, VALBONNE, France; [3]Biomed. Engin., Georgia Tech. and Emory, Atlanta, GA

Abstract: Amblyopia is a prevalent disorder characterized by reduced vision in one eye resulting from inadequate visual experience during early development. Current strategies for treating amblyopia are generally only effective if implemented at a young age, before the age of seven, and compliance with treatment is often a challenge for children and their caregivers. Thus, developing new treatment strategies for amblyopia addresses an unmet medical need. While exploring alternative ways to treat amblyopia, our lab and our collaborators made the discovery that temporary inactivation of the non-amblyopic eye enables a recovery from amblyopic rearing in mice and cats. Of particular significance, this recovery was possible in older animals when conventional treatments fail and occurred without penalty to the inactivated eye. The mechanism by which this treatment promotes recovery is currently unknown, but a potential clue comes from recordings in the dorsal lateral geniculate nucleus (dLGN) contralateral to retinal inactivation, which reveal an increase in burst mode firing for at least 48 hours. In the present study, we aimed to determine whether this increase in burst-mode firing after retinal inactivation was input specific, given that retinal inactivation as a treatment for amblyopic rearing involves the ipsilateral and not the contralateral eye. For this we used single unit recordings of ~p54 male

and female mice via acute silicone probe implants targeting the dLGN ipsilateral to intravitreal injection of either tetrodotoxin (TTX) or saline. We found ipsilateral eye inactivation significantly increases dLGN burst mode firing in contralateral eye responsive units by two hours after inactivation. We additionally found this inactivation induced increase in burst-mode firing can be reliably eliminated via knockout of the predominant low voltage activated calcium channel in the dLGN, $Ca_v3.1$. This was achieved by injecting HSV-Cre into the dLGN of both transgenic mice in which the gene encoding $Ca_v3.1$ is flanked by loxP sites and non-floxed mice. This elimination of burst firing occurs without significant impacts in firing rate statistics or PSTHs. Furthermore, Nissl staining of HSV-Cre injected and uninjected hemispheres revealed no significant changes in dLGN cell count following viral injection, suggesting elimination of burst-mode firing via $Ca_v3.1$ KO occurs without tissue damage or changes in tonic firing. This method of eliminating dLGN burst mode firing will be harnessed to determine whether dLGN burst mode firing is necessary for ipsilateral retinal inactivation induced recovery from amblyopic rearing.

Disclosures: M. Echavarri-Leet: None. **T. Chauhan:** None. **A. Thomazeau:** None. **M. Fong:** None. **M.F. Bear:** None.

Poster

PSTR284: Visual System: Plasticity

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Program #/Poster #: PSTR284.05/G12

Topic: D.06. Vision

Support: ZIAMH002909

Title: Primary visual cortex responds during spoken language comprehension

Authors: *S. LIPETZKY[1], G. EDWARDS[2], A. SEYDELL-GREENWALD[3], E. P. MERRIAM[4], R. HUBER[5], E. STRIEM-AMIT[6], C. I. BAKER[7];
[1]Lab. of Brain and Cognition, NIMH, Baltimore, MD; [2]Lab. of Brain and Cognition, NIH, Bethesda, MD; [3]Georgetown Univ. Med. Ctr., Washington, DC; [4]NIMH/LBC, NIH, Natl. Inst. of Mental Hlth. (NIMH), Washington, DC; [5]NIH, Bethesda, MD; [6]Neurosci. Dept., Georgetown Univ. Med. Ctr., Washington, DC; [7]Lab. Brain and Cognition, NIH, Bethesda, MD

Abstract: In congenitally blind populations there is increased activity in the primary visual cortex (V1) while listening to spoken language, suggestive of cross-modal plasticity. However, recent evidence of V1 activation by spoken language in sighted individuals in the 3 Tesla MRI (Seydell-Greenwald et al., 2023) suggests pre-existing connectivity between V1 and regions associated with language comprehension. Given the auditory nature of the stimulus, the V1 activation must reflect cortico-cortical feedback originating from non-visual areas. Here, we

replicate activation of V1 from spoken language in the 7 Tesla MRI. Following the method of Seydell-Greenwald and colleagues, we presented sighted participants (n=9) with 6 runs of audio recordings of forward and reversed speech in 30 second counterbalanced blocks. While listening to the audio, participants looked at a fixation cross while their eye-movements were recorded. In the forward speech condition, participants heard short sentences (e.g., “Birthday cake lights are candles”, “Something that sweeps the floor is a canoe”) and were tasked with indicating when a sentence was semantically incorrect. In the reversed speech condition, participants heard sentences played in reverse and were tasked to press a button if they detected a beep at the end of an utterance. Replicating the previous study, activation in V1 for the forward speech condition was greater than the reverse speech condition across all participants. Moreover, there was increased forward versus reverse activation in left V1, potentially reflecting the left lateralization of the frontotemporal language network. In the future we plan to employ the Vascular Space Occupancy (VASO) sequence to characterize the depth-dependent profile of feedback in V1 from non-visual stimuli.

Disclosures: S. Lipetzky: None. **G. Edwards:** None. **A. Seydell-Greenwald:** None. **E.P. Merriam:** None. **R. Huber:** None. **E. Striem-Amit:** None. **C.I. Baker:** None.

Poster

PSTR284: Visual System: Plasticity

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Program #/Poster #: PSTR284.06/G13

Topic: D.06. Vision

Support: EY035138
EY035885

Title: Ocular Dominance Plasticity and Visual Circuit Reorganization in Nogo Receptor 1 (ngr1) mutant mice

Authors: *T. BROWN, A. W. MCGEE;
Univ. of Louisville, Louisville, KY

Abstract: During development, sensory systems engage experience to aid in proper wiring. In many sensory systems, periods of heightened sensitivity to experience known as "critical periods” govern when the influence of experience is most prominent on circuit formation. Abnormal experience during critical periods can cause enduring deficits in sensory function. Extending or reopening critical periods has been a therapeutic target to treat many diseases and neurological conditions. Nogo receptor (NGR1) is a neuronal receptor and deletion of the ngr1 gene extends critical period-like plasticity for ocular dominance into adulthood. Here, we probed the neuronal mechanisms of this critical period with two-photon calcium imaging at cellular

resolution by measuring the stability of neuronal tuning properties, a phenomenon termed ‘representational drift’. By measuring representational drift in juvenile wild-type mice during the critical period, adult wild-type mice after the closure of the critical period, and adult ngr1 KO mice, we have determined how the magnitude and mechanisms of ocular dominance plasticity relates to representational drift

Disclosures: T. Brown: None. **A.W. McGee:** None.

Poster

PSTR284: Visual System: Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR284.07/G14

Topic: D.06. Vision

Title: Altered neocortical inhibitory populations in early mammalian evolution

Authors: *R. GORZEK[1], E. TRING[1], S. JAIN[2,3], J. T. TRACHTENBERG[1];
[1]Neurobio., Univ. of California-Los Angeles, Los Angeles, CA; [2]Georgia Inst. of Technol., Atlanta, GA; [3]Biological Chemistry, University of California-Los Angeles, Los Angeles, CA

Abstract: Recent technological strides in transcriptomic profiling of single neurons (RNA-seq) have yielded novel insights into the diversity of neocortical cell types in vertebrates across the evolutionary tree. Previous studies indicate that the fundamental cell classes and subclasses are largely conserved between mice and primates, but the transcriptomic landscape of neocortex in species that diverged early in mammalian evolution has not been characterized. This would aid in identifying cell types that consistently shifted in proportion or transcriptomic complexity throughout mammalian evolution, which are likely to play a role in supporting enhanced cortical representations of the sensory environment and higher-order cognition.
The gray short-tailed opossum, *Monodelphis domestica*, is a marsupial that diverged from placental mammals around 160 million years ago and is considered a good model of the ancestral mammalian brain. We used comparative single-nucleus transcriptomics to characterize cortical cell types in opossums and mice. While the relative abundance of glutamatergic subclasses is largely preserved between these species, the balance of GABAergic subclasses shifted dramatically. Specifically, we identified a decrease in parvalbumin-positive (PV) GABAergic neurons from opossums to mice across neocortical regions. In more rostral areas, this was accompanied by an expansion of GABAergic subclasses derived from the caudal ganglionic eminence (CGE), including vasoactive intestinal peptide-positive (VIP) GABAergic neurons. Importantly, although CGE-derived GABAergic subclasses preferentially occupy the upper neocortical layers, the glutamatergic composition of layer 2/3 is unchanged between opossums and mice. Thus, it appears that during mammalian evolution, alterations in GABAergic populations preceded well-characterized glutamatergic changes between mice and primates,

including upper-layer expansion. We posit that these GABAergic changes improved the quality of cortical representations of the environment. This would improve evolutionary fitness and may be a recurring motif in mammalian neocortical evolution.

Disclosures: R. Gorzek: None. **E. Tring:** None. **S. Jain:** None. **J.T. Trachtenberg:** None.

Poster

PSTR284: Visual System: Plasticity

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Program #/Poster #: PSTR284.08/G15

Topic: D.06. Vision

Support: NIH R00 EY029326

Title: Transient inactivation of ON signaling drives pathway-specific cortical plasticity in mice

Authors: *R. D. LAMPRECHT, D. DOWE, A. KOTAR, M.-F. FONG;
Biomed. Engin., Georgia Tech. and Emory, Atlanta, GA

Abstract: Early visual processing is broadly organized into two independent signaling pathways that respond to increments (ON) and decrements (OFF) in luminance. The ON and OFF signaling pathways emerge in the retina, operate in parallel through the thalamus, and combine within the primary visual cortex (V1). We previously discovered that transient bilateral inactivation of the retinas led to a long-lasting potentiation of visual cortical responses in mice. In this study we asked whether visual enhancement could similarly be achieved via selective blockade of the ON retinal pathway. To inactivate the ON pathway, we used intravitreal injections of the mGlu6 antagonist, L-2-amino-4-phosphonobutyric acid (APB), to block depolarization of ON bipolar cells in both eyes. We also recorded visually-evoked potentials in the binocular zone of V1 before, during, and for several days after ON pathway blockade. Immediately after APB injection, we observed a pronounced disruption of visual responses across a range of visual stimuli. However, upon recovery of ON pathway signaling in subsequent days, we observed an increase in the magnitude of the visually-evoked responses to ON stimuli that exceeded baseline values; meanwhile responses to OFF and grating stimuli returned to baseline. While multiple forms of visual deprivation have been shown to drive homeostatic plasticity in V1, our observation that ON pathway blockade selectively drives potentiation of ON responses suggests that the compensatory response to visual deprivation may be pathway-specific.

Disclosures: R.D. Lamprecht: None. **D. Dowe:** None. **A. Kotar:** None. **M. Fong:** None.

Poster

PSTR284: Visual System: Plasticity

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Program #/Poster #: PSTR284.09/G16

Topic: D.06. Vision

Support: CIHR
VHRN
FRQS

Title: Longitudinal monitoring of visual functions after stroke in the mouse visual cortex as a new preclinical model of cortical blindness using calcium imaging

Authors: *C. ALBERT[1], B. O. DE SOUZA[2], J. ROYEA[3,4], J.-F. BOUCHARD[2], M. VANNI[2]; [1]Visual Neurosciences, École d'optométrie de l'Université de Montréal, Montréal, QC, Canada; [2]Sch. of Optometry, Univ. of Montreal, Montreal, QC, Canada; [3]Cell. and Mol. medecine, Univ. of Ottawa, Pointe-Claire, QC, Canada; [4]School of Optometry, University of Montreal, Montreal, QC, Canada

Abstract: As the global population grows older, ischemic stroke (IS) prevalence increases. IS induce disabilities varying as a function of which cortical areas are injured. Following an IS, spontaneous functional reorganization generally occurs but remains limited. Mostly studied in the motor cortex, functional reorganization mechanisms include connectivity modifications and adaptation of functional properties of neighbouring cortical areas. Here, we sought to investigate whether similar mechanisms were present within the visual cortex. Indeed, visual disabilities following IS, such as cortical blindness, are far less studied compared to motor impairments. Cortical blindness occurs when the visual cortex is injured and can no longer interpret visual information. In the absence of preclinical models, our goal is to develop a cortical blindness mouse model using widefield calcium imaging.
By implanting adult Thy1-jrGECO1a mice with cortical windows (n=12), we observed fluorescence fluctuations associated to neuronal calcium activity over multiple weeks in response to visual stimulations or during rest. After 3 weeks of baseline recordings, photothrombotic strokes were induced in the primary visual cortex (V1). Specifically, following an intraperitoneal injection of Rose Bengal, a green laser was targeted on the cortex to induce an IS in V1, imitating cortical blindness. One week after stroke induction, we observed a strong reduction of visual evoked responses (baseline stroke mice DF/F = 0.042 ± 0.002% vs sham 0.045 ± 0.002%, one week after for stroke mice 0.017±0.004% vs sham 0.032±0.002%). After this transient impairment, a tendency towards functional recovery of calcium activity and evoked contrast response appeared four weeks after photothrombosis in comparison to sham mice (stroke mice 0.023 ± 0.003% vs sham 0.030 ± 0.004%, two-way repeated measure ANOVA: pValue = 0.085). Overall, our findings show that V1 neuronal activity is first disrupted by IS induction and then trends towards partial functional reorganization after a month of recovery. Further exploitation of

this preclinical model will improve fundamental knowledge on cortical blindness mechanisms as well as facilitate the evaluation of new treatments strategies. In the future, our findings may benefit patients by advancing vision practitioners knowledge; thereby, allowing them to improve their patient care.

Disclosures: C. Albert: None. **B.O. de Souza:** A. Employment/Salary (full or part-time):; LabeoTech Inc., Montreal. **J. Royea:** None. **J. Bouchard:** None. **M. Vanni:** None.

Poster

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Topic: D.06. Vision

Support: NIH F31 EY034400-01A
NIH RO1 EY034303

Title: Available sensory input instructs performance and sensory sampling strategy in early blind and sighted short-tailed opossums (Monodelphis domestica)

Authors: *C. R. PINEDA[1], L. A. KRUBITZER[2];
[1]Psychology, Univ. of California Davis, Davis, CA; [2]Psychology, Univ. of California, Davis, Davis, CA

Abstract: Available sensory input during early post-natal life instructs typical neocortical development, such that the loss of sensory input drastically changes neocortical organization and subsequent adult behavior. For example, congenitally blind adult humans develop new adaptive navigation and communication strategies such as cane-walking and braille reading supported by the spared somatosensory system. Despite the prevalence of vision-loss in human populations sensory-mediated compensatory strategies after sensory loss are not systematically studied. Studies in our laboratory in short-tailed opossums (*Monodelphis domestica)* that are bilaterally enucleated at post-natal day 4 (EB), before the formation of retinogeniculate and thalamocortical pathways, allowed us to examine several behaviors mediated by the spared sensory systems. For example, EB opossums have lower texture discrimination thresholds, and lower error rates in variable ladder-rung walking task, which are accompanied by postural differences (Englund et al., 2020; Rammamurthy et al., 2021). Here, we trained EB and SC opossums in a classic skilled reaching task to examine the relative contribution of the spared sensory systems to reward targeting performance. We restricted somatosensory input by trimming whiskers of the snout and chin, and restricted olfactory input with chemically induced anosmia. Animals were recorded with 3 video cameras positioned in stereo which allowed us to extract pose kinematics using DeepLabCut, a deep learning algorithm (Mathis et al., 2018). Results show that EB opossums

performed above chance, but significantly lower than SC opossums under light conditions while the performance of SC opossums decreased significantly under dark conditions. Sensory deprivation had an effect on both groups, though whisker trimming significantly decreased EB performance relative to SC opossums. Anosmia abolished targeting performance in some, but not all animals. Kinematic analysis of the extension phase of the reaching movement showed similar effects between groups across sensory deprivation types in both light and dark conditions. Kinematic analysis of the snout showed effects on snout position after whisker trimming in which the snout of EB opossums had greater peak vertical displacement compared to SC opossums, indicating differential strategies in sensory sampling between SC and EB opossums. Determining how EB opossums use the spared senses to compensate for the lack of vision allows us to see the extent to which the body and the brain adjust to sensory deficits over a lifetime.

Disclosures: C.R. Pineda: None. **L.A. Krubitzer:** None.

Poster

PSTR284: Visual System: Plasticity

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Topic: D.06. Vision

Support: NIH grant no. 1ZIAEY000570-01 to H.N.

Title: Transient morphological changes in the primary visual cortex of the thirteen-lined ground squirrel during hibernation

Authors: *A. C. FULTZ[1], C. J. JACOB[1], C. MEJIAS-APONTE[1], F. M. NADAL-NICOLAS[1], W. LI[2], H. NIENBORG[3];
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Abstract: Hibernating and seasonally breeding animals undergo seasonal neuroplasticity manifesting as changes in brain volume, dendritic arborization, and dendritic length. In the thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*), an obligatory hibernator, such morphological changes have been observed in the hippocampus, thalamus, and somatosensory cortex. We recently reported (Jacob et al. SFN 2023) that similar changes extend to layer 2/3 pyramidal cells in the primary visual cortex (V1). Here, we expand our previous results and ask whether these morphological changes reverse during the summer months. We performed Golgi-cox staining on squirrel brains collected in March (at the end of the hibernation cycle) and September (at the end of the summer months). Animals collected in March were divided into three groups (4 animals per group): awake (no hibernation, body temperature 100 °F), hibernating (torpor, body temperature 35 °F), and interbout arousal (aroused from hibernation

using gentle handling, body temperature 96.2 °F to 103 °F). Experimenters were blind to the group. Animals collected in September either did not hibernate (awake non-hibernating, 4 animals) or did hibernate (awake hibernating, 3 animals) during the previous hibernation cycle. For the animals sampled in March, we counted the number of dendritic intersections at 10-µm intervals extending from the soma (Sholl analysis) and found a significant decrease (basal $p=0.0029$, apical $p=0.0056$) during hibernation compared to when awake. We also found significant reductions in the number of dendritic nodes (basal $p<0.0001$, apical $p=0.0029$), dendritic length (basal $p=0.0016$, apical $p=0.0037$), and dendritic complexity (basal $p=0.0028$, apical $p=0.0153$). These changes demonstrated that seasonal neuroplasticity occurs in the visual brain areas. For the animals sampled in September, we completed the same analysis on the same dendritic variables we analyzed in March and found no significant differences between the awake hibernating and awake non-hibernating groups. These results show that the morphological changes during the hibernation cycle are transient. Together, our results reveal for the first time pronounced seasonal neuroplasticity in V1 of the ground squirrel that reverses before animals enter the next hibernation cycle.

Disclosures: A.C. Fultz: None. **C.J. Jacob:** None. **C. Mejias-Aponte:** None. **F.M. Nadal-Nicolas:** None. **W. Li:** None. **H. Nienborg:** None.

Poster

PSTR284: Visual System: Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR284.12/G19

Topic: D.06. Vision

Support: NSERC Grant RGPIN-2018-06506 to DB
NSERC ES-D 569471-2022 to GL
NSERC USRA to KL

Title: Stereological analysis of cholinergic and monoaminergic innervation of visual and frontal cortex in early enucleated and anophthalmic mice.

Authors: K. LAPOINTE, G. LALIBERTE, R. DUBOIS, *D. BOIRE;
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Abstract: Only a few studies demonstrate modifications of neuromodulator afferents to sensory cortices following sensory deprivation. The cholinergic and noradrenergic systems have been proposed to be involved in visual cortex plasticity. An increase in choline acetyl transferase has been observed in the primary and extrastriate cortices of dark reared kittens. Similarly, choline levels were elevated in the visual cortex of anophthalmic and early blind humans. Moreover, the basal nucleus of Meyner (NBM), a major source of cholinergic input to cortex, has a stronger

functional connectivity with the visual, language and default mode networks in early blind than in late blind and sighted subjects. This increased functional connectivity between NBM and visual cortices might suggest a strengthened anatomical projection from NBM to the visual cortex in the early blind. Moreover, the increased basal forebrain cholinergic connectivity with the aforementioned networks in the early blind could be related to the enhanced performance of early blind subjects in auditory, tactile and language tasks compared to late blind subjects. Stereological estimations of total length of VAChT and TH immunolabeled axons and varicosity density were performed in the primary visual cortex and in frontal cortices in two mouse models of early blindness, perinatal enucleation and anophthalmia. Enucleations were performed in C57B/6J mice and in mice from a cross between anophthalmic ZRDCT/An and DBA mice. This backcrossing produced mice with normal eyes and anophthalmic mice in the same litter, allowing for a direct comparison of the effects of early enucleation and anophthalmia. Density of VAChT labeled axons was greater in frontal and visual cortex in enucleated compared to intact C57BL6 mice. VAChT+ axonal density was increased only in the visual cortex of the anophtalmic hybrid mice. Linear density of VAChT labeled varicosities was lower in the frontal cortices of the enucleated C57BL6 but unchanged in their visual cortex. TH labeled axon density was similar in frontal cortices and increased in the visual cortex of blind mice. TH+ varicosity density was similar in all groups. Early loss of vision had a greater impact on cholinergic innervation of frontal and visual cortex of C57B/6J mice than on monoaminergic afferents. The precocial loss of vision can alter the development of cholinergic and monoaminergic innervation of frontal and visual cortices. Early blindness had a lesser impact on the development of these neuromodulators in ZRDCT/An X DBA hybrid mice.

Disclosures: K. Lapointe: None. **G. Laliberte:** None. **R. Dubois:** None. **D. Boire:** None.

Poster

PSTR284: Visual System: Plasticity

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Program #/Poster #: PSTR284.13/G20

Topic: D.06. Vision

Support: R01EY025613
T32MH019929

Title: Prey capture induces dynamic and persistent plasticity in binocular visual cortex (V1b)

Authors: *D. P. LEMAN[1], B. A. CARY[2], B. J. LANE[2], K. BHUT[2], M. R. SHANLEY[2], N. F. WONG[2], D. BISSEN[2], G. TURRIGIANO[2];
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Abstract: Neurons in the primary visual cortex (V1) have firing rates that span several orders of magnitude and remain stable across time, light-dark cycles, and even sensory perturbations. This work suggests that V1 neurons have an individual 'firing rate set point' that is homeostatically maintained. Here we asked whether these set points are truly 'set' or might be malleable in response to salient, ethological experiences. To investigate this, we use a prey-capture paradigm where juvenile rats learn to hunt live crickets. Rodents are opportunistic omnivores with an innate drive to hunt, but progressively improve their performance across multiple hunting sessions in one day, indicating robust learning. To determine whether this behavior depends on V1, we expressed excitatory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) in parvalbumin-positive interneurons to suppress V1 activity during hunting, which prevented the learning. Next, we used chronic, in vivo extracellular recordings in V1b to track neuronal activity during learning. Initially a small subset of neurons in V1b were significantly modulated during hunting epochs, but this fraction increased dramatically across subsequent hunting sessions, indicating that the activity of V1b neurons becomes tied to the behavior during learning. Next, we measured how learning impacts firing rates on longer timescales to determine whether firing rate set points were altered by learning. We found that V1b neurons have stable firing rates prior to hunting, but that firing rates change slowly over several days hours post-learning, leading to an overall increase that persists at both the individual neuron and population levels for at least 60 hours post-learning. This increase was observed in both regular spiking and fast-spiking neurons, suggesting that excitation and inhibition increase in a coordinated manner. Because upward firing rate homeostasis is gated by wake states, we used polysomnography to determine when this increase in firing occurs. Remarkably, we found that the increase in firing happens primarily during waking states, implicating homeostatic, rather than Hebbian, plasticity in this process. Finally, pharmacological block of homeostatic plasticity using a TNFa inhibitor administered after learning was completed significantly decreased the retention of learning several days later. Our data are consistent with a model in which learning rapidly resets the firing rate setpoint for the network, and wake-dependent homeostatic processes then slowly move the network to this new state.

Disclosures: D.P. Leman: None. **B.A. Cary:** None. **B.J. Lane:** None. **K. Bhut:** None. **M.R. Shanley:** None. **N.F. Wong:** None. **D. Bissen:** None. **G. Turrigiano:** None.

Poster

PSTR284: Visual System: Plasticity

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Program #/Poster #: PSTR284.14/G21

Topic: D.06. Vision

Support: CRSNG RGPIN-2018-06506
CRSNG ESD-2022-569471

Title: Mesoscopic calcium imaging of cortical activity in early blind mice

Authors: *G. LALIBERTE, K. LAPOINTE, D. BOIRE;
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Abstract: Early visual loss in humans triggers cortical network restructuring and heightened sensitivity to remaining sensory inputs and cross-modal activation of visual cortical areas. However, resting-state functional connectivity and anatomical projections between visual and other primary sensory cortical areas are decreased. It has been suggested that cross-modal cortical activation rely on established top-down projections and modifications of the excitatory/inhibitory balance in local cortical circuits rather than new connections linking sensory modalities. We used mesoscopic calcium imaging in sighted (n=19) and visually deprived mice (neonatal enucleation [n=17] or congenital anophthalmia [n=5]) to record cortical activity at rest and investigated functional alteration after early blindness. We recorded all cortical neuronal populations (AAV.PhP.eB-hSyn-GCaAMP6s), or selectively glutamatergic (Thy1-GCaMP6s), or GABAergic (AAV.PhP.eB-mDLX-GCaMP6s). Non-visual stimulation activated areas beyond those typically engaged in sighted controls, demonstrating cross-modal activation in mice. Resting-state functional connectivity (rsFC) spectral analysis reveals shifts in spontaneous activity within hSyn and glutamatergic groups, but not among the GABAergic population. These changes are mostly evident in low-frequency oscillations (<5 Hz), enucleation increased negative intrahemispheric correlations between medial higher visual areas (PM, AM, and A; HVA) and motor/trigeminal somatosensory regions (BC, NO, and MO) for the hSyn targeted neuronal population. Also, a reduction occurred in interhemispheric correlations between lateral (AL, LM, and RL) HVA and motor and trigeminal areas. Within the glutamatergic population a decrease in intrahemispheric correlations between lateral HVA and lemniscal somatosensory areas (HL, FL, and TR) was observed. Resting state recording of GABAergic neurons showed a decreased interhemispheric positive correlation between homotopic lateral HVA. The global network metrics were mainly unchanged by the loss of sight (network density, global efficiency, and global strength), but nodes efficiency, strength, centrality and participation coefficients reveal a reduced influence of V1 and contrasting effects between lateral and medial HVA, and among lemniscal and trigeminal somatosensory areas. Early blindness seems to have a greater effect on rsFC disruption than the congenital blindness. These results demonstrate differential effect of specific neuronal populations on cortical plasticity and their influences on the dynamics of cortical sensory networks following visual deprivation.

Disclosures: G. Laliberte: None. **K. Lapointe:** None. **D. Boire:** None.

Poster

PSTR284: Visual System: Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR284.15/G22

Topic: D.06. Vision

Title: Effect of attentional state on experience dependent visual cortical neuroplasticity of EEG contrast response functions

Authors: *P. N. LIMON[1], A. M. NORCIA[2], R. T. ASH[3];
[1]Psychiatry and Behavioral Sci., Stanford Univ., Palo Alto, CA; [2]Wu Tsai Neurosciences Inst., [3]Psychiatry and Behavioral Sci., Stanford Univ., Stanford, CA

Abstract: Passive viewing of a 2 Hz contrast-reversing stimulus has been shown to potentiate visual evoked responses in humans, allowing for quantification of experience-dependent visual cortical neuroplasticity referred to as stimulus-specific response potentiation (SSRP). Brain states like attention are known to modulate learning and plasticity, but how attentional state impacts SSRP effects has not been previously studied. We hypothesized that focusing visual spatial attention on the SSRP-induction stimulus enhances response potentiation within its retinotopic extent, while diverting attention away from the SSRP-induction stimulus will reduce response potentiation. Stimuli consisted of 2 semicircular checkerboards placed on each side of fixation (10° eccentricity), each frequency-tagged to measure EEG steady-state visual-evoked potentials, recorded with a 128-channel EGI system. Before and after SSRP-induction, participants observed contrast-sweeps of the checkerboard stimuli with sign-reversal rates are 2F1 = 6 Hz, 2F2 = 7.5 Hz (sign-reversal frequencies not expected to induce plasticity), while maintaining fixation via a letter-detection task. In the SSRP-induction block, the left checkerboard (potentiating stimulus) was presented at 100% contrast with sign-reversal rate at 2F1=2 Hz (a frequency known to induce plasticity), and the right checkerboard (non-potentiated control) was presented at 2% contrast, sign-reversed at 2F2 = 3 Hz. During the SSRP-induction block, participants deployed peripheral spatial attention to either the potentiating stimulus (Aligned SSRP + Attention) or the non-potentiated control (Unaligned SSRP - Attention) in two separate sessions, by performing a contrast increment detection task. Our preliminary data (n=26 SSRP + Attention, n=24 SSRP - Attention) suggests that post-induction neural activity in the potentiated hemifield increased regardless of attentional deployment. During the Aligned SSRP + Attention condition, both the potentiated stimulus and non potentiated control increased, whereas in the Unaligned SSRP - Attention condition, responses increased in the potentiated hemifield but decreased in the control hemifield. Our results preliminarily demonstrate SSRP induced response enhancement in the visual cortex in this study and suggest that attentional deployment extends the neuroplastic effect of SSRP beyond its retinotopic extent, affecting responses in the contralateral hemisphere.

Disclosures: P.N. Limon: None. **A.M. Norcia:** None. **R.T. Ash:** None.

Poster

PSTR284: Visual System: Plasticity

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Topic: D.06. Vision

Support: NIH R01EY023037
Picower Institute Innovation Fund
NSF Graduate Research Fellowship Program

Title: Investigating the emergence of a form of visual recognition memory stored in mouse V1

Authors: *S. K. SIMPSON[1], P. S. B. FINNIE[2], M. F. BEAR[1];
[1]MIT - Picower Inst. For Learning & Memory, Cambridge, MA; [2]Univ. of Toronto, Toronto, ON, Canada

Abstract: Briefly exposing mice to an unrewarded, oriented, phase-reversing visual grating stimulus initiates thalamocortical synaptic plasticity that later enables the animal to recognize the stimulus as familiar. In primary visual cortex (V1), stimulus-selective response plasticity (SRP) is characterized by a robust increase in the magnitude of the visually-evoked potential (VEP) recorded in layer 4 that reports summed thalamocortical and intracortical synaptic currents and correlates with increased peak firing in response to the familiar stimulus. Intriguingly, VEP potentiation does not emerge within a single stimulus presentation session but manifests by the next recording session the following day, raising the possibility that the plasticity requires a period of offline consolidation. The goal of this work was to characterize this consolidation period. We began with “deck-splitting” experiments designed to investigate if classic circuit- or cellular-level neural mechanisms of consolidation are recruited following initial stimulus presentation. We found that systemically treating mice with anesthetics, barbiturates, amnestic drugs, or a protein synthesis inhibitor after initial stimulus presentation to broadly disrupt ongoing neural or biochemical activity in the subsequent hours failed to block emergence of stimulus-selective VEP potentiation. Next, we examined the possibility that SRP requires sleep and found no difference in emergence of SRP during the day and overnight. We then directly addressed the question of how long it takes for this electrophysiological change to emerge after the mouse first sees the grating stimulus. We found that following presentation of a 1 Hz phase-reversing grating, VEP potentiation in juvenile mice reaches an asymptote within 30 minutes. However, when we presented the grating at a higher temporal frequency (4 or 7.5 Hz phase-reversals), stimulus-selective VEP potentiation emerged during initial stimulus exposure - on the timescale of minutes - reminiscent of *in vivo* thalamocortical long-term potentiation. This suggests that the emergence of SRP can be fully set into motion by the initial exposure to the grating stimulus and that neither circuit-level consolidation mechanisms nor sleep need to be recruited after the stimulus presentation for VEP potentiation to emerge.

Disclosures: S.K. Simpson: None. **P.S.B. Finnie:** None. **M.F. Bear:** None.

Poster

PSTR284: Visual System: Plasticity

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR284.17/G24

Topic: D.06. Vision

Support: R21-EY034297

Title: A mouse model to probe the limits of adult neuroprosthetic plasticity

Authors: *L. MESIK[1], H.-K. LEE[2];
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Abstract: As neural prostheses become increasingly more sophisticated, they may hit a point where they are no longer limited by the number of recording channels or stimulation electrodes, but rather by the reduced plasticity potential of adult cortical circuits. At the same time, a multitude of methods have been proposed for reopening 'critical periods' of plasticity, but whether these would work in the context of neuroprosthetics remains unknown. To bridge this gap, we developed a mouse model of a thalamic visual prosthesis in which normal vision is replaced by patterned optogenetic stimulation of dLGN using a digital micromirror device, with light delivered through a fiber bundle coupled to an implanted grin lens. In a proof of principle experiment, we trained mice on a go-nogo task based on optogenetic stimulation of 2 different spatial locations within thalamus. Most of the mice were able to learn detection of these artificial stimuli, with the speed of learning being strongly correlated with overall stimulus strength. Stimulus discrimination, however, proved much more challenging and even the mice that learned to discriminate could not do so at the levels achievable with regular visual discrimination tasks, with performance of even 'successful' mice often capped at a d' of about ~1. Learning was accompanied by several changes in the activity of L2/3 neurons monitored using calcium imaging. These include a modest increase in stimulus selectivity, decrease in response magnitude, and decrease in stimulus-evoked correlations that is at least partly explained by increased sparseness of response. We are currently examining a range of interventions to see whether we can enhance the neuroprosthetic learning in the trained mice.

Disclosures: L. Mesik: None. **H. Lee:** None.

Poster

PSTR284: Visual System: Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR284.18/G25

Topic: D.06. Vision

Title: Stimulation of primary adult pig retinal pigment epithelium culture with an alpha7 nicotinic agonist leads to neurogenesis in primary adult pig retinal cell culture

Authors: C. A. EASTMAN, E. E. VAN PELT, T. M. SABER, *D. M. LINN;
Grand Valley State Univ., Allendale, MI

Abstract: Previous work has shown that activation of the alpha7 subtype of nicotinic acetylcholine receptor (nAChR) on retinal ganglion cells (RGCs) protects from glaucoma-like damage. More recent work has shown that in addition to this neuroprotective effect, activation of the alpha7 nAChR can lead to the generation of new adult retinal cells via a multi-cellular pathway. Specifically, activation of alpha7 nAChRs (with PNU-282987) on retinal pigment epithelial (RPE) appears to induce the release of substances that cause retinal Muller glia (MG) to re-enter the cell cycle and produce new retinal cells, including RGCs. One study exposed an RPE cell line to PNU-282987 and then injected the supernatant from that culture into the eye of an adult rodent. The neurogenesis of retinal cells was confirmed by several techniques. We are attempting to expand on those studies by using primary culture of adult pig RPE and adult pig retina. Basically, we used 4 different experimental conditions and quantified cell number at the end of culture. In Condition A: Retinal cells were cultured for 5 days without any intervention and served as a control. Condition B: Retinal cells were co-cultured with untreated RPE that had been cultured for two days prior. Condition C: Retinal cells were co-cultured with RPE treated with a low dose (100 nM) of PNU-282987 for two days prior. Condition D: Retinal cells were co-cultured with RPE treated with a high dose (200 nM) of PNU-282987 for two days prior. We observed an increase in the number of retinal cells when co-cultured with RPE, and an even greater dose-dependent increase in cell number when RPE were exposed to PNU-282987. When retinal cells were co-cultured with primary RPE that had been cultured without any PNU-282987 (Condition B), we observed a 28.3% increase ($p = 0.02$, N=6). When retinal cells were co-cultured with RPE exposed to a low dose of PNU-282987 (100 nM) for two days (Condition C), we observed a 57.7% increase ($p = 0.01$, N=6). And, when retinal cells were co-cultured with RPE exposed to higher dose of PNU-282987 (200 nM, Condition D), we observed a 102.8% increase ($p < 0.001$, N=6). Preliminary data where cultures were exposed to tagged alpha-bungarotoxin shows diffuse staining through-out the RPE and punctate staining on presumptive RGCs. This is the first report, that we are aware, where control and stimulated primary RPE can induce an increase in a primary retinal cell culture numbers from an adult non-rodent large mammal model. Further experiments need to be conducted to differentiate between potential neuroprotective and neurogenerative mechanisms activated via the alpha7 nAChR.

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Poster

PSTR284: Visual System: Plasticity

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Program #/Poster #: PSTR284.19/G26

Topic: D.06. Vision

Support: U01EY025858

Title: A new large dataset using a macular degeneration model for human visual plasticity

Authors: *K. VISSCHER[1], P. D. STEWART[2], L. L. FLEMING[3], P. DEMIRAYAK[4], M. K. DEFENDERFER[5], S. A. SIMS[6];
[1]Univ. of Alabama, Birmigham, Birmingham, AL; [2]Univ. of Alabama, Birmingham, Birmingham, AL; [3]Psychiatry, Mclean Hosp./Harvard Med. Sch., Atlanta, GA; [4]Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL; [5]Grad. Biomed. Sci., Univ. of Alabama At Birmingham, Calera, AL; [6]MUSC, Charleston, SC

Abstract: We present a new dataset, the Macular Degeneration and Plasticity (MDP) project, which is part of the Human Connectome Project (HCP), now publicly available through the NIMH Data Archive. Macular degeneration (MD) encompasses pathologies resulting in the loss of photoreceptors in the macula, causing central vision loss. Patients with MD often have a specific portion of spared visual field that they preferentially use in place of the fovea. This pseudofovea, also known as a preferred retinal locus (PRL), is an area of increased use in the periphery of vision. While some MD patients never fully develop a PRL, all participants with central vision loss take advantage of areas of spared visual field to compensate for their vision deficit. Since many areas of the brain are retinotopically mapped, data from participants with MD allows for an examination of increased and decreased usage of brain regions within the same participant. Thus, observing how the brain adapts to loss of central vision loss can provide useful insights on mechanisms of plasticity in the visual system. The MDP project is made up of 38 participants with central vision loss due to MD and 30 controls. Data including MRI, visual exams, and behavioral data was collected across 6 sessions. MRI data includes T1/T2 anatomical scans, multiple diffusion scans, as well as 150 hours of curated, cleaned BOLD data across all participants. BOLD data includes over 40 hours each of eyes-open resting state data and eyes-open resting state data acquired in complete darkness, as well as 4 movie watching scans and 4 HCP task scans collected for most participants. Additionally, a full-field stimulation scan, a scan designed to stimulate the PRL region, and 4 retinotopy scans were collected. Visual exams performed for all participants includes fundus photography, Optical Coherence Tomography (OCT), Macular Integrity Assessment (MAIA), and tests for visual acuity and contrast sensitivity, the Farnsworth color test, and completion of the Visual Function Questionnaire (VFQ). Behavioral data includes a full HCP behavioral workup, including NIH toolbox, the Penn Computerized Neuropsychological Test Battery (Penn-CNB), and daily functioning and personality exams. Additionally, we present the results of several analyses of the dataset, showing its utility for examining plasticity in an ecological model in humans. We believe this dataset has potential to help the field better understand the scope and nature of neural plasticity in the visual system.

Disclosures: K. Visscher: None. **P.D. Stewart:** None. **L.L. Fleming:** None. **P. Demirayak:** None. **M.K. Defenderfer:** None. **S.A. Sims:** None.

Poster

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR284.20/G27

Topic: D.06. Vision

Support: NIH Grant EY034092

Title: Impairments to sensorimotor behavior from long-term monocular deprivation can be explained by a degraded representation of disparity in the visual cortex

Authors: T. BROWN[1], C. BARR[2], A. W. MCGEE[3], ***J. SAMONDS**[2];
[1]ASNB, Univ. of Louisville, Louisville, KY; [2]Univ. of Texas at Austin, Austin, TX; [3]Univ. of Louisville, Louisville, KY

Abstract: Amblyopia is the most frequent cause of vision loss in children aside from refractive error, and impaired stereopsis is a principal symptom of amblyopia. Understanding the development, refinement, and disruption of the neural circuits for binocular depth perception may lead to rational therapies to improve or recover stereoscopic perception. We recently demonstrated that mice possess binocular depth perception, and that the disparity information from populations of neurons in visual cortex is sufficient to explain the performance of naïve adult mice. Here we probe the emergence and refinement of binocular depth perception in mice during development, as well as the impact of early abnormal vision, long-term monocular deprivation (LTMD), on depth perception, binocular alignment, and disparity tuning in primary visual cortex. Using the pole descent cliff task (PDCT), we observe that depth perception requiring binocular vision is not fully mature until around postnatal (P) day 80. We also detect a deficit in binocular depth perception with the PDCT in mice receiving LTMD from P22-52 followed by 6 weeks of binocular vision compared to control mice. Interestingly, the sensitivity of binocular depth perception to LTMD extends beyond the maturation of visual acuity as mice receiving LMTD from P70-98 also display deficits in performance. Disruptions of sensory input such as amblyopia may also lead to abnormal binocular alignment, referred to as sensory strabismus. Remarkably, mice receiving LTMD had a standard deviation of binocular alignment that was twice as large as control mice (6 vs 3 degrees of visual angle) and equal to that observed in mice at P22. In order to explain their behavioral deficits, we examined the disparity-dependent responses in the visual cortex of LTMD mice to see if they differed systematically from control mice. At first glance, visual responsiveness to disparity did not appear to be disrupted in these mice. In fact, mice that underwent LTMD had larger responses across all disparities, more visually responsive neurons, a higher percentage of significantly disparity-tuned neurons, and no clear differences in disparity selectivity compared to control mice. When we fit Gabor functions to disparity-tuned neurons in LTMD mice though, they had spatial frequencies that were half as

large as those measured in control mice (0.02 vs 0.04 cycles/degrees). In addition, preferred disparities in LTMD mice were spread out over a broader range of disparities. This suggests that LTMD degraded the representation of disparity, a circuit disruption that could explain the associated sensorimotor deficits.

Disclosures: T. Brown: None. **C. Barr:** None. **A.W. McGee:** None. **J. Samonds:** None.

Poster

PSTR284: Visual System: Plasticity

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Program #/Poster #: PSTR284.21/G28

Topic: D.06. Vision

Support: R01AG064067

Title: Structural excitatory and inhibitory synaptic remodeling elicited by repeated visual experience

Authors: *J. DODERER[1], J. SUBRAMANIAN[2];
[2]Dept. of Pharmacol. and Toxicology, [1]Univ. of Kansas, Lawrence, KS

Abstract: Structural excitatory and inhibitory synaptic remodeling elicited by repeated visual experience**Julia J. Doderer,** Jaichandar Subramanian; Department of Pharmacology and Toxicology, School of Pharmacy, University of Kansas, Lawrence, KS 66045, USA
AbstractRepeated visual stimulus exposure leads to robust recognition memory, facilitates perceptual learning of specific details, and enables generalization to novel similar stimuli. Repeated passive visual experience of orientation grating stimuli for multiple days modulate neural responsiveness to familiar and non-familiar stimuli. How repeated exposure to a specific visual stimulus alters the excitatory and inhibitory synaptic adaptation is not fully understood. Here, we present the structural synaptic adaptations associated with repeated visual experiences of orientation grating stimuli in ~4-6 month old B6SJL mice. Using multicolor two-photon *in vivo* synaptic imaging of visual cortical neurons fluorescently labeled with PSD-95 and gephyrin, we found both excitatory and inhibitory synapse dynamics are modulated by repeated passive visual experience. Furthermore, the modulation of inhibitory synapses differs based on their dendritic location. We also test whether the plasticity of these synapse types and their coordinated dynamics is affected in a mouse model of Alzheimer's disease. These results provide insight into the synaptic plasticity mechanisms of visual recognition memory.

Disclosures: J. Doderer: None. **J. Subramanian:** None.

Poster

PSTR284: Visual System: Plasticity

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Topic: D.06. Vision

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Title: Treatment induced neural plasticity in convergence in sufficiency: A longitudinal resting-state study

Authors: *F. HAJEBRAHIMI[1], S. GOHEL[2], A. SANGOI[1], M. SCHEIMAN[3], E. M. SANTOS[1], T. ALVAREZ[1];
[1]New Jersey Inst. of Technol., Newark, NJ; [2]Rutgers Univ., Newark, NJ; [3]Salus Univ., Philadelphia, PA

Abstract: Introduction: Convergence Insufficiency (CI) is the most common dysfunction in binocular vision, impacting quality of life, especially for daily tasks involving close visual work such as reading. CI participants show reduced resting-state functional connectivity (RSFC) compared to those with normal binocular vision. To date, there is a lack of longitudinal studies exploring RSFC changes following Vergence and Accommodative Therapy in CI participants. This study aimed to investigate the neural effects of Office-Based Vergence and Accommodative Therapy (OBVAT) on RSFC in participants with CI.
Methods: The study included fifty-one CI participants aged 18-35, randomly assigned to receive either 12 sessions of OBVAT or a placebo treatment. Baseline and post-treatment RSFC assessments were conducted using resting-state functional magnetic resonance imaging alongside clinical evaluations. A Region of Interest (ROI) analysis was conducted on 9 areas of the oculomotor vergence network, including the cerebellar vermis (CV), frontal eye fields (FEF), supplementary eye fields (SEF), parietal eye fields (PEF), and primary visual cortices (V1). RSFC changes were evaluated within each group using paired t-tests. A linear regression analysis was employed to explore behavioral correlations in significant ROI pairs at the group level analysis.
Results: Following OBVAT, increased RSFC was observed in 10 ROI pairs compared to the placebo treatment group ($p<0.05$, False Discovery Rate corrected). These pairs included Left (L)-SEF - Right (R)-V1, L-SEF - CV, R-SEF - R-PEF, R-SEF - L-V1, R-SEF - R-V1, R-SEF - CV, R-PEF - CV, L-V1 - CV, R-V1 - CV, and L-V1 - R-V1. Significant behavioral correlations were noted between the RSFC strength of the R-SEF - R-PEF pair and visual function parameters of positive fusional vergence and near point of convergence ($p<0.05$).
Conclusion: While OBVAT led to increased RSFC in specific ROIs of the oculomotor vergence network in CI participants, correlating with improvements in clinical measures, placebo treatment did not significantly change RSFC.

KeywordsResting-state functional connectivity, resting-state, fMRI, convergence insufficiency, binocular vision

Disclosures: F. Hajebrahimi: None. **S. Gohel:** None. **A. Sangoi:** None. **M. Scheiman:** None. **E.M. Santos:** None. **T. Alvarez:** None.

Poster

PSTR284: Visual System: Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR284.23/Web Only

Topic: D.06. Vision

Support: TÜBİTAK 1001 Grant 121K902

Title: Visual Brain Regions in the Blind Become Multiple Demand and Not Language Regions

Authors: *A. VAROL[1], S. KURT[3], T. GEZICI[1], A. A. FAROOQUI[2];
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Abstract: The fate of occipital cortices in the blind has been of key interest in neuroscience to investigate if and how much brain regions change with experience. Neuroimaging shows that occipital cortices in the blind activate during all kinds of non-visual tasks. We recently showed that the entire occipital regions in the blind become multiple-demand (MD) regions and activate in response to any kind of control demand. We suggested that this may account for the numerous existing observations of these regions activating during non-visual tasks.
However, some past studies have also shown activations of foci within these regions to language tasks and have suggested a hemispheric specialization within these regions, with left occipital regions predominantly taking up language functions. It is unclear, however, if left occipital regions in the blind do indeed take up language functions or if their activation is due to control demands immanent in many language experiments.
We had 19 blind and 15 sighted participants do two language fMRI tasks. The language blocks of the first one required participants to hear a sentence with a missing last word and then generate and verbalize the missing word. In the non-language blocks of this task, participants heard a pseudoword sentence and then repeated the last pseudoword. The language and non-language blocks of the second task required listening, respectively, to passages from Alice in Wonderland and degraded incomprehensible sentences. The first language task, requiring participants to select and articulate the missing word, had high domain-general control demands, while the second one, which only required listening and comprehending passages, was a passive language task with barely any control demand. These participants also did a tactile decision-making task requiring size judgments on tactile shapes. These judgements could be easy or hard.

We found that while both language tasks activated the language network, the first task additionally activated MD regions – pre-supplementary motor area, inferior frontal junction and frontal eye fields – that typically activate to control demands. Crucially, only the first language task, but not the second, activated occipital regions in the blind. That this occipital activation was due to the domain-general control demand was also suggested by the fact that both the MD regions and the occipital regions activated by the first language task also activated to the control demands of the tactile task.
All of these suggest that blind occipital regions primarily become MD regions, and their activation during language tasks is limited to those that require domain-general cognitive control. (121K902)

Disclosures: A. Varol: None. **S. Kurt:** None. **T. Gezici:** None. **A.A. Farooqui:** None.

Poster

PSTR284: Visual System: Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR284.24/G30

Topic: D.06. Vision

Support: TÜBİTAK121K902

Title: Regions within V1 of the sighted activate to non-visual control demands but still are categorically different from V1 of the blind

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Abstract: If and how much brain regions can change from their biologically determined functions is a key question in neuroscience.
In this regard, the fate of visual regions in the blind has been a focus of many studies that have tried to show that visual regions in the blind behave in ways very different from those in the sighted and activate to tasks in other sensory modalities. But it remains unclear if these regions are indeed showing distinct functions, or are just showing multi-sensory responses already present in these regions.
We recently showed that all visual-occipital regions in the blind become multiple-demand (MD) regions and, like the canonical frontoparietal MD regions, activate to all kinds of cognitive-control demands. In contrast, most visual-occipital regions in the sighted either showed a reliable absence of such activation or deactivated to such control demands. However, we did find a small cluster of voxels in the primary visual cortex (V1) of the sighted that activated to different non-visual control demands, much like the visual-occipital regions of the blind.

In the current study, we investigated if this V1 voxel-cluster in the sighted behaves identically to the analogous voxels in the blind. Both participant groups did two fMRI experiments. The first involved easy and hard blocks of an auditory n-back task that required participants to continually update items in their working memory. The second involved easy and hard blocks of a tactile decision-making task.
We found that even though this voxel-cluster activated to control demands in both participant groups, it did behave very differently in the blind compared to the sighted, especially during the auditory n-back task: (1) it showed more intense activation to control demands in the blind than in the sighted; (2) the pattern of its activity across the three junctures of a task block – beginning, main block, and completion – were different across the blind and the sighted; (3) its time-course of activity across a task block was also different between these groups.
Thus, even though this small V1 cluster did activate to non-visual control demands in the sighted, its behavior was categorically different from that in the blind, suggesting that even this visual region developed into a categorically different brain region in the blind. (121K902)

Disclosures: E. Oymagil: None. **A.A. Farooqui:** None. **S. Kurt:** None.

Poster

PSTR284: Visual System: Plasticity

Location: MCP Hall A

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Program #/Poster #: PSTR284.25/G31

Topic: D.06. Vision

Support: FRQS - 312876
NSERC
CIRCA

Title: Mapping the spontaneous brain activity in adult mice after vision loss

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Abstract: Most people who lose their vision do so in adulthood, when the brain is less plastic than in youth. In these people, functional magnetic resonance imaging has shown that the spontaneous resting brain activity of visual regions is affected. Functional connectivity between the primary visual cortex and other areas of the visual network, as well as with the primary motor and somatosensory cortex, is weaker than in sighted people. These changes may be associated with cross-modal plasticity, where in the case of blindness, neurons in the visual cortex start to represent other sensory modalities. However, it’s unclear how this plasticity induced by bilateral vision loss develops in the adult brain. Furthermore, we don't know how functional connectivity is altered in active behavioral states. To answer those questions, we are using the mouse, a

popular model for studying the spontaneous brain activity and plasticity in the visual system, and where there is evidence of cross-modal plasticity occurring in the adult brain. We used mesoscopic calcium imaging of the dorsal cortex on 24 transgenic mice Thy1-jRGECO1a to measure the cortical activity during head-fixed spontaneous behavior. After the surgical implantation of a chronic imaging window, mice were group-housed to maximize the multisensory experience. The mice were habituated to being head-fixed on a running wheel under the imaging system for 1 week. Then we started the baseline imaging sessions. The spontaneous activity sessions lasted 20 min, during which the mice were free to run on the wheel and their behavior was captured by a camera under infrared illumination. From the face and paw movements, the behavior was classified into rest and locomotion. The longitudinal protocol started with 4 weeks of baseline measures. Then 18 mice were bilaterally enucleated, while the 6 others remained sighted as a control. Imaging sessions continued at 1, 2, 3, 5, 7, and 10 weeks after the vision loss. We found that, during the resting state, the primary visual cortex (V1) has an increase in activity that reaches its maximum 3 weeks after vision loss. At that time, the standard deviation of the signal was 28% higher than baseline. After 3 weeks, the level of activity slowly decreases to near baseline levels at 10 weeks after vision loss. While the activity increases, V1 decreases its functional connectivity with the other regions. However, during locomotion, V1 has an opposite temporal dynamic. To conclude, it seems that the spontaneous brain activity is affected by vision loss in a state-dependent manner. This ongoing project will help to better understand how plasticity shapes the functional brain architecture after vision loss.

Disclosures: I. Djerourou: None. **M. Ptito:** None. **M. Vanni:** None.

Poster

PSTR284: Visual System: Plasticity

Location: MCP Hall A

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Topic: D.06. Vision

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Institute for AI and Beyond
JSPS KAKENHI (JP19H05642, JP20H05917)

Title: Orientation representation is functionally reorganized after eye opening in the mouse primary visual cortex.

Authors: *F. KISHINO[1,2,3], T. YOSHIDA[1,2,3], M. UEMURA[4], S. TRÄGENAP[5], M. KASCHUBE[5], K. OHKI[1,2,3];

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Abstract: Information representation in the visual cortex changes after the onset of visual experience. In the mouse primary visual cortex (V1), the number of visually responsive neurons, particularly those preferring oblique orientations, increases after eye opening (Rochefort et al., 2011; Hoy et al., 2015; Hagihara et al., 2015). Thus, the neuronal population matures to represent all orientations, but the process remains unknown. One possibility is that neurons that initially prefer horizontal or vertical orientations change to prefer oblique orientations. Another possibility is that neurons that gain responsiveness after eye opening predominantly prefer oblique orientations. To test these possibilities, using longitudinal two-photon calcium imaging, we tracked the responses of the same neurons to drifting gratings in the mouse monocular V1 every other day for one week after eye opening. Among the tracked neurons, only a small proportion of neurons were consistently responsive across imaging days. The majority of neurons either gained or lost their responsiveness from day to day, highlighting the daily reorganization of visual responsiveness after eye opening. In addition, the proportion of neurons that maintained their responsiveness between two consecutive imaging days increased. Thus, while the visual responsiveness of individual neurons was dynamically reorganized, their visual responses gradually stabilized. Under this reorganization, the proportion of oblique-preferring neurons increased rapidly from day 1 to day 3 after eye opening. This increase was mainly contributed by both neurons that changed their preferred orientations from cardinal to oblique and those that gained responsiveness on day 3. These results indicate that the orientation representation in the neuronal population matures under the daily reorganization of both visual responsiveness and orientation tuning in the mouse V1.

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Poster

PSTR284: Visual System: Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR284.27/G33

Topic: D.06. Vision

Support: DoD #W81XWH-21-1-0884
NIH EY032948-01A1

Title: Mild traumatic brain injury to mouse V1 affects the network mediating the suppressive surround

Authors: *D. C. LYON;
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Abstract: Visual dysfunction is a common disability in people with traumatic brain injury (TBI), but underlying mechanisms remain largely unknown. In a previous study (Frankowski, Foik et al, 2021), we reported ~35% reduction in inhibitory neurons and impaired responsivity to visual stimuli, including broader orientation and size tuning, following mild controlled cortical impact to mouse primary visual cortex (V1). Here, we studied the effects of TBI on extra-classical surround suppression in mouse V1. We delivered a mild unilateral injury centered over the rostral end of V1 in young-adult mice at P60 (N=6) from both sexes. Three months post-injury, animals underwent in-vivo single-unit recording in V1 under isoflurane anesthesia. We compared the orientation tuning of neurons to drifting gratings presented at the optimally sized classical receptive field (CRF; smaller aperture which elicits the largest response) and the full-field suppressive surround (FF; size larger than the optimal which results in fewest spikes/s). In control animals we found that response rates were lower to FF compared to CRF, approximately 30 vs 40 spikes/s, similar to our results shown previously in cat V1 (e.g. Liu, Hashemi-Nezhad, Lyon, 2015). Conversely, in TBI animals there was no difference between FF and CRF response rates and both were significantly lower than controls at ~12 spikes per second under both stimulus conditions. In addition, our preliminary data suggests in controls FF suppression results in sharper orientation tuning (measured as the half-width at half-height; HWHH) compared to CRF stimuli, as previously shown (ibid.) In TBI, there was no suppressive FF effect and orientation tuning not only broader than controls, but showed the opposite effect of controls in the tuning was worse under FF compared to CRF. Surround suppression has been shown to result, at least in part, from long range excitatory inputs (from within V1 and from higher visual areas) synapsing onto local inhibitory neurons. This long-range mediated suppression leads to sharpening of orientation tuning by suppressing responses to non-preferred orientations more strongly than the preferred. We detected this suppressive effect in controls, but not in animals suffering TBI to V1. These results suggest local inhibition is markedly reduced following mild TBI to V1, consistent with a loss of inhibitory neurons following injury.

Disclosures: D.C. Lyon: None.

Poster

PSTR285: Eye Movements: Saccades

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR285.01/G34

Topic: E.01. Eye Movements

Support: JSPS KAKENHI Grant-in-Aid for Scientific Research (C) (Grant Number JP24K14586)

Title: Early saccade strategy for rapid prediction of moving targets: The role of right MT and premotor low beta EEG

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Abstract: This study aimed to elucidate the mechanisms that enable participants to quickly shift their gaze to the predicted arrival position of a parabolically moving target that becomes an invisible mid-trajectory, and to successfully execute the Saccade Strategy Task (SST). The participants' electroencephalography (EEG) and gaze signals were recorded while they engaged in the SST. The participants performed a task to predict the final arrival position and the time of a target moving parabolically from the lower left to the lower right of the screen. For the SST, participants were instructed to move their eyes directly to the presumed final arrival point, which was at almost the same height as the starting point, as soon as they predicted it. The participants were then instructed to keep their eyes at the final arrival point (almost the same height as the starting point) at the bottom of the display, and to respond by pressing a button with their right thumb when they predicted that it had been reached. Within 1 s of the target movement onset, the x-coordinate of the eye position of six participants changed rapidly (indicating a saccade to the arrival position of the moving target), and these participants did not track the parabolic moving target (the y-coordinate of the eye position was below a certain standard point). These participants were categorized as the Early Saccade Strategy Group (ESSG), while the remaining ten participants were grouped into the Late Saccade Strategy Group (LSSG). In the right premotor, low beta EEG activity was found to be significantly higher in ESSG than in LSSG at 0.517-0.594s after the start of target movement ($p<0.05$). This low beta EEG activity in the right premotor in ESSG may reflect visuomotor transformation. In addition, in the right middle temporal (MT) visual area, low beta EEG activity was significantly higher in ESSG than in LSSG at 0.723-0.810s after the initiation of target movement ($p<0.05$). This low beta EEG activity in the right MT visual area in ESSG may reflect the detection of moving target speed information. Conversely, in the right parietal region, low beta EEG activity was significantly higher in the LSSG than in ESSG at 1.714-1.757s after target movement started ($p<0.05$). The low beta EEG activity in the parietal region in LSSG may reflect visual attention to moving targets in late period. Overall, our study suggests that it is necessary to quickly detect the speed and direction of a moving target in the right MT visual area and perform a visuomotor transformation in the right premotor area, thereby performing an Early Saccade Strategy.

Disclosures: R. Koshizawa: None. **K. Oki:** None. **M. Takayose:** None.

Poster

PSTR285: Eye Movements: Saccades

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR285.02/G35

Topic: E.01. Eye Movements

Support: Boehringer Ingelheim Fonds PhD Fellowship

Title: Saccade-associated responses in zebrafish optic tectum

Authors: *L. BAUER, J. C. DONOVAN, H. BAIER;
Genes - Circuits - Behavior, Max Planck Inst. for Biol. Intelligence, Planegg, Germany

Abstract: Saccadic eye movements are fundamental to vertebrate visual perception, yet it is still unclear how visual circuits smoothly handle the resulting shifts in visual input. The zebrafish model, with its genetic and optical tractability, offers an ideal system for investigating the underlying sensorymotor circuits. We combined eye tracking and two-photon calcium imaging to investigate the neuronal correlates of saccades in larval zebrafish (6-8 dpf). We used a modified two-photon microscope equipped with a remote focusing path to enable rapid multi-plane imaging. This provides single-cell resolution neuronal activity across six planes of the optic tectum and parts of the hindbrain at 5 volumes per second, correlated with the changes in eye angles. Even in the absence of visual stimuli we find neurons in the optic tectum and hindbrain that display increased activity associated with spontaneous saccades. To investigate how visual stimuli are integrated, we recorded from the same neurons during various visual stimulus paradigms. We are in the process of modeling how both visual scene and eye motor information influence the responsiveness of these neurons. Furthermore, we plan to follow up our imaging experiments with third generation *in situ* hybridization chain reaction, aiming to correlate functional and transcriptional cell types on a single-cell basis. Leveraging the ability to look at single cell information across the entire circuit, we aim to contribute to our understanding of fundamental principles governing sensorymotor integration.

Disclosures: L. Bauer: None. **J.C. Donovan:** None. **H. Baier:** None.

Poster

PSTR285: Eye Movements: Saccades

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR285.03/G36

Topic: E.01. Eye Movements

Support: Brain/MINDS grant 19dm0207093h0001

Title: The Effect of Frontal Eye Field Inactivation by Muscimol and Optogenetics in Common Marmosets.

Authors: *W. AMLY[1], C.-Y. CHEN[2], H. ONOE[4], T. ISA[3];
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Abstract: For over a century, researchers have identified the frontal eye field (FEF) as a crucial region for eye movement control in macaque monkeys. However, it wasn't until the late nineties that a series of studies employing reversible inactivation of the FEF unveiled substantial impairments in both saccadic and smooth pursuit eye movements. Our laboratory has been investigating the potential FEF regions in marmosets using electrical stimulation and tracer injection. However, to conclusively determine whether these areas correspond to the FEF and exhibit similar functions in controlling eye movements as in macaques, acute, reversible inactivation is needed.To target the putative FEF, we conducted MRI scans of 2 marmosets and designed a chamber covering the lateral areas 45, 8aV, 8C, 8aD, 6Va, 6DR, 6DC, and the medial areas 8b, and 6M based on our previous results. We injected 0.5µl of 5mM muscimol and one hour post-injection, marmosets performed visually guided saccade (VGS) and free viewing tasks, while their gaze was recorded. Muscimol was administered every other day to ensure recovery and to obtain control data. After confirming the affected area with muscimol, we injected 0.8 µl of the red-shifted Halorhodopsin, (AAV2.1-TRE3G-Jaws-KGC-GFP-ER2) and (AAV2.1-Thy1-stTA) in area 6Va. One month later, we started optogenetics inactivation of this area at the time of target onset while the marmoset performed the same tasks.Consistent with previous observations in macaques, muscimol injection into lateral frontal areas (6DR, 8C, 8aV,6Va) resulted in prolonged saccade latency and duration, more scattered saccade endpoints, even an inability to generate contralateral saccades in both tasks. Optogenetics inactivation showed similar results. On the contrary, muscimol injection into medial frontal areas (8b, 6M) did not affect saccadic behaviour in VGS task but it did result in a decrease in fixation in the ipsilateral visual field while preserving the capacity to generate saccades during free viewing task. In conclusion, our study demonstrates that marmosets possess a fully functional FEF akin to macaques. Furthermore, our results highlight distinct effects on saccades following inactivation of lateral versus medial frontal areas. We also show that optogenetics can work successfully in marmosets.

Disclosures: W. Amly: None. **C. Chen:** None. **H. Onoe:** None. **T. Isa:** None.

Poster

PSTR285: Eye Movements: Saccades

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR285.04/G37

Topic: E.01. Eye Movements

Support: CIHR Grant MOP-FDN-148418
Ontario Brain Institute

Title: Saccade abnormalities across prosaccade, antisaccade, and dynamic video-viewing tasks illuminate multiple maladapted neural processes in Parkinson's disease

Authors: *H. C. RIEK[1], D. C. BRIEN[1], B. J. WHITE[2], B. C. COE[3], D. A. GRIMES[4], A. LANG[5], C. MARRAS[5], D. P. MUNOZ[1];
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Abstract: Saccade behaviour is underpinned by well-established circuitry spanning diffuse brain regions. Therefore, patterns of saccade alteration caused by Parkinson's disease (PD) can provide insight into inter-individual and longitudinal variation in pathology location and severity. Understanding links between pathology and abnormal patterns of behaviour will facilitate the development of objective behavioural disease markers that could form the basis of future automated disease screening and diagnostic tools. These patterns can be measured using video-based eye tracking, which provides objective and precise measurements of saccade behaviour. 119 PD patients recruited from the Ontario Neurodegenerative Disease Research Initiative and 104 healthy age-matched controls completed an interleaved pro- and anti-saccade task (IPAST) and instruction-free viewing of rapidly changing video clips (FV). We compared behaviour between groups and across tasks to determine the effects of pathology and explore underlying neural mechanisms. PD demonstrated voluntary saccade control deficits across tasks (e.g., increased antisaccade errors, reduced FV saccade rate during voluntary epoch), indicating heightened inhibitory input to the superior colliculus (SC) from the substantia nigra pars reticulata (SNr). PD also displayed faster visually-driven prosaccades: they made more correct express prosaccades (90-140ms latency) and faster prosaccades relative to controls, but these parameters did not correlate with voluntary control deficits, suggesting a different causal process such as a mechanism compensating for impaired movement initiation by enhancing responses to visual stimuli. Saccade amplitude was reduced in PD across tasks but was uncorrelated with measures of voluntary control and fast visually-driven processing, suggesting a third discrete dysfunctional mechanism. Finally, PD made fewer short-latency microsaccades than controls following changes between video clips during FV, which might imply a fourth process impacted by PD pathology. Together, these results illuminate several independent PD-affected neural processes that can be indexed by eye tracking, which may have implications for the development of screening or diagnostic tools that quantify behavioural irregularities and underlying neural dysfunction.

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Poster

PSTR285: Eye Movements: Saccades

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Topic: E.01. Eye Movements

Support: CIHR MOP-FDN-148418
CIHR PJT-190028

Title: Transient post-saccade pupil response suggests saccade and pupil premotor circuits share common signals

Authors: T. PONESSE[1], B. J. WHITE[2], B. C. COE[3], G. BLOHM[2], ***D. P. MUNOZ**[4];
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Abstract: It is well known that the superior colliculus (SC) in the midbrain coordinates the orienting reflex to novel stimuli, including both saccades and changes in pupil size. However, the mechanisms of this coordination are not fully understood. This study aimed to determine if a temporal correlation existed between saccade initiation and pupil size changes. We used video-based eye tracking data collected on an Eyelink 1000 (SR Research) during a 10-minute naturalistic scene viewing experiment to investigate this. Horizontal (n=40,000) and vertical (n=15,000) saccades of 98 participants aged 20-25 were analyzed. Sex differences were not investigated. Post-saccadic pupil responses were aligned on saccade onset to examine the temporal relationship of saccade execution and accompanying pupil size changes. Approximately 200-400ms after horizontal and upwards saccades, there was a small, consistent transient pupil constriction that returned to baseline around ~600ms after the saccade. The speed of the constriction increased systematically with saccade amplitude ($p = 0.02$). Interestingly, the velocity of this transient constriction was smaller after rightward saccades, compared to amplitude-matched leftward saccades ($p = 0.0001$). Downwards saccades produced different pupil dynamics than the other directions. Around ~500ms after downward saccades, a transient dilation started, instead of a constriction. The dilation velocity increased with saccade amplitude, though this was not statistically significant. The results found for horizontal saccades, including the difference in pupil constriction velocity between right and leftward saccades, were replicated using data collected from the same participants performing the Interleaved Pro- and Anti-Saccade task, in which subjects make 10-degree horizontal saccades across different points on the screen. Further investigation is required to determine how these discrepancies between different saccade directions are affected by geometrical issues related to the camera and infrared light source, which were located below the computer screen. This study provides important preliminary evidence that a correlate of the saccade burst command is also present as a post-saccadic pupil response, indicating a shared pathway between saccade and pupil motor circuitry that likely includes the superior colliculus.

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Poster

PSTR285: Eye Movements: Saccades

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Topic: E.01. Eye Movements

Support: National Natural Science Foundation of China

Title: The role of prefrontal and posterior parietal cortex in generating multiple step saccades

Authors: *W. MA[1], M. ZHANG[2];
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Abstract: Objective Multiple step saccades (MSS) has been treated as a non-physiological behavior and occurred more frequently in patients with Parkinson's disease (PD). It has been reported that the incidence of MSS in reflexive and voluntary saccadic tasks could serve as the complementary biomarker for the diagnosis of PD. Thus, MSS has attracted increasing attention in the clinical research. However, it remains largely unclear regarding the neural mechanisms underlying the generation of MSS. The objective of the present study is to explore the role of prefrontal and posterior parietal cortex in the generation of MSS.**Methods** There are two subsequent studies. Each study recruits fifteen male and female right-handed healthy subjects. In study one, the brain activity is recorded by electroencephalogram (EEG) while the subjects performing the visually guided gap saccade task. In study two, we use single pulse transcranial magnetic stimulation (TMS) to transiently disrupt neuronal activity in the prefrontal cortex (PFC) or posterior parietal cortex (PPC) of the participants while they perform the same saccadic task.**Results** In study one, there are significant differences in time-frequency power and Granger causality coefficients between trials with MSS and without MSS. In study two, the incidence of MSS increases more after TMS on PFC than on PPC. **Conclusions** Both PFC and PPC are involved in the generation of MSS, and PFC plays more important role than PPC does.

Disclosures: W. ma: None. **M. Zhang:** None.

Poster

PSTR285: Eye Movements: Saccades

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR285.07/H3

Topic: E.01. Eye Movements

Support: NIH R01 NS09295001
NIH T32 HD07418

Title: Beneficial effects of antiparkinson medication on memory-guided saccades

Authors: *M. J. MUNOZ[1], J. L. REILLY[2], A. DOMINGUEZ-RUIZ[1], Y. M. RIVERA[3], L. VERHAGEN METMAN[4], G. D. PAL[5], L. C. GOELZ[6], M. P. TREVARROW[1], D. M. CORCOS[1], F. J. DAVID[1];
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Abstract: There is a prevailing view that internally-driven movements, such as memory-guided saccades, are more impaired in people with Parkinson's disease (PwP) compared to externally-driven movements. Internally-driven eye movements are thought to be more reliant on the basal ganglia and PwP have shown greater deficits during memory-guided saccades compared to visually-guided saccades. PwP tend to have increased memory-guided saccade latency, decreased gain, and decreased peak velocity compared to age-matched healthy individuals. The deficits in saccade latency and gain tend to be greater during memory-guided saccades compared to visually-guided saccades. Despite this, no study has determined the effect of antiparkinson medication on memory-guided saccades using participant's clinical dosages. If memory-guided saccades are more reliant on the basal ganglia, then we hypothesize that antiparkinson medications will improve memory-guided saccade performance.
Thirty-three PwP and 14 healthy controls completed a memory-guided saccade task. Participants fixated on a central fixation point even when a peripheral target appeared for 50ms. Participants were told to memorize the location of this target using peripheral vision. After a delay period (0.5 or 5 seconds), the central fixation point disappeared, which was the cue to make a saccade to the remembered target location. The PwP performed this task over 2 days: 1 day off medication after overnight withdrawal and 1 day on usual medications. The order of medication condition was randomized. Healthy controls performed the task once. The outcome measures were saccade latency, gain, peak velocity, and duration.
We found that medication significantly decreased latency back towards healthy control performance. Additionally, medication had no effect on saccade gain, but significantly increased peak velocity and decreased duration towards healthy control performance. Overall, antiparkinson medication is improving memory-guided saccade performance. This is in contrast to the previously reported detrimental effects of medication on visually-guided saccade performance.

Disclosures: M.J. Munoz: None. **J.L. Reilly:** None. **A. Dominguez-Ruiz:** None. **Y.M. Rivera:** None. **L. Verhagen Metman:** F. Consulting Fees (e.g., advisory boards); AbbVie, Abbott, Neuroderm, and Supernus. **G.D. Pal:** F. Consulting Fees (e.g., advisory boards); Guidepoint and

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Poster

PSTR285: Eye Movements: Saccades

Location: MCP Hall A

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Program #/Poster #: PSTR285.08/H4

Topic: E.01. Eye Movements

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NIH Grant T32-DC000023
NIH Grant 1UF1-NS111695

Title: Disruption of limb, head, and gaze stability after vestibular loss is reduced by prosthetic stimulation in locomoting macaques

Authors: *O. R. STANLEY, R. WEI, K. E. CULLEN;
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Abstract: Stable and accurate control of gaze - the sum of the head's position and orientation in space with the orientation of the eyes within the head - is integral to activities of daily living, including for guiding locomotion. The vestibular system makes critical contributions to both postural and visual stability via vestibulo-spinal and vestibulo-ocular reflexes. Patients with vestibular deficits must thus contend with symptoms including postural instability, vertigo, and blurred vision.
To better understand how nonhuman primates adapt to vestibular dysfunction during locomotion, we investigated differences in gait and gaze control of two normal rhesus macaques and two with bilateral vestibular loss (BVL) implanted with vestibular prostheses. Animals walked on a treadmill at varied speeds and along a linear walkway at a self-selected pace. Data were captured using single-eye video-oculography via head-mounted camera, a head-mounted 6D inertial measurement unit and marker-based tracking systems mounted to the head and trunk, and markerless tracking using synchronized cameras set around the behavioral apparatus.
Normal animals maintained stable limb and head movements across paradigms. During slow phase periods (i.e., between gaze shifts), compensatory reflexes kept the animals' gaze stable in space. Gaze shifts made by these animals were more frequent during self-paced overground walking compared with experimenter-paced treadmill walking. In contrast, BVL animals exhibited variable head and limb movements. Mean gaze velocity during slow phases was significantly higher in BVL animals, indicating reduced gaze stability. Additionally, gaze shifts made by BVL animals were larger rather than more frequent during overground walking. The degree of phasic modulation of gaze shift activity with the step cycle increased from treadmill to overground locomotion across both groups. Finally, we found that when head-coupled electrical

stimulation was delivered by a vestibular prosthesis, head and limb kinematic instability decreased while VOR gain increased, reducing observed differences between BVL and normal animals.
Taken together, these findings demonstrate a change in gaze control strategy following BVL and establish that the vestibular system provides a foundation of postural and gaze stability. This work thus advances our understanding of a critical preclinical model of vestibular loss during more dynamic, natural behavior than traditionally studied. Further research will be needed to specifically elucidate how adaptations to this loss occur at the neural and circuit level.

Disclosures: O.R. Stanley: None. **R. Wei:** None. **K.E. Cullen:** None.

Poster

PSTR285: Eye Movements: Saccades

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Program #/Poster #: PSTR285.09/H5

Topic: E.01. Eye Movements

Support: NIH grant R01NS079518
NIH grant R01NS129608

Title: Superior colliculus circuitry for precise saccadic eye movements

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Abstract: Saccades, or rapid orienting eye movements, occur during spatial navigation and are essential for gathering visual information. The intermediate and deep layers of the superior colliculus (SC) integrate input from several brain regions to select spatial targets and initiate saccades to them. In addition, the SC is thought to convey predictive information about future saccade targets to regions responsible for motor error signaling like the inferior olive (IO). However, the functional coupling between saccade initiation and error signaling, particularly at the level of specific SC cell types, is unclear. Here, we focus on Pitx2-expressing pre-motor output neurons in the SC. These neurons are critical for orienting head movements and project to the IO, suggesting a potential role in linking orienting movements with motor error signaling. However, the role of Pitx2+ neurons in initiating saccades, and whether they convey spatial information to IO sufficient for error correction, is unknown. We hypothesize that Pitx2+ neurons directly initiate saccades and project topographically to the IO. Using *Pitx2-Cre* mice and DREADDs, we silenced Pitx2+ neuron activity in head-fixed mice during a paradigm eliciting saccades. Comparing saccade parameters between sessions with DREADD and saline administration, we aim to determine the necessity of Pitx2+ neuron activity for saccade initiation. Within the same mouse line, we employed cell-type-specific anterograde viral tracers

to map Pitx2+ neuron projections in the IO. Preliminary results suggest that inhibiting Pitx2+ neurons affects saccades, while tracing experiments confirm their projection to the IO. Ongoing experiments and analyses will quantify the relationship between the activity of Pitx2+ neurons and saccades, as well as the role of their projection to the IO in error signaling. Ultimately, this research seeks to elucidate integrated functional circuitry governing precise movements during active vision.

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Poster

PSTR285: Eye Movements: Saccades

Location: MCP Hall A

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Topic: E.01. Eye Movements

Support: NIH Grant EY029438
NIH Grant P51 OD011106
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Title: Wireless eye tracking for 3D gaze estimation in head-free and freely moving macaques

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Abstract: To explore their environment, primates commonly use saccadic eye movements to bring objects of interest into focus. In natural settings, this visual exploration requires the scanning of a three-dimensional (3D) space, and therefore disjunctive saccades in which the two eyes differentially rotate. To monitor such binocular eye movements, various eye-tracking techniques have been implemented, including conventional sclera-embedded search coils and optical tracking. In either case, calibration typically relies on the estimation of offsets and gains that are applied to linearly map the measured signals onto screen coordinates at a fixed distance. These methods are best suited for head-fixed conditions and are not readily adaptable to head-free or freely moving paradigms. To perform accurate, real-time measurement of 3D gaze endpoints in head-free and freely moving macaques, we devised a novel wireless face-mask-mounted eye tracking device. First, a face mask is 3D printed based on 3D scans of the animal's head. The mask is designed to have a low-profile that minimizes the occlusion of the visual field. Within the mask, two small cameras are embedded for optically measuring the movements of each eye. An additional scene camera and LIDAR camera are embedded at the level of the brow. The mask and camera assembly are rigidly secured to a cranial implant that further allows for

neurophysiological recordings. Images of each eye are transferred by separate single-board computers over a wireless network and fit with a computational model to estimate the rotation of the eyes inside their orbits. A small battery pack secured to the assembly powers the single-board computers. The 3D kinematics of the head are further incorporated by using a 3D motion capture system to track reflective markers attached to the assembly. The eye rotation and head pose information are then combined to determine 3D gaze end points within the environment. To calibrate the tracker, the animal's head is initially fixed while a computer numerical control machine precisely positions fixation targets at locations distributed throughout the 3D visual field. At its current specifications, this head-free wireless binocular eye tracker acquires eye position measurements within an average angular error of 0.87° at a 120 Hz sampling rate for each eye. This eye tracker is therefore well-suited to support 3D gaze tracking across a wide range of head-free and freely moving non-human primate experimental paradigms.

Disclosures: B. kim: None. **R. Doudlah:** None. **A. Rosenberg:** None.

Poster

PSTR285: Eye Movements: Saccades

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR285.11/H7

Topic: E.01. Eye Movements

Support: The Max Planck Society

Title: Eye saccades align optic flow with intended heading during object pursuit in freely moving mammals

Authors: *D. J. WALLACE[1], K.-M. VOIT[2], J. SAWINSKI[3], D. S. GREENBERG[4], P. STAHR[3], F. B. ROSSELLI[5], D. FITZPATRICK[6], J. N. KERR[7];
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Abstract: For predatory mammals with retinal specializations, the visual system accurately tracks erratically fleeing objects, such as prey, whilst simultaneously providing information about the environment being coursed through. While the visual system contains a complex set of eye-rotation and compensatory mechanisms that could potentially achieve this, how the visual system enables simultaneous navigation and object tracking is not known. By computational reconstruction of the visual fields of freely moving ferrets during pursuit of an unpredictably

moving target, we found that rapid eye saccades, exclusively elicited during curved trajectories, did not track the target. Instead, saccades aligned features of the optic-flow fields generated during motion, used for navigation in many species, with the ferret's intended direction of travel and retinal specializations. The mechanisms underlying this were tightly synchronized head and eye rotation kinetics, with matched head and eye rotation velocities and amplitudes in individual saccades. Lastly, we show the same tightly synchronized and matched head and eye rotations in freely moving tree shrews and rats, suggesting this is a generalized visual mechanism for navigation during free motion.

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Poster

PSTR285: Eye Movements: Saccades

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR285.12/H8

Topic: E.01. Eye Movements

Support: JST Grant JPMJFR2044

Title: Neural substrates for inhibiting and triggering saccadic eye movements

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Abstract: Saccadic eye movements are the fastest and most accurate movement among all kinds of body movements, and are vital for vision and represented in wide areas of the central nervous system. Saccades shift gaze to objects of interest in the visual field, moving their image to the central retina, where it is maintained for detailed examination (eye fixation). During such fixation, all eye movements to other targets are suppressed. It is known that neurons in the frontal eye field (FEF), the rostral pole of the superior colliculus (SC), and the raphe interpositus, have tonic activity that suppress high gain saccade burst neurons during fixation, and that is inhibited before and during saccades, but the neural mechanisms for triggering saccades by suppressing raphe neurons have not been analyzed. Recently, we identified the neural mechanisms for suppressing saccades (fixation) and triggering saccades, using intracellular recording and staining methods in anesthetized cats.The SC has a functional difference between rostral and caudal parts. The rostral pole of the SC contains neurons whose discharge increases during fixation and decrease just before and during saccades. The more caudal SC contains saccade-related neurons that show burst activity at the onset of saccades. In the saccade generating system in the brainstem, one group of neurons stops firing during saccades in all

directions (OPNs, omnipause neurons) and shows tonic activity between saccades. Stimulation of the OPN area in the midline pons could prevent saccade occurrence in all directions, suggesting that pontine excitatory (EBNs) and inhibitory burst neurons (IBNs) may be under inhibitory control by OPNs so that a cessation of activity of OPNs is necessary before burst neurons can discharge. Many researchers have tried to identify inhibitory interneurons that inhibit OPNs, but neural substrates for triggering saccades was still controversial. This study was to provide an electrophysiological and morphological evidence that IBNs project to OPNs and help to trigger saccades. The results showed that OPNs received monosynaptic excitation from the rostral SC and disynaptic inhibition from the caudal SC, and this latter disynaptic inhibition was mediated via IBNs. Microstimulation of the IBN area evoked monosynaptic inhibition in OPNs and intracellular staining of single IBNs with HRP demonstrated that they projected to the contralateral OPNs. These findings suggested possible roles of IBNs for triggering horizontal saccades by actively inhibiting the tonic activity of OPNs at the onset of saccades and also maintaining saccades by continuously inhibiting OPN activity during saccades.

Disclosures: M. Takahashi: None. **Y. Shinoda:** None.

Poster

PSTR286: Cerebellum: Non-Motor Functions

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR286.01/H9

Topic: E.02. Cerebellum

Support: NSF GRFP
NIH R01 NS132926

Title: Exploring non-motor prediction errors in the human cerebellum

Authors: *J. E. TRACH, S. D. MCDOUGLE;
Psychology, Yale Univ., New Haven, CT

Abstract: The cerebellum has long been considered a crucial hub for sensorimotor learning. In this domain, the cerebellum is thought to act as an internal forward model, making predictions about the outcomes of motor commands and computing prediction errors based on discrepancies between predicted and actual outcomes. The prediction errors calculated in the cerebellum are then used as teaching signals to refine actions and reduce error in subsequent attempts. Numerous studies have contributed to our understanding of the cerebellar circuitry that supports motor learning and highlights how the cerebellum is optimized for prediction error-driven learning. More recently, there has been growing interest in the role that the cerebellum might play in nonmotor learning, particularly in learning situations that also use prediction and prediction error. For example, reinforcement learning (RL) involves using reward feedback and

reward prediction errors (i.e., discrepancies between predicted and actual reward) to learn about the environment and guide choice behavior. Might the human cerebellum play a role in prediction-based learning in nonmotor domains as it does in motor learning? In this project, we investigate the role of the cerebellum during RL in humans using fMRI. In particular, we are interested in neural correlates of reward processing, reward anticipation, and reward prediction error in the human cerebellum. Recent work in animals models provides strong evidence that the cerebellum is indeed involved in core neural computations associated with RL. Still, the role of the cerebellum in RL in humans has not been rigorously studied and extant work often cannot rule out sensorimotor explanations for cerebellar activation during RL. To address this, we scanned adults as they engaged in a RL task. During the task, participants chose between stimuli that were associated with different reward probabilities and were instructed to use reward feedback to optimize their choices. We then used model-based fMRI analyses to find regions of the brain where activity was associated with reward prediction and outcome evaluation, with a particular focus on the cerebellum. Data collection is ongoing so results are preliminary. However, pilot analyses provide initial evidence that the human cerebellum is involved in both reward processing and reward prediction in RL, echoing to its role in error-based motor learning. These results suggest that the cerebellum might performed more generalized error-based learning in both motor and nonmotor domains, rather than serving narrowly for motor learning.

Disclosures: J.E. Trach: None. **S.D. McDougle:** None.

Poster

PSTR286: Cerebellum: Non-Motor Functions

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR286.02/H10

Topic: E.02. Cerebellum

Support: Marie Skłodowska-Curie grant agreement No 956414

Title: Characterisation of the cerebellar-midbrain connectivity and interactions in a rat model of SYNGAP1 haploinsufficiency.

Authors: *S. COUTO OVEJERO, P. RIGNANESE, S. TILL, T. WATSON, P. C. KIND; Simons Initiative for the Developing Brain, Ctr. for Discovery Brain Sciences, Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: The cerebellum is increasingly implicated as a key structure for the processing of emotions. In particular, distributed cerebellar circuits are critical to fear extinction learning processes (Apps and Strata, 2015). Interestingly, many behavioural and pathological changes in autism, such as atypical adaptive behaviour, are also associated with cerebellar circuit dysfunction. Furthermore, changes in fear recall and extinction learning are present in both

animal models and people with autism (Powell et al., 2016). Thus, aberrant connectivity/activity within extended networks formed between the cerebellum and other fear-related brain regions, such as the ventrolateral periaqueductal gray or the superior colliculus, may contribute to core features of autism. *SYNGAP1* haploinsufficiency is associated with intellectual disability and autism (Berryer et al., 2013). A recent study using a novel rat model of *SYNGAP1* haploinsufficiency identified impairments in extinction of a conditioned fear response (Katsanevaki et al., 2020). To understand the contributions of distributed cerebellar network function to the fear extinction deficits in this model, we are using a range of behavioural, anatomical, electrophysiology/circuit-manipulation techniques. As a first step, we used retrograde adeno-associated virus tracing to characterise and compare the cerebello-midbrain monosynaptic connectivity patterns for both wildtype and *Syngap*$^{+/\Delta\text{-}GAP}$ rats. We have also charted the immediate early gene c-Fos activation patterns in this network at various time points across the fear-learning paradigm. In addition, we are using *in vivo* opto-tagging and neural recording techniques to compare cerebello-midbrain neural dynamics across the fear paradigm in both genotypes.

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Poster

PSTR286: Cerebellum: Non-Motor Functions

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR286.03/H11

Topic: E.02. Cerebellum

Title: Ablation of Endocannabinoid Synthesizing Enzyme DAGLa from Cerebellar Purkinje Cells Affects Mitochondria Morphology and Function

Authors: *E. G. GRANT[1], A. KALINOVSKY[2], K. MACKIE[3];
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Abstract: The endocannabinoid signaling system (ESC) plays a key role in neuromodulation andneurodevelopment. ESC consists of small signaling lipids, their synthesizing and degrading enzymes, andtheir receptors. Diacylglycerol lipase alpha (DAGLα) is responsible for the synthesis of about 80% of themajor neuronal endogenous cannabinoid, 2-Arachidonoylglycerol (2-AG). Mutations in DAGLα areassociated with neurodevelopmental disorders, seizures, and cerebellar ataxia. In order to elucidate thepathological mechanisms associated with mutations in DAGLα, we generated cerebellar Purkinje cellspecific DAGLα mutant mice (PC-DAGLα-KO). Our transcriptomics analysis of Purkinje cells in PC- DAGLα-KOs revealed dramatic changes in the expression of mitochondrial proteins, including the components ofthe electron transport chain

and the regulators of mitochondria size and shape. A defect in mitochondriacould lead to a decreased availability of ATP or to the deregulation of cytoplasmic calcium concentration,therefore interfering with neuronal activity. Another important aspect of mitochondria that previousresearch has seen to be affected by endocannabinoids is mitochondrial fusion. Both hypo and hyper-fusion have been seen in endocannabinoid knockout models, with fusion playing an important role inmitochondrial health and maintenance. To determine if endocannabinoids could play a potential role inmitochondrial morphology, the net voxel volume of mitochondria was measured between a DAGLαknockout and littermate to observe any differences. Immunohistochemistry was used to stain for TOM20and IMARS software was used to quantify voxel amount. By looking into specific mitochondrial functionsthat are affected by DAGLα, we can determine endocannabinoids function within Purkinje cells overall.

Disclosures: E.G. Grant: None. **A. Kalinovsky:** None. **K. Mackie:** None.

Poster

PSTR286: Cerebellum: Non-Motor Functions

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Program #/Poster #: PSTR286.04/H12

Topic: E.02. Cerebellum

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NHLBI Grant 114474 to MRD & AMG

Title: Sex Chromosomes Affect Density of Rat Cerebellar Granule Cells

Authors: S. PRIESTLEY[1], X. CHEN[2], M. R. DWINELL[4], A. M. GEURTS[5], V. HARLEY[6], A. P. ARNOLD[7], ***W. GRISHAM**[3];
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Abstract: Using MRI scans, Corre et al., (2014) noted that the cerebellar vermis was a region affected both by sex chromosome complement and gonad in Four Core Genotypes mice. In FCG-like rats, we previously found that Purkinje cell density differs due to gonadal sex as well as across lobules in the cerebellar vermis. Here we examine granule cell density across vermal lobules to see if it differs either with gonadal sex or sex chromosomes using a five-group design. This design includes XX gonadal females (FXX, n = 9), XY females in which the *Sry* gene was disrupted so they developed ovaries (FXY, n = 7), XY gonadal males (MXY, n = 10), XX males

in which the *Sry* gene had been inserted into an autosome so they developed testes instead of ovaries (MXX, n = 6), and XYTG gonadal males that had *Sry* genes both on the Y chromosome and also inserted into an autosome (MXYTG, n = 5). Cerebelli were cut at 40µm in the sagittal plane and stained with thionin. Between 3-6 images per vermal lobule, each spanning 330µm, were taken at 50x. Since granule cells are quite small, we measured the relative optical density (ROD) using FIJI to measure the density of the granule cell layer as well as the adjacent molecular cell layer on the same section. The largest possible ROI was marked out within each layer and the mean density of the two layers subtracted for each lobule. This procedure controlled for variation in staining or illumination. All measures were made blind to gonadal sex and genotype. An ANOVA having 5 groups x 11 lobules revealed significant effects of lobule, $F(10, 32) = 4.615$, $p < 0.001$, $\eta^2 = 0.034$, and a lobule by group interaction $F(40, 32) = 1.475$, $p < 0.05$, $\eta^2 = 0.044$, but no main effect of group. Posthoc Holm analysis (corrected for 231 comparisons) revealed significant differences across lobules within the MXY group that did not occur in other groups. Most differences were because MXY lobules 6a and 6b had a higher ROD than other lobules within the MXY group (lobules 1, 9, and 10). Subsequent analyses further revealed that the MXX group had lower ROD than did the MXY group in Lobule 6a, $F(1,16) = 4.613$, $p < 0.05$, $\eta^2 = 0.224$. This latter difference suggests that granule cell numbers differ between two of our male gonadal phenotypes and are influenced by their sex chromosome complement. Notably, lesions of Lobule 6a result in impaired sexual performance and other motor problems in male rats (Ortiz-Pulido et al., 2010). Thus, a rat FCG-like model and the mouse FCG model both show sex chromosome effects on cerebellar morphology.

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Poster

PSTR286: Cerebellum: Non-Motor Functions

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR286.05/H13

Topic: E.02. Cerebellum

Title: Investigating Cerebellar Representations of Reward Signals

Authors: *B. A. FILIO III[1,2], M. J. WAGNER[1];
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Abstract: The cerebellum is traditionally recognized for its role in sensorimotor learning and body coordination, but recent anatomical and behavioral studies demonstrate its involvement in higher cognitive functions such as reward expectation and nonmotor skill learning. From previous studies, it known that in an operant task where mice push a manipulandum to receive

delayed water reward, a subset of cerebellar granule cells in Lobule VI activate in anticipation of expected water reward. Simultaneously, climbing fibers from the inferior olive spike just after water reward is given. This activity becomes more robust and precise as the mice learn the timing of the task. Importantly, this activity cannot be explained by licking to consume the water or any other extraneous body movements. This demonstrates that the cerebellum may play a role in encoding nonmotor aspects of reward.
Despite these findings, it is still unknown whether this activity generalizes across different reward contexts, such as in compulsive reward-seeking behavior which is a key component of addiction-related disorders. The question remains whether cerebellar circuits are engaged by reward seeking or because consuming water rewards requires movement. To investigate these questions, we are training head-fixed mice in an operant paradigm where they push a manipulandum in exchange for delayed stimulation of the midbrain. At the same time, we are monitoring activity of cerebellar granule cells and climbing fibers chronically as the mice learn the task. This study will enhance our understanding of cerebellar encoding of reward signals and widespread brain circuitry changes caused by midbrain activity. This will broaden the field's understanding of how cerebellar activity contributes to addiction and other related neurological disorders.

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Poster

PSTR286: Cerebellum: Non-Motor Functions

Location: MCP Hall A

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Program #/Poster #: PSTR286.06/H14

Topic: E.02. Cerebellum

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Title: Exploring the role of inhibitory interneurons of the posterior vermis in drug-induced conditioned preference

Authors: *I. MELCHOR EIXEA, P. CARRETERO, M. MIQUEL;
Univ. Jaume I, Castellón de la Plana, Spain

Abstract: Previous findings consistently have shown that the expression of a preference for drug-related cues correlates with increased neuronal activity in the posterior cerebellar vermis. Cerebellar manipulations that reduce the inhibitory control of the cerebellar vermis over the interposed and lateral deep cerebellar nuclei (DCN) facilitates the acquisition of conditioned preference for drug-related cues. Furthermore, chemogenetic inhibition of the DCN can prevent the facilitating effect over drug-induced conditioning.
Here, we addressed two studies to further investigate the role of posterior vermis in drug-induced associative learning using a biased conditioned place preference (CPP). In the first study, we expressed AAV5-hSyn-hM3/hM4D-mCherry DREADDs in lobule VIII. Only inhibitory interneuronal populations, Golgi and molecular interneurons, were infected and expressed the viral construct. DREADD activation by IP CNO administration was accomplished 30 minutes before every conditioning trial. Activation of inhibitory interneurons encouraged in all rats the acquisition of CPP and increased the magnitude of the preference for cocaine-associated compartment (CS+). As expected, their inhibition prevented the acquisition of conditioned preference. Then, we wondered whether the activation of these inhibitory interneurons would be rewarding per se and capable to induce preference conditioning in absence of drug effects. To this end, we expressed the DREADD in lobule VIII and injected CNO before every conditioning trial. Unexpectedly, activation of Golgi and molecular interneurons reduces the time spent in CS+. These findings strongly suggest a key role of the posterior cerebellar vermis in modulating cocaine-induced Pavlovian learning and indicate that cocaine-induced changes such as granule cell hyperactivity are required for cerebellar modulation on drug effects.
Keywords: Cerebellum, cocaine, DREADD, Conditioned Place Preference.

Disclosures: I. Melchor Eixea: None. **P. Carretero:** None. **M. Miquel:** None.

Poster

PSTR286: Cerebellum: Non-Motor Functions

Location: MCP Hall A

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Topic: E.02. Cerebellum

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Title: Cerebellar regulation of fear extinction through the limbic thalamus

Authors: *A. G. IGLESIAS[1], K. VLASOV[1], M.-R. MBAH[1], S. CAMARERO[1], S. JUNG[1], E. G. ANTZOULATOS[1,2,3], D. FIORAVANTE[1,2];
[1]Ctr. for Neurosci., [2]Dept. of Neurobiology, Physiol. and Behavior, [3]Dept. of Psychology, Univ. of California, Davis, Davis, CA

Abstract: The cerebellum is an established regulator of motor control and has recently garnered novel focus for its modulation of affective processes. We previously characterized cerebellar projections to the limbic thalamus, including the parafascicular nucleus (PF), which classically modulates orienting responses and behavioral alertness. We also identified an anatomical circuit between the deep cerebellar nuclei (DCN) and the basolateral nucleus of the amygdala (BLA) through the limbic PF. Here we provide functional characterization of this circuit and examine whether and how the circuit might regulate affective processing. We show that optogenetic stimulation of DCN-PF projections induces *c-Fos* expression in limbic PF and modulates BLA neural activity, which establishes functional connectivity. To test the behavioral relevance of the circuit, we optogenetically inhibited the DCN-PF projections during distinct phases of auditory fear conditioning. We find that inhibition during training does not affect acquisition or expression of learned fear 48 h later. However, optoinhibition during extinction training results in faster extinction. This effect is only observed when inhibition is applied during both cue and omitted shock, but not during cue alone or omitted shock alone, indicating that the circuit may encode information other than stimulus salience or prediction error. Interestingly, the effect on extinction appears to be specific to the DCN-PF projection: optoinhibition of cerebellar projections to the centromedial thalamic nucleus (CM), which is also an intermediary node between the cerebellum and amygdala, does not appear to modulate fear acquisition or extinction. Anatomical work reveals largely segregated populations of PF- and CM-projecting DCN neurons. Ongoing work is targeting these neural populations in order to capture neural signatures of fear regulation. Overall, our findings reveal a novel functional divergence between limbic projections of the cerebellum and establish future targets for exploring affective processes of relevance to neuropsychiatric disorders.

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Poster

PSTR286: Cerebellum: Non-Motor Functions

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR286.08/H16

Topic: E.02. Cerebellum

Support: Arizona Alzheimer's Consortium
Institute for Social Science Research
Institute of Mental Health Research
Arizona State University Start-up Funds

Title: Amelioration of social behavior and alteration of cortical neural activity markers following adolescent cerebellar nuclei manipulation in mice

Authors: *T. LYLE[1], H. VIEIRA[1], J. L. VERPEUT[2];
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Abstract: The cerebellum, predominantly known for its influence in motor behavior, has recently been identified to be active during non-motor tasks, notably social behavior (Badura et al. 2018; Stoodley et al. 2017). Individuals with autism spectrum disorder (ASD) show decreased connectivity between the cerebellar nuclei (CN) and regions of the prefrontal cortex, including the anterior cingulate (ACC) and orbitofrontal cortex (OFC) when performing social tasks (Olivito et al. 2017). Additionally, a recent monosynaptic pathway has been identified between the cerebellum and the ventral tegmental area (VTA) that is active during social interactions (Carta et al., 2019). However, it is still unclear how CN development shapes social and reward circuits during CN developmental critical periods. Moreover, CN perineuronal nets (PNNs), specialized extracellular matrix structures whose appearance are associated with the end of the critical period of plasticity, are largely understudied within the CN. Therefore, we hypothesized that typical maturation of CN PNNs are required to shape the cerebello-neocortical circuit for social behavior.
In the following experiments, lateral CN activity was manipulated in adolescent mice using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to enhance (AAV2-hSyn-hM3D(Gq)-mCherry) or reduce (AAV2-hSyn-hM4D(Gi)-mCherry) cerebellar output. Males (n=20) and females (n=17) were exposed to the DREADD receptor ligand, clozapine-N-oxide (CNO; 10 mg/kg), in drinking water from postnatal day (PND) 21-35. Social behavior was assessed using a classic three-chamber assay at PND 65-70 and analyzed using SLEAP (SLEAP Estimates Animal Pose). Neural activity via the immediate-early gene, c-Fos, was quantified in the ACC, OFC, and VTA to understand relationships between CN development and social behavior. In the three-chamber task, we found both CN excitation ($p=0.04$) and inhibition ($p=0.01$) resulted in indifference for male social preference compared to controls. However, in females, no group differences in social preference were observed ($p = 0.41$). Additionally, CN PNN intensity had no relation with social preference ($R2 = -0.03$, $p = 0.84$). However, c-Fos counts in the VTA revealed a positive correlation with social preference ($R2 = 0.82$, $p < 0.001$, $n = 12$). These results suggest an early-life CN manipulation can impact adolescent social preference, potentially through changes in CN-VTA activity and/or distal cortical regions. These findings can guide clinical applications targeting development in individuals with neurodevelopmental disorders, such as ASD.

Disclosures: T. Lyle: None. **H. Vieira:** None. **J.L. Verpeut:** None.

Poster

PSTR286: Cerebellum: Non-Motor Functions

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Program #/Poster #: PSTR286.09/H17

Topic: E.02. Cerebellum

Support: Arizona Alzheimer's Consortium
Psi Chi Undergraduate Research Grant

Title: Aging and Autism: Modulation of the cerebellar nuclei during critical periods of development to assess social changes with age in mice

Authors: *V. TRUONG[1], T. LYLE[1], J. L. VERPEUT[2];
[1]Psychology, Arizona State Univ., Tempe, AZ; [2]Psychology Dept., Arizona State Univ., Tempe, AZ

Abstract: Autism Spectrum Disorder (ASD) is a heterogeneous condition dynamic to the aging process, yet little research focuses on the relationship between aging and ASD in regards to health outcomes. Social deficits, a hallmark symptom of ASD, limit essential healthcare engagement, which can vary between neurotypical and neurodivergent individuals as they age. Older individuals with ASD are more likely to be diagnosed with early-onset dementia (Vivanti et al., 2021), which can worsen both cognitive and social function. The cerebellum is commonly atypical in ASD and plays a role in social behavior possibly due to cerebellar nuclei (CN) connections to reward pathways, including the ventral tegmental area (VTA), but it is unknown how the cerebellum may contribute to social decline in aging. Since social deficits precede the presentation of cognitive decline (Boyer et al., 2019), social deficits may serve as an early detection criterion for cognitive deterioration, which may present differently for individuals with or without ASD.

To assess rates of social decline, we inhibited the CN-VTA pathway in male mice (n=16 test and n=16 controls) using chemogenetics. The CN-VTA pathway was inhibited with an inert ligand, clozapine-N-oxide (CNO), during postnatal days 21-35 (P21-35), followed by a washout period (5 days). Behavioral tests, including the open field, 3-chamber social preference, olfactory habituation and dishabituation, and the Y-maze test, were used to evaluate social and cognitive ability. Videos were analyzed using Social LEAP Estimates Animal Poses (SLEAP). A separate subset of animals was analyzed for changes in dendritic complexity from juvenile to aged using Golgi-cox staining. Comparison between juvenile animals (P40) and middle-aged animals (10 months) allows for cross-sectional comparisons, while a subset of animals tested at P40 and retested at 10 months allows for longitudinal comparisons. Furthermore, in-situ hybridization assays probing for genetic markers involved in ASD and aging (RLN, IL-6, and GAD1) will be correlated with behavior metrics.

In typical aging mice (juvenile to middle-aged) we found longitudinal changes in cognition,

revealing decreased acquisition ability ($p=0.004$) and slower response time ($p<0.001$) in a touchscreen learning task. Aged animals also revealed decreased dendritic complexity ($p<0.001$) in male animals. These results suggest that aging and reductions in neural complexity contribute to cognitive decline and future experiments will reveal aging and CN impacts on social changes in aging. This will be important in understanding how social behavior can be a key factor for early detection of cognitive deficits.

Disclosures: V. Truong: None. **T. Lyle:** None. **J.L. Verpeut:** None.

Poster

PSTR286: Cerebellum: Non-Motor Functions

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR286.10/H18

Topic: E.02. Cerebellum

Support: School of Life Sciences Undergraduate Research (SOLUR) Program at Arizona State University, Tempe
Arizona State University

Title: Social memory and interactive behaviors are sex-dependent and modulated by the cerebellum in mice

Authors: M. R. NELSON, *J. VERPEUT;
Psychology, Arizona State Univ., Tempe, AZ

Abstract: Social behavior is an interaction between two or more organisms and is theorized to incorporate multiple brain regions, including the cerebellum. Studying cerebellar-social circuits, which can connect to reward pathways, are important for understanding how social behavior develops. In this study, machine learning tools were utilized to understand sex differences in social behavior in a pair of freely moving mice. Then, the effect of chemogenetic excitation of crus I on social behavior was analyzed, as inhibition of crus I in the cerebellum has been shown to negatively impact social preference (Badura et al., 2018). All mice completed the three-chamber task, consisting of social discrimination and social memory components, and a novel freely moving social interaction test. Sex differences were examined in male (n = 8) and female (n = 8) C57BL/6J mice. In the three-chamber social discrimination task, both male ($p = 2.31 \times 10^{-6}$) and female ($p = 0.017$) mice spent significantly more time in the chamber with the conspecific, compared to the object. Yet, only male mice spent significantly more time ($p = 0.04$) and traveled a greater distance ($p = 0.034$) in the chamber with the novel versus familiar mouse. To explore the role of the cerebellum on social behavior, bilateral crus I molecular layer interneuron (MLI) activity was increased using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs, AAV8-hSyn-hM3D(Gq)-mCherry) in male C57BL/6J mice (n =

4). Mice received either clozapine-N-oxide (CNO, 1 mg/kg) or vehicle (0.9% saline) prior to testing in a within experimental design. MLI excitation significantly increased the time spent with the social partner during both the social discrimination ($p = 0.0083$) and social memory ($p = 0.027$) components of the three-chamber test. In addition, MLI excitation significantly increased anogenital sniffing ($p = 0.013$) and side-side contact ($p = 0.031$) in the freely moving social interaction test. All mice, regardless of sex and treatment, showed an interest in exploring a conspecific over an object during the three-chamber test. However, untreated male mice were more inclined to investigate the novel conspecific during the social memory component, while cerebellar modulation led male mice to spend more time with the familiar conspecific. Examining the naturalistic behaviors of mice with machine learning can aid in uncovering the role of sex and the cerebellum on social behavior.

Disclosures: M.R. Nelson: None. **J. Verpeut:** None.

Poster

PSTR286: Cerebellum: Non-Motor Functions

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR286.11/H19

Topic: H.08. Learning and Memory

Support: R21MH129041

Title: Investigating a possible role for the cerebellar interposed nucleus in habit behavior

Authors: *D. JUNG, M. MIQUEL, K. KHODAKHAH, S. M. NICOLA;
Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Habits, defined as automatic behaviors triggered by stimuli, involve neural mechanisms distinct from those regulating goal-directed actions. While habits are crucial for efficiency, their imbalance with goal-directed control underlies disorders like OCD and addiction. Prominent theories attribute habit formation to dopamine-mediated reinforcement learning and cortico-striatal plasticity. Although studies of dorsal striatum support this, neurophysiological evidence is deficient. Neuronal activity in the cerebellum connects individual actions to form sequences, suggesting that it could also connect cues to actions to form habits. Anatomical studies show that the interposed nucleus (IP) of the cerebellum has reciprocal mono- and polysynaptic connections with the infralimbic cortex, a structure required for habit behavior. Therefore, we explored whether chemogenetic disruption of IP disrupts habit learning and execution. We trained male and female Long-Evans rats in a discriminative stimulus task. A discriminative stimulus (S) informs the subject that a reward is available. In response to S presentation, the subject enters a reward port (A) and a sweet liquid reward (R) is delivered. This sequence must occur in order (S-A-R). We first gained evidence that the task is acquired via

habit learning by showing that responses in over-trained animals are resistant to LiCl-induced devaluation of the reward, while in contrast this effect was not observed early in training. Using this behavioral paradigm, we then tested our hypothesis that IP contributes to habit learning and expression. We injected the inhibitory hM4D(Gi) DREADD virus bilaterally into IP. After 3 weeks, virus-injected and control rats received an intraperitoneal injection of clozapine-N-oxide 30 minutes prior to each daily training session. The time taken to learn the task in IP-suppressed rats was not different from that in control rats. Furthermore, in a devaluation test after habit formation, there was no difference in responding between the control group and the IP-suppressed group.These results indicate that IP may not contribute to behavioral learning and habit formation in the discriminative stimulus task, although this conclusion should be considered tentative because we have not yet verified the effectiveness of DREADD inhibition of IP.

Disclosures: D. Jung: None. **M. Miquel:** None. **K. Khodakhah:** None. **S.M. Nicola:** None.

Poster

PSTR287: Dexterous Motor Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR287.01/H20

Topic: E.04. Voluntary Movements

Title: Characterization of manual exploration in early infancy: A sequence analysis approach

Authors: *J. J. BURNS, M.-H. LEE;
Kinesiology, Michigan State Univ., East Lansing, MI

Abstract: Manual exploratory behaviors provide infants with a crucial opportunity for acquiring knowledge about object properties, including size, shape, and texture. Understanding how infants engage with and explore objects offers potential insights into the developmental mechanisms underlying object manipulation. However, prior work has focused mainly on ‘frequency’ of behaviors and paid little attention to how these behavioral action sequences are organized. Here, we aim to investigate how infants explore objects with different properties over time, specifically focused on action sequence. Infants, aged 6-12 months, participated in a longitudinal exploration of eight distinct objects characterized by differences in size (2” vs. 4”), shape (cube vs. sphere), and texture (hard vs. soft). We categorized infant exploration into three main actions – fingering, rotation, and transportation. Our results show three main findings: first, there was a tremendous range of observed behavioral action sequences across infants and objects, suggesting that these behaviors are highly exploratory. Second, of the three properties that were varied, changes in object size elicited the widest range of behavioral sequences. Third, with development, infants demonstrate a progression from simple fingering exploration to more complex actions, such as transporting objects through throwing or bringing them to their mouths. Understanding the

evolution of these action sequences provides insight into the complex interplay between perception, cognition, and motor development during this critical period of early childhood. Furthermore, identifying atypical exploration sequences may also have implications for clinical applications, aiding in the early identification of children who may be at risk for movement disorders or autism spectrum disorder.

Disclosures: J.J. Burns: None. **M. Lee:** None.

Poster

PSTR287: Dexterous Motor Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR287.02/H21

Topic: E.04. Voluntary Movements

Support: NIH Grant: 5R01HD107730-02, PI: Heathcock

Title: Developmental trajectories for object retrieval in children ages 4 to 38 months

Authors: *M. R. ROSALES, J. HEATHCOCK;
Sch. of Hlth. and Rehabil. Sci., The Ohio State Univ., Columbus, OH

Abstract: Introduction: Motor assessment is vital for the understanding of pediatric development and neurorehabilitation. The ability to assess reaching, grasping, and the manipulation of objects using a repeatable task that could be paired with neuroimaging (e.g. EEG); would aid in gauging a child's progress during an intervention, and guide future studies aimed at understanding the neural correlates of motor recovery. Here we present the preliminary data from a longitudinal study that aims to develop an assessment tool for gauging the reaching and grasping behaviors of children. Specifically, we describe the developmental trajectory for an object retrieval task in a wide age range of children. Methods: N=37 children with typical development participate in this project. During 4 visits (baseline and 1-month, 3-months, and 7-months following baseline), children (4- 38 months) were asked to retrieve a toy from an opaque box (task 1), a clear box (task 2), and then the clear box nested inside the opaque box (task 3). Verbal cues and assistance were provided at standardized time points, and the task was defined as complete when the child grasped the toy in their hand. Using video recordings, trained behavioral coders identified the start and completion of each task, each hand contact of the boxes and toys, and the amount of assistance provided. Pearson correlations were used to test for correlations between age, task duration, amount of assistance provided, number of toy contacts, and the proportion of bilateral contacts during each task. Results: With age, children take less time to complete each task ($r > -0.6$, $p < 0.01$ for all three task), require less assistance ($r > -0.39$, $p < 0.01$ for all three task), contact the test materials a fewer amount of time ($r > -0.6$, $p < 0.01$ for all three task), and the proportion of bilateral contacts increases ($r > 0.5$, $p < 0.01$ for all three task). Conclusion:

Developmental trends show that as children age, they can retrieve the toy quicker, with more independence, and more efficiently. The object retrieval task used can be collected in a wide age range and should be used to study children with neurodevelopmental disorders. We have started to collect these data in children with perinatal stroke, and plan to present findings related to their neurorehabilitation once our clinical trial is completed. Future results will guide research directed towards understanding the neurorehabilitation of object retrieval skills in children.

Disclosures: M.R. Rosales: None. **J. Heathcock:** None.

Poster

PSTR287: Dexterous Motor Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR287.03/H22

Topic: E.04. Voluntary Movements

Support: NIH Grant R15AG067792

Title: Effect of simple hand motor repetition on balancing a virtual inverted pendulum in young and older adults

Authors: K. KIANI, *Q. FU;
Mechanical and Aerospace Engin., Univ. of Central Florida, Orlando, FL

Abstract: The human hand is critically important for performance of many activities of daily living. It remains unclear what type of motor repetition is most beneficial for training sensorimotor control of the hand. This gap in our knowledge limits the extent to which motor rehabilitation programs can be optimized to ensure maximal gains in training and generalization to a wide variety of untrained motor tasks. This study compared two types of motor repetition paradigms that require generating either discrete force pulses (FD) or continuous motion (MC) with participants' hands in the supination and pronation directions. We assessed how these repetition training can affect the ability to control a complex motor task in which a virtual inverted pendulum must be balanced by either left or right hands. Both young and older adults were tested (n = 29 and 27, respectively). We found that the FD training enabled better performance of the pendulum balance task than the MC training in both hands of older adults. In contrast, young adults showed better pendulum balance performance with their dominant hands when MC training, but not FD training, was used. Lastly, we also evaluated how performing the virtual pendulum task may alter the postural balance control by measuring the center of pressure (CoP) trajectory during quite standing both before and after the pendulum task. Interestingly, older adults showed a decreased CoP velocity in the mediolateral direction after the pendulum task during eyes-open but not eyes-close conditions, suggesting an improved visuomotor control of postural balance.

Disclosures: K. Kiani: None. **Q. Fu:** None.

Poster

PSTR287: Dexterous Motor Control

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Topic: E.04. Voluntary Movements

Support: NIH Grant R01AG069227

Title: Probing cortico-cortical interactions in the orofacial cognitive-sensorimotor network in aged macaques

Authors: *F. I. ARCE-MCSHANE, L. FAVOUR, V. HOSACK, J.-S. LI, S. PUNACHA, B. SADEGHI, D. TANG;
Univ. of Washington, Seattle, WA

Abstract: Age-related oral health problems have been associated with Alzheimer's Disease (AD), yet the pathophysiological link between oromotor dysfunction and AD is still unknown. Understanding the cortical underpinnings of such association in a non-human primate (NHP) model is an important step in filling this knowledge gap. Specifically, the ventrolateral prefrontal cortex (vPFcx) has been shown to exert cognitive influences on the primary motor (MIo) and somatosensory (SIo) areas of the orofacial sensorimotor cortex (OSMcx). We hypothesize that cortico-cortical interactions are altered in the orofacial cognitive-sensorimotor networks in a NHP model of AD-related dementia. In our study, we investigate the interactions between the activity of populations of neurons in OSMcx and vPFcx during natural feeding behavior in aged rhesus macaques (*Macaca mulatta*). We performed simultaneous recording of 3D tongue and mandible kinematics with neuronal activity in multiple cortical areas (Brodmann areas 44, 45, 4, 3a/3b, 1/2) of an aged male macaque (21 years, 12 kg). We show successful recordings from 320 microelectrodes (i.e., three Utah arrays (Blackrock, Salt Lake City, UT) and two Floating Microelectrode Arrays (Microprobes, Gaithersburg, MD)), which were surgically implanted using Brainsight Vet Robot and workflow (Rogue Research, Montreal, Quebec, Canada). Using functional connectivity analyses, we characterize the cortico-cortical interactions within the orofacial cognitive-sensorimotor neuronal networks during feeding (i.e., chews and swallows) and how these may be differentially affected by memory deficits. The aberrant connectivity patterns between cognitive and sensorimotor networks may relate to disrupted interactions between cognitive or sensory inputs and motor outputs (i.e., tongue and jaw movements) and/or compensatory mechanisms. These have important implications for understanding how cortico-cortical interactions may be altered in healthy aging and in AD.

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Poster

PSTR287: Dexterous Motor Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR287.05/H24

Topic: E.04. Voluntary Movements

Title: Two Aspects of Feed-Forward Control of Action Stability: Effects of Action Speed andUnexpected Events

Authors: *S. D. DE[1], S. AMBIKE[2], M. L. LATASH[1];
[1]Dept. of Kinesiology, Penn State Univ., University Park, PA; [2]Hlth. and Kinesiology, Perdue Univ., West Lafayette, IN

Abstract: Changes in stability of a steady-state action in preparation to a quick change of the salient performance variable have been addressed as anticipatory synergy adjustments (ASAs). We explored two types of ASAs during accurate four-finger total force (F_{TOT}) production task. The first type is a change in the index of total force stability (ΔV) quantified using the framework of the uncontrolled manifold (UCM) hypothesis when a person is expecting a signal to produce a quick F_{TOT} change, which is seen even when the signal does not come (steady-state ASA, ASA_{SS}). The other type is the drop in ΔV prior to a planned change of F_{TOT} starting at a known time (transient ASA, ASA_{TR}). The subjects performed a task of steady F_{TOT} production at 10% of maximal voluntary contraction (MVC). This phase was followed by a ramp F_{TOT} change to 20% MVC over 1 s, 3 s, and as a step function (0 s). In another task, in 50% of the trials during the steady-state phase, an unexpected signal could come requiring a quick force pulse to 20% MVC (0-surprise). Inter-trial variance in the finger force space was quantified per dimension within the UCM (V_{UCM}) and orthogonal to the UCM (V_{ORT}). A synergy index ΔV was computed as the normalized difference (V_{UCM} - V_{ORT}) and log-transformed (ΔV_Z). We observed significantly lower ΔV_Z values during the steady state in the 0-ramp trials (1.19 ± 0.327) compared to the 1-ramp and 3-ramp trials (1.36 ± 0.326 and 1.33 ± 0.271). There were also larger ASA_{TR} during the 0-ramp trials (median drop in ΔV by about 0.2) compared to the 1-ramp and 3-ramp trials (median drops of 0.024 and 0). In the 0-surprise condition, ΔV_Z was significantly higher compared to the 0-ramp condition (without surprise targets) whereas the ASA_{TR} was significantly larger (median drop in ΔV_Z of 0.37). The former effect was due to both larger V_{UCM} and smaller V_{ORT}. The finding of ASA_{TR} scaling is of importance for clinical studies, which commonly involve populations with slower actions, which can by itself lead to smaller ASA_{TR}. The higher V_{UCM} in the 0-surprise trials suggests that the participants varied the sharing pattern of F_{TOT} across trials more in the series with “surprises”. This effect remains

enigmatic and in need of further exploration. This was coupled to more attention to precision of performance, i.e., inter-trial deviations from the target as reflected in V_{ORT}, possibly reflecting higher concentration on the task, which the participants perceived as more challenging compared to a similar task without surprise targets.

Disclosures: S.D. De: None. **S. Ambike:** None. **M.L. Latash:** None.

Poster

PSTR287: Dexterous Motor Control

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Program #/Poster #: PSTR287.06/H25

Topic: E.04. Voluntary Movements

Title: Yank and endpoint accuracy of ballistic goal-directed isometric contractions

Authors: *R. J. MALIK[1], J. J. KIM[1], S. DELMAS[1], J. HUBBARD[1], B. YACOUBI KEYHANI[2], E. A. CHRISTOU[3];
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Abstract: Yank is defined as the time derivative of force, which is an essential metric for quantifying changes in force over time. Existing literature has used yank to quantify the instantaneous rate of force development and steadiness of constant force tasks. To our knowledge, no studies exist that examined if yank control is essential for the accuracy of goal-directed contractions. In this study, we aimed to investigate the significance of yank in modulating the accuracy of goal-directed ballistic contractions across varying force levels. Eighteen healthy young adults (26.3 ± 6.4 years, 9 women) performed ballistic abduction of the index finger in isometric contractions towards a targeted force. Participants performed 50 trials at seven randomly assigned force levels (2%, 15%, 30%, 50%, 70%, and 85% of maximum voluntary contraction force, MVC). The first 10 trials were excluded (adjustment to the targeted force) and the last 40 trials were analyzed. We found a significant reduction of absolute error (63%) and bias error (86%) from 2% to 15% MVC, indicating that very low forces are challenging. To understand which yank variables associated with the reduction of error from 2% to 15% MVC, we quantified the change in the following yank metrics from 2% to15% MVC: the magnitude of the whole yank as root mean square (RMS) yank, the time duration from yank onset to its positive peak (termed as Time to Peak Yank), Positive Peak and Negative Peak of yank, Area of the Positive Yank. In addition, we examined the trial-to-trial variability across the 40 trials of each yank parameter with the coefficient of variation (CV). We found that the reduction in absolute ($p<0.05$, $r2=0.33$) and biased ($p<0.05$, $r2=0.23$) error at 15% relative to 2% MVC associated with the reduction in the CV of the RMS yank. All other yank variables were weakly or not associated with endpoint accuracy. The findings provide novel evidence that

greater inaccuracy at very low force levels relates to greater variance in yank amplitude, highlighting the importance of yank regulation during targeted ballistic contractions.

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Poster

PSTR287: Dexterous Motor Control

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Program #/Poster #: PSTR287.07/H26

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Title: Control, Variability and Embodiment of Complex Tools: Hitting a Target with a Bullwhip

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Abstract: SCOPE: When hammering a nail into a wall, the hand can move along multiple trajectories even if the hammer always hits the nail. This variability in tasks with redundancy is even more complex when the actions involve non-rigid tools. Examples are abundant and range from tying shoelaces to spreading a tablecloth. Control of those objects is challenging, which is particularly evident in motor-impaired individuals. Our research explores interaction with an extreme example of complex tools, a bullwhip, with a focus on variability in its underactuated degrees of freedom. METHODS: One expert (E) and 16 novices (N) used a 1.6-m whip to hit a target at 2m distance repetitively in five blocks, 30 trials per block. Eight additional novices (NP) practiced the same task in 35 blocks over a month. Arm and whip kinematics were recorded via motion capture. Analysis focused on the throw interval defined from the instance when the hand was farthest from the target until when the whip was closest to the target. Trajectories from each marker (4 on the arm, 10 on the whip) were rescaled by their mean arclength. For each marker, variability across trials was computed as the cross-section of the trajectory bundle. RESULTS: Expectedly, E hit the target 90% of the trials, while N scored 5-35% and NP scored 25-55%. Results indicated an emerging spatiotemporal structure of variability across the arm and whip markers: (1) E showed smaller variability in the whip than N and NP. (2) In all subjects, whip variability decreased by the end of the throw interval, while hand variability increased. (3) In less

accurate N16, variability increased from the shoulder to the tip of the whip, while more accurate N and NP showed no change; variability in E decreased. (4) The more accurate N, NP, and E showed a cascade of low variability propagating from the hand towards the tip as the whip unfolded towards the target. INTERPRETATION: These results demonstrated a channelling of variability with the least variability at the tip of the whip when close to the target, the location and time most critical for task success. Counter to findings on manipulating rigid tools, hand variability was not necessarily decreased, suggesting a shift of control attention from the hand, the end-effector, towards the task-relevant tip of the whip. The low-variability cascade propagating from the hand to the tip indicates that participants discovered "natural" modes of the whip, consistent with its dynamics. Taken together, these findings demonstrate effective control and embodiment of even such complex tools as a bullwhip.

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Poster

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Title: Brain Activity before Target Presentation Predicts Arm Choice

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Abstract: Arm choice, whether to reach an object with the left or right arm, is an unconscious decision frequently made in daily life. When a target is provided on the right or left side of the space, the probability of choosing an arm on the same side is very high (Schweighofer 2015; Oliveira 2010). However, when a target is provided near the center space between the left and right arms, the probability of choosing either arm is approximately 50% (equivalence point). A previous forced binary choice study reported that the state of brain activity before option presentation was related to subsequent choice when the choice was at the equivalence point (Bode et al. 2012). Here, using a task in which participants were required to select one arm quickly to reach targets that appeared at a variable location on a semicircular array, we examined whether brain activity, as recorded by EEG before the target was presented, could predict arm choice. We measured whole-head 64-channel EEG (Biosemi) while participants executed an

arm-choice reaching task with 13 healthy right-handed individuals. Signal source estimation was performed using the preprocessed EEG data during a fixation period of 1 second before target representation using the Brainstorm software. We performed sLORETA to compute the source activity and investigated 79 cortical regions of interest (ROI) that were parcellated using the Brodmann Area map and delineated using the PALS-B12 atlas. We then classified arm selection with a linear support vector machine (SVM) using the 79 EEG features for center and peripheral targets and calculated the classification accuracy with ten-fold cross-validation. The classification accuracy of the center target condition (58.8 ± 1.9%) was significantly higher than the chance level (50%; $P < 0.05$) and that of the peripheral target condition (51.2 ± 1.8%; $P < 0.05$). In addition, we conducted a weight analysis to examine the highly related cortical areas for the SVM classification. The activities of the posterior parietal and premotor cortices showed high weights for the classification. These results suggest that the state of brain activity before target presentation can influence subsequent arm choice. Furthermore, some previous studies have suggested activities in the posterior parietal and premotor cortices to be important regions in arm choice (Hirayama 2021; Fitzpatrick 2019; Hamel-Thibault 2016), and the present results support those findings.

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Poster

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Topic: E.04. Voluntary Movements

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Title: Role of Movement Time Estimation and Discounting in Arm Choice

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Abstract: Reaching movements post-stroke are variable, effortful, and slow. Our previous work (Kim et al., 2022; Schweighofer et al., 2015) showed that paretic arm choice is influenced by success rates (variability), and (physical) effort. Here, we considered the potential impact of longer movement time (*MT*) on choice. The duration of fast movements, like eye saccades and arm reaches, has been proposed to act as an implicit delay during reward acquisition (Berret et al., 2022; Shadmehr et al., 2016). We conducted a two-day experiment to assess the effect of movement time on arm choice. On day 1, to evaluate discounting, targets were presented at

various distances, sizes (Fitts' law), and angles to produce multiple *MT*s. Participants were shown two targets at each trial, one for each arm, and were instructed to choose one arm to reach the corresponding target. Arm positions were concealed, represented only by cursors. Arm choice was modeled as a competition between internally estimated action values assigned to each arm, which combine a reward term discounted by *MT* and an effort term estimated using inverse dynamics. Hierarchical Bayesian methods were used to estimate the steepness of the discounting function (*k*), the trade-off between reward and effort, the randomness of choice, and the handedness bias.Discounting depends on accurate internal estimation of *MT*. On day 2, we evaluated how the internal estimates MT^* for each arm are updated and how it affects choice. To achieve this, the right arm was abruptly slowed by manipulating the visible cursor, altering the relationship between the target and *MT*. Determining the value of moving with the right arm requires re-estimation of MT^* by learning the new relationship between movement and target. Predictions of MT^* are based on internal simulations of the arm movement (Papaxanthis et al., 2012). We assumed that the CNS estimates MT^* with an internal (linear) model that expands Fitts' Law. A Kalman filter updates the internal model coefficients using the error between predicted MT^* and actual *MT* when the movement is selected and executed. Data from healthy, young participants show nearly equal selection of the right and left arms on day 1. Parameter estimation shows that *MT* affects decisions via a (positive) discounting parameter *k*, validated by parameter recovery in simulations. On day 2, right arm choice gradually decreased after the cursor manipulation slowed movements, indicating that the participants gradually re-estimated MT^*. Our results suggest that the commonly observed "non-use" phenomenon post-stroke, where individuals can move the paretic arm but choose not to, may partly result from the slow movements made with the paretic arm.

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Poster

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Topic: E.04. Voluntary Movements

Title: Effects of Augmented Feedback on Hand Selection Behavior

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Abstract: Hand selection behavior has been shown to be associated with interlimb asymmetries in sensorimotor performance. Understanding the neural mechanisms underlying this association is crucial for developing effective training and rehabilitation strategies for upper extremities. In

this study, we investigate how various forms of augmented feedback affect hand selection behavior. Healthy young individuals aged 21-25 years, all right-handed, performed 70 point-to-point hand selection reaching movements to 14 targets sampling frontal space in each of the five conditions. These conditions included baseline (veridical feedback), left arm promoted by distance (30% reduction in distance to target), right arm challenged by distance (30% increase in distance to target), left arm promoted by efficiency (50% reduction in trajectory deviation from a straight line to the target), and right arm challenged by efficiency (100% increase in trajectory deviation from a straight line to the target). Each experimental condition was preceded and followed by the baseline condition, ensuring consistency and comparability across all trials. Preliminary data analysis reveals systematic shifts in hand selection patterns towards greater utilization of the non-dominant arm across experimental conditions. These shifts are transient and washed out when participants returned to the baseline condition, indicating a highly dynamic process of hand selection. This observation aligns with our previous findings when occluding visual feedback to promote more use of the non-dominant arm. Interestingly, the magnitude of these shifts in hand selection patterns varied among conditions, suggesting an association with individual interlimb asymmetries in sensorimotor performance. These insights advance our understanding of the role of various forms of augmented visual feedback and interlimb asymmetries in hand selection behavior and provide a foundation for the development of innovative approaches to upper limb training and rehabilitation.

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Poster

PSTR287: Dexterous Motor Control

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Topic: E.04. Voluntary Movements

Support: Penn State startup funds

Title: Movement-related beta desynchronization during motor planning and execution of interception movements

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Abstract: In one's environment we frequently encounter moving targets and move our limbs to interact with them. To do this, we must accurately estimate the relative motion between the

target and our body. Previous studies have shown that during movement planning, there is a decrease in beta band power in motor regions, a phenomenon known as movement-related beta desynchronization (MRBD). Studies have highlighted the significance of beta band activity in the frontal cortex for planning movements aimed at intercepting targets. Our study aimed to investigate how beta-band activity in the frontoparietal networks is affected by target speed during the planning and execution of interception movements. We concentrated on the activity recorded from parietal (P3) and frontal (Fc1 and C3) electrodes. Participants wore a 64-channel EEG cap (Bittium Inc) and intercepted a virtual moving target using a Kinarm robot. Interception trials rials were randomized with the target moving at three different speeds (25cm/s, 30cm/s, 35cm/s) in a medial-lateral plane. In a control condition, they performed reaching movements to static targets. Participants were young, healthy adults recruited from the University (20.2 ± 0.5 years). A paired t-test revealed significant differences in cortical beta band activity between interception and reaching actions at P3 around target onset ($p < 0.05$, $t=2.8152$). We also found significant differences in event-related potentials (ERP) at Fc1 between the slowest and fastest target speeds ($p<0.05$). Preliminary results suggest potential differences in N170 and P300 amplitudes in relation to target speed. We found strong stimulus related beta band desynchronization at Fc1 for both interception and reaching movements. Our study provides new insights into how beta band activity in frontoparietal networks encode target motion and speed to facilitate planning and execution of interception and reaching movements.

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Poster

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Topic: E.05. Brain-Machine Interface

Support: NSF Grant 1849213

Title: Gradations in Isometric Finger Extension Captured by Event-Related Changes in the EEG Mu-Beta Sensorimotor Rhythm

Authors: *S. GARCIA PAVA, S. SUNDERAM;
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Abstract: Abstract: Brain-computer interfaces (BCIs) offer a revolutionary approach to assist individuals with disabilities from neuromuscular injuries or neurodegenerative diseases by directly translating brain signals into commands for external devices. BCIs commonly rely on non-invasive electroencephalography (EEG) to determine user intent. But the limited ability of

EEG to differentiate between graded levels of effort beyond simple binary commands (e.g., hand movement vs. rest), poses a significant challenge. To address this limitation, we investigated EEG correlates of graded motor effort associated with a finger extension task, which is critical for individuals with hand impairment. With prior IRB approval and informed consent, 12 right-hand dominant subjects without neurological or physical impairments were asked to extend their fingers to a certain target level (no-go/rest, low, medium, or high) when prompted by a visual cue. Each session comprised 12 runs of multiple trials, alternating between the two hands. Each target was presented 4 times in random order within each run to prevent subjects from anticipating the task. Data from 32 EEG electrodes and bipolar EMG from both extensor carpi radialis muscles were recorded at 256 Hz. Event-related desynchronization (ERD) of the mu-beta (8-30 Hz) EEG power during each movement trial relative to the median of all pre-trial periods was computed at each scalp location. Then, the norm of the resulting ERD vector was computed as a scalar measure of the strength of the ERD for each target level of extension. A test was performed to assess whether the ERD strength increased monotonically with the target extension level in each subject. The mu-beta ERD strength increased monotonically from no-go to high finger extension in 7/12 participants on the left hand, a proportion greater than chance ($p=0.0143$), and in 6/12 participants on the right hand ($p=0.054$). These trends underscore the potential for deriving graded volitional signals indicative of fine motor control, linked to a progressive increase in cortical recruitment correlated with extensor activity. However, they also emphasize the necessity for personalized BCI therapies to accommodate individual differences.
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Poster

PSTR287: Dexterous Motor Control

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Topic: E.04. Voluntary Movements

Support: NIH Grant R01NS132926

Title: Repelling effects of distractors persist in imitated movements

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Abstract: Similar actions can be taken in different contexts. For example, we may make similar reaching movements towards a glass of water either when water is the only target in front of us, or with a distractor next to it (e.g., a friend's glass of water). Does the presence of a distractor in the workspace interfere with goal-directed action, even when the action is imitated? Experiment 1 reported a robust repelling effect of distractors on spontaneous movements: When participants were asked to move their mouse to reach a goal in the workspace, their movement trajectories were repelled towards the goal when a distractor appeared at the opposite side of the target, echoing previous findings. Next, we asked whether such distractor-induced biases can be overcome by imitating visually-observed movement trajectories. To address this, we algorithmically generated 10 videos, each showing an agent's hand following a unique trajectory to a target. In Experiment 2, participants were instructed to closely observe how the agent moved their mouse, and then replicate the trajectory they observed by moving their own mouse in the same way. Surprisingly, though participants were asked to only imitate an agent's movements, their reproduced trajectories were still repelled by the simple presence of a distractor. Experiments 3-5 ruled out the possibilities that the persistent effects in imitated movements were driven by different types of memory biases and goal uncertainty, providing further evidence for a stubborn effect of distractors in goal-directed actions. Across all five experiments (N=500, pre-registered), we also found that the repelling effects emerged at very early stages of movement (<100ms), suggesting that motor planning was significantly influenced by the distractors in the workspace. Follow-up studies explored intrinsic properties of distractors that may alter their repelling effects on goal-directed movements, including learned stimulus value and inferred stimulus mass. In sum, this work explored the effects of task-irrelevant distractors on both spontaneous and imitated movements, revealing an obligatory repelling effect of distractors in shaping movement plans.

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Poster

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Topic: E.04. Voluntary Movements

Support: NIH 5R01NS112424-04
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Title: Cross-area dynamics during reach-to-grasp control and recovery

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Abstract: Despite physical therapy, about 50% of stroke survivors have impaired hand function which strongly impacts daily living. While research addressing stroke recovery has predominately focused on restoring damaged descending motor pathways, dexterous hand function is also reliant on ascending sensory input to somatosensory cortical areas. However, how somatosensory and motor areas interact on a systems level to facilitate dexterous coordination of hand function is not well understood.

Using dual chronic 64-channel microwire electrode arrays (Tucker-Davis Technologies), we recorded extracellular electrophysiology data simultaneously from dorsal premotor cortex (PMd) and area 2 of somatosensory cortex of non-human primates (rhesus macaques) recovering from a primary motor cortex (M1) stroke lesion. We then monitored arm and finger kinematics (DeepLabCut[1]) while animals performed reach-to-grasp tasks that are likely dependent on touch and proprioception of the hand and arm.

To analyze the coordination and shared signals between PMd and area 2, we approached the problem from a neural manifold perspective. Specifically, by using two-area dimensionality reduction methods (e.g. canonical correlational analysis, DLAG[2]), we are able to find a low-dimensional representation of the neural space of shared signals between the two regions on a single-trial basis. By comparing these cross-area factors to the simultaneous kinematics, we show evidence that these cross-area factors correspond to different features of reach-to-grasp behavior, and we found that similar temporal-behavioral factors were identifiable across days and across subjects. Crucially, we show evidence that these cross-area factors were weak or missing early after M1 stroke, but they became increasingly detectable over the course of recovery of reach-to-grasp behavior. Specifically, cross-area factors gradually emerged over days to weeks during recovery, but they became only fully present in later days when the animal had behaviorally recovered from stroke.

Given these results, we hypothesize that the time-varying activation of these cross-area factors may aid the time-sensitive coordination of sensory and motor regions that must cooperate to dexterously complete sensorimotor tasks.

(References: [1] Nath et al., “Using DeepLabCut for 3D Markerless Pose Estimation across Species and Behaviors.” [2] Gokcen et al., “Disentangling the Flow of Signals between Populations of Neurons.”)

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Poster

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Title: Disentangling sensorimotor communication during mouse forelimb reach to grasp movements

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Abstract: Accurate sensory-guided forelimb movements require ongoing motor commands to be closely coupled to sensory feedback. Interactions between the richly interconnected primary motor (M1) and somatosensory (S1) cortices may support this function, yet how these areas communicate during behavior is not well understood. To characterize M1-S1 communication, we developed a forelimb reach-to-grasp task where head-fixed mice made sound-cued reaches to grasp water rewards from 15 spout locations in 3D space. During this behavior, we recorded layer 2/3 excitatory cells of contralateral forelimb M1 and S1 simultaneously at 30 Hz using dual independent scan engine two-photon (Diesel2p) calcium imaging in GCaMP6f transgenic mice. To coarsely characterize differences between areas in our preliminary data, we trained linear classifiers to identify the area from which a cell was recorded using its PSTH. Each cell's source area could be decoded at 60-70% accuracy (cross validated SVM), consistent with commonalities in movement tuning. We then assessed whether kinematic signals differed across areas by linearly decoding individual joint angles from size-matched populations of M1, S1, and M1+S1 cells. Combining data across areas did not improve performance over decoding from a single area (39.2% VAF for M1, 32.2% S1, 35.4% M1+S1). This further supports our previous observations of a broadly shared linear representation of kinematics across these areas. Leveraging our simultaneous recordings, we computed pairwise correlations within and across populations, finding that M1-M1 correlations are higher than S1-S1 or M1-S1 for total, noise and kinematic-residual correlations (using deconvolved spiking events, 0.045 M1, 0.039 S1, 0.035 M1-S1 mean total Pearson correlation). Further, the autocorrelation of single cells computed from PSTHs was longer in M1 than in S1, indicating that M1 activity varied more smoothly than S1 activity (median 190.6 ms M1, 159.9 ms S1). Finally, we used reduced rank regression to characterize the communication between these regions. We found that M1 predicts S1 better than S1 predicts M1 (median 2.4% vs 1.8% VAF, mean matched populations) and that within-area prediction is higher in M1 than in S1 (2.2% vs 1.4%). Together these results confirm that movement signals are broadly distributed across the mouse sensorimotor cortex, and open a path forward for understanding communication between these areas.

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Poster

PSTR287: Dexterous Motor Control

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Topic: E.04. Voluntary Movements

Support: R01 NS117406
R01 NS112424
I01RX001640

Title: Conserved role of primary motor cortex in skilled reach-to-grasp control in mice and macaques

Authors: *K. GANGULY[1], F. APARICIO[2], P. KHANNA[3], S. BARATI[4], H. GHUMAN[4]; [1]UCSF, San Francisco, CA; [2]Neurosci., Univ. of California San Francisco Neurosci. Grad. Program, Chatsworth, CA; [3]Neurol., UC Berkeley, Berkeley, CA; [4]Neurol., UCSF, San Francisco, CA

Abstract: An important goal of neuroscience is to establish principles that can guide comparative studies across species. If we specifically compare rodents and macaques, there are fundamental differences in motor network anatomy. From a functional perspective, there are also a growing number of studies which indicate that in rodents, extensive skill training can lead to a 'disengagement' of M1 from movement control; after a period of initial deficits, movements appeared to be identical and relied only on subcortical structures. However, classic studies indicate that cortical networks are essential for prehension and perhaps less important for proximal control. However, the exact amount of task training was not clear and detailed kinematics were not performed. Thus, it remains unclear precisely how gross and fine motor control might change with a M1 lesion in primates. Here we aimed to measure changes in skilled gross and fine motor control after a M1 lesion in both macaques and mice. In macaques, we ensured that animals were well trained; we also independently measured performance in a gross motor skill and a reach-to-grasp skill. In mice, we also performed long-term monitoring of layer 5 M1 projection neurons, i.e., pathways we know are more likely to reflect movement control signals from M1. Across species, we performed detailed kinematic monitoring to quantify changes in performance, kinematic variability and transitions between sub-movements. Together, our results indicate that there is a common and preserved functional principle after the loss of M1. Even in primates, there is rapid restoration of gross movement control after a period of deficits; strikingly similar to what is reported for rodents. In contrast, for a task involving prehension, we noted prolonged deficits in prehension and changes in the transition reliability of reach to grasp. Lastly, we also do not find any evidence of neural disengagement when considering layer 5 projection neurons, pathways that are known to be critical for reach to grasp skills. Interestingly, the shared trajectory post-lesion across the two species underscores a

commonality in the disruption of smooth transition probabilities that is replaced by a mosaic of fragmented movements during a task that is comprised of reaching and prehension.

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Poster

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Title: Continuous Offline Force Decoding from Human M1 Population Activity

Authors: *I. GONZALEZ[1,4], G. H. BLUMENTHAL[2,4,5], E. OKOROKOVA[8], S. M. CHASE[6,7,5], B. M. DEKLEVA[2,4,5], M. L. BONINGER[2,4,3], J. L. COLLINGER[2,3,6,5,4]; [2]Physical Med. and Rehabil., [3]Bioengineering, [1]Univ. of Pittsburgh, Pittsburgh, PA; [4]Rehab Neural Engin. Labs, Pittsburgh, PA; [5]Ctr. for Neural Basis of Cognition, [6]Biomed. Engin., [7]Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA; [8]Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: Paralysis from spinal cord injury leads to loss of upper-limb motor function, and brain-computer interfaces (BCIs) are a promising approach for restoring this functionality. The ability to modulate grasp force is a critical component of dexterity, requiring both static force production and the ability to dynamically adjust grasping force. Non-human primate studies have identified neural correlates of static and dynamic grasping forces at the single-neuron level (Cheney and Fetz 1980; Evarts et al., 1983; Maier et al., 1993). We quantified how well we can predict continuous force output from populations of neurons in human primary motor cortex (M1) as it can be used to restore hand function with a BCI. Two participants with tetraplegia had intracortical microelectrode arrays implanted in their M1 and somatosensory cortex as part of an ongoing clinical trial conducted under an FDA Investigational Device Exemption. Neural data was recorded while participants performed a force-matching task comprised of static (unchanging force over time) and dynamic (changing force over time) periods. The participants were unable to execute overt grasps, but were able to perform overt isometric wrist extension to match force targets while we recorded electromyography (EMG) activity from the forearm. Using M1 population activity as the input into a long short-term memory (LSTM) network, we attempt to continuously predict target force and EMG RMS amplitude offline. The model did

well predicting target force (R^2 = 0.7) and EMG (R^2 = 0.8-0.9) during static force periods. During dynamic force periods, the ability to predict target force was reduced (R^2 = 0.4-0.5), but the model still performed well when predicting EMG (R^2 = 0.7) suggesting that target force may not reflect the participants' actual force output during the dynamic periods. This has implications for BCIs that typically rely on attempted, rather than overt, movements for calibration. Complex and dynamic actions may be challenging to perform as instructed making the target force a less reliable proxy for their true intention. Ultimately, we hope to restore the ability to modulate grasp force using a BCI. We found that the decoding accuracy was greatest during static force production tasks or when there is a true behavioral correlate (i.e. EMG) of the participant's intention. Simple calibration paradigms based on achieving static force targets may be sufficient for restoring simple, planned grasps but the ability to manipulate objects and generate dynamic forces will require new calibration strategies that provide for better alignment between the instructed action and neural correlates of the participant's intention.

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Poster

PSTR287: Dexterous Motor Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR287.18/H37

Topic: E.04. Voluntary Movements

Support: 915027201
1415181023

Title: Causal relationships between local-field potentials in frontoparietal networks during motor prediction errors

Authors: *M.-K. KIM[1], J.-W. SOHN[2];
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Abstract: It is hypothesized that forward models anticipate movements following a motor command by simulating the consequences of efferent output to the motor plant, which is thought

to play a pivotal role in motor control. Several studies have indicated the involvement of the posterior parietal cortex (PPC) and prefrontal motor cortices in realizing this hypothesis in the brain. Researches suggest that the PPC is associated with the translation of predictive signals into awareness. Meanwhile, the supplementary motor area appears to be crucial in suppressing overshoot movements in the primary motor cortex (M1), resulting in inhibitory outputs on subsequent efferent commands. It has also been reported that these processes are likely to involve the encoding of prior motor intentions in the dorsolateral prefrontal cortex (DLPFC). However, the mechanism of information exchange between these areas is not yet fully understood. In this study, we examined neural responses of the DLPFC, PPC, and M1 under challenging motor prediction conditions. We recorded LFPs from two rhesus macaques performing a five-radial target center-out reaching task with abrupt target changes. Both DLPFC and M1 showed significant responses to abrupt target changes, with M1 responding later than DLPFC. In contrast, PPC represented movement direction without significant differences between conditions. Causality between these regions was significantly higher in the condition with abrupt target changes compared to no changes. Based on our results, it is proposed that frontoparietal networks might execute organized neural processes to correct unpredictable motion errors.

Disclosures: M. Kim: None. **J. Sohn:** None.

Poster

PSTR287: Dexterous Motor Control

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR287.19/H38

Topic: E.04. Voluntary Movements

Support: NIH Grant R21AR081636

Title: Manipulation and grasp forces are differentially sensitive to predictable and unpredictable changes in object properties

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Abstract: The human hand’s impressive dexterity allows for interaction with the environment through complex sensorimotor interactions, such as the manipulation of objects through sensory feedback and elaborate coordination of finger forces. However, the coordination of digit forces to simultaneously prevent object slip and control object position and orientation (pose) remains unknown.
We have applied manipulation and grasp force decomposition (MGFD), a force analysis tool developed for robotic applications, to identify sensorimotor mechanisms underlying the control

of dexterous manipulation. MGFD decomposes digit forces into Grasp Force (FG), the force required to prevent object slip, and Manipulation Force (FM), the force required to control object pose. We have found that slip prevention and manipulation rely on feedforward and feedback mechanisms, respectively. Nevertheless, how object properties influence the modulation of FG, FM, and their coordination remains to be investigated.
We recruited 20 participants to perform a dexterous task that requires simultaneous object slip prevention and pose control with two-digit precision grasping. We instructed participants to reach and grasp an inverted-T shape object using the thumb and index fingertip, lift the object vertically while preventing it from tilting, hold the object, and set the object back down. We systematically changed the object's mass or moment of inertia to vary the requirements for object slip prevention and pose control, respectively. We also addressed the effect of predictability of object property by using either blocked experimental conditions, consisting of consecutive trials with the same object property, or employing a pseudorandomized design across trials.
We found that FG increased in the heavier mass and decreased for higher moment of inertia conditions. However, FM was modulated selectively to object pose control requirements. Furthermore, FG was controlled in an anticipatory fashion at object lift onset, whereas FM was modulated following acquisition of somatosensory and visual feedback of object's dynamics throughout object lift. These findings were found regardless of object property predictability. Together, the present results point to differential sensitivity of FG and FM to task requirements while providing novel insights into how the central nervous system controls digit forces to attain two functionally distinct goals of dexterous object manipulation.

Disclosures: W.P. Noll: None. **Y. Wu:** None. **M. Santello:** None.

Poster

PSTR287: Dexterous Motor Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR287.20/H39

Topic: E.04. Voluntary Movements

Support: NIH R00 NS124748

Title: A paradigm to study the role of tactile contact events in learning and execution of object manipulation behavior

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Abstract: In everyday life, using and handling objects requires learning sequences of coordinated grip, transport, and manipulation movements. These movements are punctuated by multi-modal sensory events termed “contact events”. Theoretical work proposes that contact events serve as intermediate sub-goals that indicate submovement completion and serve as a starting point for control of the subsequent submovement (Flanagan, 2006). Humans are incredibly deft at learning and executing different sequences of object manipulation movements, as long as their somatosensory system is intact. With disruptions to somatosensation, learning of new manipulation skills (Palvides, 1993, Sakamoto, 1989), statistics of exploratory manipulation movements (Binkofski, 2001), and rapid adaptation of movement to new environmental forces (Mathis, 2017) are disrupted. Together, this suggests that somatosensory feedback generally, and perhaps the somatosensory component of contact events specifically, are critical for learning and executing manipulation skills. In this study, we develop a paradigm to study how contact events contribute to shaping the learning and execution of object manipulation movements. Human participants are presented with a sensorized object to manipulate in a two-dimensional plane, through an unseen maze, to a final rewarded location. Participants must rely on contact events to navigate through the maze. We study the dynamics of first, how contact events become more predictable with practice, and second, how object movement aligned to predicted contact events is refined with extended practice of a single maze. Finally, after participants have learned the maze configuration, we study how object movement is adjusted when contact events are relocated by adjusting the maze boundaries. This test identifies whether and how contact events are still relied upon after task learning. Overall, our paradigm enables the study of how tactile contact events are used to influence control of movement during the learning and execution of object manipulation behaviors. Future work will leverage this paradigm to study the somatosensory-motor cortex neurophysiology underlying the updating of movement following contact events.

Disclosures: W. Kim: None. **S. Chaudhary:** None. **T. Oppenheim:** None. **P. Khanna:** None.

Poster

PSTR287: Dexterous Motor Control

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Program #/Poster #: PSTR287.21/H40

Topic: E.04. Voluntary Movements

Support: NIH 1-R01-NS096083

Title: Reaching vigor tracks learned prediction error

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Abstract: Previous inquiry has shown that individuals move faster towards goals or targets associated with greater reward. A potential explanation for how this may come to be lies in the neurotransmitter dopamine (DA), which is implicated in both the representation of value as well as the control of movement vigor. If vigor is indeed a reflection of value, then vigor should track the learning of value as well. And if dopamine is indeed the bridge between value and movement vigor, then vigor should track phasic dopamine release coincident with learning and reward prediction error. In this study, we sought to investigate whether human kinematic response to probabilistic rewards would mirror the characteristic reward prediction error response by mesencephalic dopaminergic neurons (DAN) as well as tonic DA response correlated with reward history on a sub-second timescale. To test these hypotheses, we performed two experiments, each of which involved human subjects performing (n=42; n=22 respectively) out-and-back reaching movements to a probabilistically rewarding target (p(Reward) = 0, 0.33, 0.66, or 1). These two experiments differed in that in one, reward frequencies were explicitly stated, and in the other, they were left unknown and to be learned by the participants. Furthermore, individuals in the second experiment were incentivized to learn the relative reward frequencies due to the inclusion of choice trials and potential monetary bonus contingent on performance. In the explicit environment, movement vigor (characterized by outgoing peak velocity and relative return peak velocity) increased with both reward expectation and the prediction error (R - E[R]). In the second experiment, where reward frequencies were initially unknown, vigor, in both the outgoing and return portions of the arm reach, was better characterized by a trial-to-trial learned utility estimate, modeled with a Bayesian hierarchical delta-rule, that integrated both rewards and biomechanical efforts. In both experiments, outgoing reach vigor increased with greater amounts of reward received in recent history, modeled as a leaky integration over several trials. In conclusion, we demonstrate that reach vigor tracks canonical variables of learning and motivation across time scales ranging from milliseconds to minutes. These results point to the potential neural mechanisms by which dopamine can explain and serve as the bridge between decision making and movement control.

Disclosures: C. Korbisch: None. **A.A. Ahmed:** None.

Poster

PSTR287: Dexterous Motor Control

Location: MCP Hall A

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Program #/Poster #: PSTR287.22/I1

Topic: E.04. Voluntary Movements

Support: Whitehall Foundation
NSF Grant 2145412

Title: Leveraging adaptation and learning to optimize reward in neurofeedback tasks: insights from non-human primates and artificial neural networks

Authors: *R. OSUNA OROZCO, Y. ZHAO, H. M. STEALEY, H.-Y. LU, E. CONTRERAS-HERNANDEZ, S. R. SANTACRUZ;
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Abstract: Adaptation and learning have been observed to contribute to the acquisition of new motor skills and are used as strategies to cope with changing environments. However, it is hard to determine the relative contribution of each when executing goal-directed motor tasks. This study explores the dynamics of neural activity during a center-out reaching task with continuous visual feedback under the influence of rotational perturbations. Results for a brain-computer interface (BCI) task performed by two non-human primate (NHP) subjects are compared to simulations from reinforcement learning agents performing an analogous task. We characterized baseline activity and compared it to the activity after rotational perturbations of different magnitudes were introduced. We employed principal component analysis (PCA) to analyze the spiking activity driving the cursor in the NHP BCI task as well as the activation of the neural network of the reinforcement learning agents. Our analyses reveal that both for the NHPs and the reinforcement learning agent, the task-relevant neural manifold is isomorphic with the task. However, for the NHPs the manifold is largely preserved for all rotational perturbations explored, and adaptation of neural activity occurs within this manifold as rotations are compensated by reassignment of regions of the neural space in an angular pattern that cancels said rotations. In contrast, retraining a reinforcement learning agent to reach the targets after rotation results in modifications of the underlying neural manifold. Our findings demonstrate that NHPs adapt their existing neural dynamic repertoire in a quantitatively precise manner to account for perturbations of different magnitudes. They do so in a way that obviates the need for extensive learning.

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Poster

PSTR287: Dexterous Motor Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR287.23/Web Only

Topic: E.04. Voluntary Movements

Title: The Effects of Anodal tDCS over the Frontoparietal Cortex on Reaction Time and Intramanual Transfer learning

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Physiotherapy, Sch. of Primary and Allied Hlth. Care, Fac. of Medicine, Nursing and Hlth. Sciences, Monash University,, Melbourne, Australia

Abstract: Effects of anodal tDCS over the frontoparietal cortex on reaction timeand intramanual transfer Learning

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Reaction time is one of the most important sensory-motor behavioral outcomes that canbe improved through practice. A significant reduction in reaction time after training isassociated with neuroplasticity in different areas of the brain. Given the benefits of non-invasive brain stimulation approaches to identify the role of different regions of the brainin sensorimotor behaviors such as reaction time, we stimulated three different areas ofthe frontoparietal cortex in healthy participants to elucidate the most effective stimulationsites for enhancing reaction times during a sequential visual isometric pinch task (SVIPT)and transfer of learning to the untrained hand. This study was a parallel randomizedsingle-blind sham-controlled study. A total of 48 right-handed healthy individuals fromMonash University took part and were randomly assigned to one of the four a-tDCSgroups: 1) left primary motor cortex (M1), 2) left the dorsolateral prefrontal cortex(DLPFC), 3) left posterior parietal cortex (PPC), and 4) sham stimulation. Anodaltranscranial direct current stimulation (a-tDCS) (0.3 mA, 3 cm2, 20 min) was appliedconcurrently with SVIPT, in which the participants accurately controlled their forces toreach seven different target forces from 10 to 40% of maximum voluntary contraction(MVC) presenting on a computer monitor with the right dominant hand. The ratio ofreaction time was measured at baseline and 15 minutes after the intervention with eitherhand. Our results showed significant elongations between M1-Sham groups in the ratioof reaction time for the right trained hands for target forces of 15% and 30 % of MVC.This deleterious effect was also transferred into the left untrained hand. Our findingsrevealed that a-tDCS over M1 resulted in a significant increase in reaction time withinSVIPT at the first stage of learning, while DLPFC and PPC stimulation did not modifythis temporal variable. Our findings suggest that not only enhancement but also thereduction of behavioral outcomes with one hand can be transferred to the opposite hand.Further research is required to detect the optimum stimulation sites for tDCS stimulationto improve the temporal variables such as reaction time and intramanual transfer acrosshands within a force control task such as SVIPT.

Disclosures: F. Hashemirad: None. **M. Zoghi:** None. **S. Jaberzadeh:** None.

Poster

PSTR287: Dexterous Motor Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR287.24/I2

Topic: E.04. Voluntary Movements

Title: The influence of dual source premotor cortex of transcranial direct current stimulation on muscle fatigue in hand muscles

Authors: *R. YOUNG[1], V. CONTINI[1], E. CLINTON[1], E. W. WILKINS[1], J. A. PARK[1], J. MOURADIAN[1], Z. A. RILEY[2], B. J. POSTON[1];
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Abstract: Single source transcranial direct current stimulation (tDCS) has been shown to increase the time to task failure (TTF) of fatiguing contractions when applied to the dorsolateral prefrontal cortex (DLPFC) and to the primary motor cortex (M1). The primary purpose was to determine whether dual source tDCS delivered bilaterally over the premotor cortices (pmc-tDCS) could increase the TTF of a fatiguing contraction performed by hand muscles. A double-blind, randomized, SHAM-controlled, crossover design was used for this study. Thirteen young adults (8 males, 5 females) performed two experimental sessions held on separate days a week apart. The only difference between the two sessions was the type of stimulation (pmc-tDCS or SHAM; counterbalanced) applied concurrent with the fatiguing contraction. In each experiment, the fatiguing contraction was performed by gripping a manipulandum with the index finger and thumb. This was accomplished by using a precision grip and matching an isometric target equal to 15% of the maximum voluntary contraction (MVC) for as long as possible until task failure. The main findings were: 1) both the TTF and the percentage decline in MVC force did not significantly differ between the pmc-tDCS and SHAM conditions ($P = 0.104$ and $P = 0.985$, respectively); 2) the rates of increase in electromyographic (EMG) activity ($P = 0.253$), force error ($P = 0.532$), and standard deviation (SD) of force ($P = 0.792$) were not significantly different between the pre-tDCS and SHAM conditions; and 3) transfer of motor skill under fatigue was similar between the two stimulation conditions ($P = 0.622$). Collectively, these results suggest that pmc-tDCS does not decrease the rate of muscle fatigue during a sustained isometric contraction of the muscles of the hand.

Disclosures: R. Young: A. Employment/Salary (full or part-time):; UnitedHealthCare. **V. Contini:** None. **E. Clinton:** None. **E.W. Wilkins:** None. **J.A. Park:** None. **J. Mouradian:** None. **Z.A. Riley:** None. **B.J. Poston:** None.

Poster

PSTR287: Dexterous Motor Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR287.25/I3

Topic: E.04. Voluntary Movements

Title: The influence of bilateral transcranial direct current stimulation of primary motor cortex on muscle fatigue progression

Authors: *E. CLINTON[1], A. CHAUHAN[1], R. J. YOUNG[1], E. W. WILKINS[1], J. PAMARAN[1], S. D. KARAVADIA[1], Z. A. RILEY[2], B. POSTON[1];
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Abstract: Transcranial direct current stimulation (tDCS) applied unilaterally to the primary motor cortex (M1) can significantly prolong the time to task failure (TTF) of a fatiguing contraction. The primary purpose was to examine the influence of bilateral dual source tDCS (ds-tDCS) applied over the left and right M1s on the TTF of a precision grip task. This was accomplished through the utilization of a double-blind, randomized, SHAM-controlled, within-subjects design. Fourteen young adults (6 males, 8 females) completed two experiments (ds-tDCS and SHAM stimulation conditions) with a seven-day washout period between sessions. Each experiment involved the performance of a sustained isometric fatiguing contraction using a precision grip (index finger and thumb) of the right hand while either ds-tDCS or SHAM stimulation was applied to the left and right M1 by two separate stimulation devices. Participants were directed to match a target force equivalent to 15% of the maximum voluntary contraction (MVC) force for as long as possible (TTF). The main findings were that both the TTF (P = 0.570) and the percentage decline in MVC force (P = 0.456) were not significantly different between the ds-tDCS and SHAM stimulation conditions. In addition, the force error (P = 0.413), standard deviation (SD) of force (P = 0.356), and EMG activity (P = 0.998) were all not significantly different between the ds-tDCS and SHAM stimulation conditions. These findings suggest that ds-tDCS does not reduce the rate of progression of muscle fatigue in a sustained submaximal isometric contraction of hand muscles.

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Poster

PSTR287: Dexterous Motor Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR287.26/I4

Topic: E.04. Voluntary Movements

Title: Investigating the Impact of Cerebellar Transcranial Direct Current Stimulation on Bimanual Force Production: A Novel Approach to Enhance Motor Function

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Abstract: The cerebellum plays a crucial role in prehensile tasks, including object manipulation and bimanual coordination, making it a target for neuromodulatory interventions to improve motor function. While some studies show the potential positive behavioral results of cerebellar transcranial direct current stimulation (ctDCS), the inconsistent effects on motor performance have questioned its usefulness as a valuable neuromodulatory tool. The impact of ctDCS is frequently evaluated at the performance-level variables (i.e., performance error, reaction time) that do not capture possible alternative (compensatory) mechanisms at the level of motor elements (i.e., finger and hand forces.), neglecting valuable aspects of individualized traits of movement coordination. Thus, the possible source of inconclusive results of ctDCS may be the inadequate assessment and monitoring of motor effects.
Here, we evaluated the effects of ctDCS on dynamic stability and bimanual coordination in a bimanual force production task. Four young adults matched the target total force by cyclically pressing on force sensors with the index and middle fingers of two hands. We used the uncontrolled manifold (UCM) method that separates inter-cycle variance of hand forces into variance that affects (VORT) and does not affect (VUCM) the performance (i.e., total force). We quantified the dynamic stability and bimanual coordination by calculating the index of stability (ΔV) as a difference between VUCM and VORT, normalized by the total variance.Then, we applied bootstrapping analysis within each subject to explore the individualized effects of ctDCS. A group analysis revealed that ctDCS increased ΔV more than sham stimulation (0.49 ± 0.17 vs. 0.23 ± 0.07, for cTDCS and sham, respectively), but these changes were non-significant. The individual analysis, however, showed positive effects of ctDCS in 3 out of 4 subjects, with significant ΔV changes for two subjects (0.7 [95% CI: 0.11, 1.28] and 0.91 [95% CI: 0.34, 1.26]) and borderline significant ΔV change for the third subject (0.52 [95% CI: -0.08, 1.07]), suggesting individualized effects of ctDCS. This study paves the way for developing personalized assessments and targeted rehabilitation strategies to improve bimanual sensory-motor impairments and potentially advance our understanding of cerebellar function in dexterous movements.

Disclosures: S. Solnik: None. **D. PIscitelli:** None. **C. Cuadra:** None.

Poster

PSTR287: Dexterous Motor Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR287.27/I5

Topic: E.04. Voluntary Movements

Title: The influence of transcranial alternating current stimulation application on contralateral primary motor cortex excitability

Authors: *B. POSTON[1], E. W. WILKINS[2], R. DAVIDSON[2], R. KRIDER[2], G. ALHWAYEK[2], F. SHIHADY[2], K. YI[2], R. J. YOUNG[2];
[1]Univ. of Nevada, Las Vegas Neurosci. Grad. Program, Las Vegas, NV; [2]Univ. of Nevada Las Vegas, Las Vegas, NV

Abstract: Transcranial alternating current stimulation (tACS) delivered to the primary motor cortex (M1) has shown the ability to increase M1 excitability and improve motor performance in relatively simple tasks performed with the hand and arm system. However, the effects of tACS on the excitability of the M1 contralateral to which it is applied has not been determined. The purpose was to determine the effect of tACS application on contralateral M1 excitability. The study implemented a double-blind, randomized, sham-controlled, within-subjects, crossover experimental design. Eleven young adults performed two experimental sessions in counterbalanced order and separated by a one-week washout. Transcranial magnetic stimulation (TMS) was used to quantify cortical excitability of the contralateral M1 to which a single 20-minute application of tACS (current strength: 1 mA; frequency 70 Hz) was delivered. TMS testing was conducted in 5 blocks (Pre, T5, T10, T15, and Post) with the Pre and Post TMS blocks being performed immediately before and after tACS application. In contrast, the TMS blocks performed during tACS or SHAM stimulation were conducted at the 5, 10, and 15-minute time points. The primary dependent variable was MEP amplitude, which served as an index of excitability of M1. MEP amplitude was analyzed with a 2 *Condition* (tACS, SHAM) x 5 *Test* (Pre, T5, T10, T15, Post) within-subjects ANOVA. The main effect for *Condition* ($P = 0.613$) and *Condition* x *Test* interaction ($P = 0.085$) were not statistically significant. There was a significant main effect for *Test* ($P = 0.002$), however, post hoc analysis with Bonferroni adjustments revealed that none of the pairwise comparisons reached statistical significance ($Ps > 0.072$). These findings suggest that tACS does not significantly impact the excitability of the M1 opposite to which it is applied.

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Poster

PSTR288: Motor Control of Free, Imagined, and Augmented Actions

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR288.01/I6

Topic: E.05. Brain-Machine Interface

Title: Distinct yet overlapping neural spaces explain local field potentials activity in motor execution and imagination

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Abstract: Recent studies revealed that neural activity lies on low-dimensional manifolds defined by co-variation patterns. This finding offered insights into motor behaviors, highlighting the orthogonal relationship between preparatory and executive motor neural activities, identifying stable neural spaces across movements, and exploring interactions among executed, imagined, or observed movements. However, these results have primarily relied on multi-unit firing rates, often recorded in non-human primates. If similar conclusions about the structure of neural dynamics apply to the mesoscale level of local field potentials (LFP) in humans remains uncertain. Also, exploring this methodology for LFP would offer considerable translational value due to the increased availability of these signals in clinical contexts. Here, we sought to elucidate the link between motor execution and imagination in a participant with incomplete tetraplegia due to spinal cord injury implanted with the electrocorticography WIMAGINE [1] device as part of the clinical trial NCT02550522. This implant featured 32 recording channels placed over the primary motor and sensory cortices. Due to some retained motor skills, the participant could engage in imagery and execution trials of reaching and wrist extension movements. To capture the whole information contained within LFP signals, we concatenated the envelope of established frequency bands along the channels dimension. Similar to [2], we identified the neural subspaces using dimensionality reduction techniques and computational optimization. We were able to decompose the overall neural activity into three distinct orthogonal subspaces: one exclusive for motor execution, another solely for motor imagery, and finally, a shared subspace comprising co-variations accounting for aspects of both conditions. Moreover, we confirmed that the variance captured by these neural spaces was task-relevant by successfully classifying the two movements after projecting the original data onto the low-dimensional manifolds. Our findings provide evidence that motor execution and imagination exhibit overlapping neural patterns while also maintaining distinct processes. Furthermore, the presence of a neural manifold exclusive to imagination suggests the potential for decoding imagined activity by isolating it from ongoing execution processes. This could have significant implications, particularly for patients with residual motor activity, enabling them to utilize imagination to control external interfaces concurrently with ongoing motor activities.[1] Mestais et al., IEEE TNSRE, 2015[2] Dekleva et al., Nat Hum Behav, 2024

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Poster

PSTR288: Motor Control of Free, Imagined, and Augmented Actions

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR288.02/I7

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Title: Exploring neural correlates of motor imagery of biological and extra limbs for motor augmentation

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Abstract: Motor augmentation (MA), achieved through the integration of extra limbs, presents promising avenues for expanding human capabilities. One of the challenges lies in controlling new interfaces without hindering existing motor functions. As outlined in Dominijanni, 2021, this can be accomplished by tapping into the body's physiological redundancies, which create what is known as muscular, kinematic, and neural task null spaces. This study focuses on the latter, particularly investigating participants' capacity for imagining motor actions involving a third arm. Distinguishing between the motor imagery (MI) of a third arm and that of biological limbs could reveal similarities and differences in the neural patterns, paving the way for neural-driven control of external interfaces for MA in the future. Thirty participants underwent pre-conditioning, conditioning, and post-conditioning phases while performing motor imagery tasks of reaching movements visualized in a virtual environment. The extra arm was positioned on the chest to avoid laterality bias. For a selected group, we introduced tactile feedback during the conditioning phase. Electroencephalographic (EEG) activity was continuously recorded throughout the tasks, using a system with 61 channels with 10-10 electrodes placement. We estimated the event-related spectral perturbance maps to study the desynchronization signatures during the task execution. Moreover, we employed a decoding approach to see if we could distinguish the neural activity for each involved arm employing a Riemannian decoder, using a 5-fold cross-validation. This decoding strategy offers several advantages, including robustness to noise, minor preprocessing, and efficient utilization of high-dimensional data. EEG data analysis

revealed significant differences in event-related desynchronization (ERD) in β and α rhythms depending on the imaged limb (biological vs. extra). The decoder trained to classify left, right, and extra arm MI achieved a balanced accuracy exceeding 0.6 on average across participants. No significant effect of tactile feedback on neural activity was observed, likely related to the simplicity of the task. The findings of this study showcase the brain's ability to produce different MI patterns between virtual extra limbs and biological counterparts, as evidenced by distinct neural signatures in ERD. This study also represents a step towards the exploitation of a neural null space, which is less investigated than its muscular and kinematic counterparts.

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Poster

PSTR288: Motor Control of Free, Imagined, and Augmented Actions

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Title: Movement initiation and termination in a limb motor imagery task

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Abstract: Brain-computer interfaces (BCIs), as rehabilitative devices, help restore communication for individuals with severe speech and motor impairments such as those with locked-in syndrome from neurological disorders and neurodegenerative diseases such as amyotrophic lateral syndrome (ALS). To be successful, motor-based BCIs must accurately decode motor imagery content (e.g., which limb was imagined) and motor initiation (e.g., when was the limb imagined). In our previous study, we evaluated a speech output BCI controlled using motor imagery, i.e., the motor imagery content. In this current study, we focus on motor initiation by examining additional time frequency components of EEG signals collected in our prior limb-motor imagery brain-computer interface (BCI) paradigm to identify new features useful for predicting and decoding onset and termination of imagined movement. The results of our current study found increases in theta-band amplitude (3-7 Hz) across all participants (N=18) at the beginning and ending of motor imagery periods (approximately 500 ms after the onset cue and 500 ms after the termination cue). We then tested the utility of these features in BCI decoding paradigm by designing a Kalman Filter to predict movement initiation and termination. The Kalman filter decoder was trained on theta-band power in a calibration data dataset without any feedback to the participant, then tested on novel experimental EEG data recorded from each participant while they received feedback of the speech decoder. Initial results show the Kalman

filter was able to predict onset and termination from unseen data that correlates with increases in theta-band amplitude. Our findings, when combined with our prior formant frequency decoding, will lead to improvements in overall BCI performance by focusing the decoder only on time intervals during which participants are actively engaged in the BCI task.

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Poster

PSTR288: Motor Control of Free, Imagined, and Augmented Actions

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Title: Compositional encoding of hand-eye coordinated movements in Primary Motor and Posterior Parietal Cortices of quadriplegic humans

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Abstract: Many complex motor behaviors require extensive interplay between the brain's sensory and motor control systems. For instance, visuomotor behaviors such as driving a car or catching a ball require the performance of precise hand movements in simultaneity with rapid gaze shifts necessary for sensory feedback. Quadriplegic humans, with intact visual sensory systems, can have their visuomotor pathway restored with neuro-prosthetics that can actuate their attempted movements. However, the complicated neural encoding of simultaneous hand and eye movements potentially limits the effectiveness of neural decoders and thus the efficacy of neuro-prosthetics during complex visuomotor behaviors. Here we report new insight into the neural encoding of hand-eye coordinated movements in two human participants as part of a brain-machine interface (BMI) clinical trial seeking to use intracranially implanted Neuroport arrays to restore motor control to patients with quadriplegia. Participant 1 has two implanted arrays in the hand knob of the Primary Motor Cortex (M1), one implanted array in superior parietal lobule (SPL) of the Posterior Parietal Cortex (PPC), and one in the supramarginal gyrus (SMG) of the

inferior parietal lobule of PPC. When participant 1 performs congruent simultaneous hand and eye movements, we find that approximately 70% of individual neurons recorded in M1 and PPC, exhibit a tuning curve that is a linear combination of the tuning curves for the hand and eye movements performed separately. This result is verified in participant 2 who has two implanted Neuroport arrays, one in the hand knob of the (M1) and another in SPL of PPC. In participant 2 single neuron activity has degraded but local population activity features also demonstrate compositional tuning curves during congruent movements. However, in both participants, during simultaneous but incongruent hand and eye movements we find that significantly more single neurons and local neuron population signals exhibit nonlinear compositionality. This increase in nonlinear compositionality in both single neurons and local neuron population signals during incongruent movements provides evidence that more sophisticated algorithms will be required to decode complex visuomotor behaviors. However, for neurons that are linearly composable we propose a mathematical framework for how future decoders can disentangle component hand and eye movements during coordinated behaviors.

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Poster

PSTR288: Motor Control of Free, Imagined, and Augmented Actions

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Program #/Poster #: PSTR288.05/I10

Topic: E.05. Brain-Machine Interface

Title: Online myoelectric gesture recognition in patients with tetraplegia during attempted movements

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Abstract: Impairment of motor functions due to neurological diseases such as spinal cord injury (SCI) can significantly affect a person's quality of life and make even basic daily activities challenging. Body-machine interface (BoMI) technology offers a promising solution for individuals with motor impairments to control assistive or rehabilitation devices [Muceli et al., Handbook of Neuroengineering, 2021]. One promising BoMI technique that has demonstrated to be effective is myoelectric pattern recognition based on residual electromyographic (EMG) activity. This approach can be used both in cases of incomplete SCIs [Lu et al., Journal of Neural Engineering, 2019], as well as in the so-called discomplete SCIs, where despite the absence of

observable movement, low-level EMG signals are present and can be used for decoding [Ting et al., Journal of Neurophysiology, 2021]. To enable the practical use of devices with myoelectric pattern recognition control in SCI patients, there is a need to validate the efficacy of this approach in real-time. To address this challenge, we conducted a study involving five participants with incomplete tetraplegia due to SCIs at levels C4-C6. During the study, participants engaged in a virtual grasping task based on decoding from the surface EMG signals of their partially paralyzed forearm muscles. Specifically, we designed a virtual reality protocol in which users controlled a virtual hand in real-time with the goal of executing five distinct types of grasping movements while interacting with various virtual objects. We included movement types that are commonly used in daily life to emulate the actions that are most relevant to restore in paralyzed patients. Before starting the online task, each participant performed several repetitions of attempted movements, which we used to train a deep-learning model for grasp classification. Following this calibration phase, participants executed the online decoding task: based on a displayed instruction, they attempted the movement; if the indicated grasp pattern was successfully decoded, the virtual hand performed the movement accordingly. We achieved decoding accuracy above 70% for all types of grasps in all study participants despite different impairment levels. Our results demonstrate successful online decoding of different grasp patterns in individuals with incomplete SCI using a non-invasive EMG interface. These findings highlight the potential utility of non-invasive BoMI interfaces for controlling assistive or rehabilitation devices, facilitating potential daily use by SCI patients.

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Poster

PSTR288: Motor Control of Free, Imagined, and Augmented Actions

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Title: Neural representation of grasps and objects in the posterior parietal cortex and motor cortex of a tetraplegic human

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Abstract: The act of grasping requires creating a goal, planning hand geometry, and executing the movement. To accomplish these tasks, we rely on the sensorimotor processing capabilities of the brain. The posterior parietal cortex (PPC) is involved in early stages of movement planning, with specific sub-regions playing crucial roles in grasp and object processing. The cortical grasping network (CGN) includes the supramarginal gyrus (SMG) of the PPC and the primary motor cortex (M1). Here, we use human electrophysiology to reveal how grasps and objects are encoded in SMG and M1. A tetraplegic participant was implanted with microelectrode arrays in M1 and SMG. Single-unit neural activity was recorded during a motor imagery task, where the participant was shown one of three sets of images: hand shape for four different grasps (Hand only, H), a hand grasping one of four objects (Hand+Object, HO), or each object alone (Object only, O). For each set, the participant is asked to imagine performing the displayed grasp/interacting with that object. We observed SMG neurons encoding grasp and object information, separately and in combination throughout the trial. We see consistent, high classification accuracy of the grasp/object type in SMG, with the HO condition yielding the highest classification accuracy (84%, chance level = 25%), followed by H (70%), then O (54%). This pattern persists when investigating the percentage of total units tuned to each condition, with the highest tuning for HO (49%), then H (36%), then O (22%). We also see overlap among the tuned units, such that ~20% of units are tuned for all 3 conditions. Taken together, these analyses suggest that SMG encodes information about grasp/objects and exhibits partial mixed selectivity encoding. Unexpectedly, neurons in M1 behaved similarly to those observed in SMG. Neurons encoded grasp and object information prior to execution of the imagined motor action, and during execution. We see high percentages of tuning for each condition (HO: 70%; H: 62%; O: 44%), along with high classification accuracies (HO: 89%; H: 84%; O: 55%; chance = 25%), similar to SMG. Prior work in our lab found that when M1 is not engaged in an ongoing action that preparatory activity related to other inputs (such as planned next actions) are present. These results suggest that M1 activity may be context dependent. In conclusion, this work shows that both M1 and SMG contain activity for grasp/objects. Further research will be required to determine if the grasp/object M1 activity is context dependent.

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Poster

PSTR288: Motor Control of Free, Imagined, and Augmented Actions

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Topic: E.05. Brain-Machine Interface

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Ralph W. and Grace M. Showalter Research Trust

Title: Decoding multi-limb trajectories of naturalistic running from calcium imaging using deep learning

Authors: *S. PARK[1], M. LIPTON[2], M. C. DADARLAT[2];
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Abstract: Decoding neural activity into behaviorally-relevant variables such as speech or movement is an essential step in the development of brain-machine interfaces (BMIs) and can be used to clarify the role of distinct brain areas in relation to behavior. Two-photon (2p) calcium imaging provides access to thousands of neurons with single-cell resolution in genetically-defined populations and therefore is a promising tool for next-generation optical BMIs. However, decoding 2p calcium imaging recordings into behavioral variables for use in real-time applications has traditionally been challenging due to the low sampling rate of the signal as well as the indirect and non-linear relationship between the underlying neural activity and the slow fluorescent signal. Here, we show an approach using deep learning to decode the naturalistic multi-limb trajectories of running mice from neural recordings made with 2p calcium imaging over the sensorimotor cortex in a single hemisphere. The work demonstrates the feasibility of using deep learning methods to identify and characterize populations of neurons that encode behaviorally-relevant variables. This approach will be critical in the future implementation of neural decoding for next-generation optical BMIs that will improve the lives of patients suffering from neurological injury and disease.

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Poster

PSTR288: Motor Control of Free, Imagined, and Augmented Actions

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Program #/Poster #: PSTR288.08/I13

Topic: E.05. Brain-Machine Interface

Title: Predicting spontaneous behaviors in freely moving monkeys from intracortical recordings

Authors: *E. RONDONI[1], F. LANZARINI[2,3], M. MARANESI[2], D. ALBERTINI[2], L. BONINI[2], A. MAZZONI[1];
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Abstract: Decoding brain signals associated with motor intention and action planning is a key challenge for prosthetics, assistive and rehabilitative technologies. Subjects with motor impairments due to neurological conditions, wherein neural motor control at cortical level is preserved (e.g., in spinal cord injury), benefit from such technologies. However, decoding from neural activity unconstrained and spontaneous movements remains an important challenge to be addressed. Moreover, a decoder endowed with predictive capabilities is essential for enabling efficient prosthetic control. This work aims to design a decoding algorithm that uses preparatory neural activity for the prediction of a variety of movements involving the mouth, the forelimbs and axial body parts in unconstrained and close-to-natural settings. The proposed decoding algorithm was tested on two monkeys (Macaca mulatta), whose neuronal activity was wirelessly recorded from the motor and premotor cortical areas using floating multielectrode arrays. During the recordings, the monkeys were freely behaving in a 2x2x1.8m^3 plexiglass room filled with enrichment items. Single- and multi-units' spikes, occurring immediately prior to the events, were extracted with an automatic sorting algorithm and binned to obtain a spike count array for each unit. Principal component analysis was performed for dimensionality reduction, and the resulting predictors were used to train and validate a support vector machine classifier. Finally, the spike count algorithm was temporally moved backward with respect to the events. The classification accuracy for imminent events ranges from 50% to 80%, with a number of classes per session that varies from 6 to 10 (respectively ~16.67% and 10% theoretical chance level). In all sessions, the prediction accuracies are significantly higher than chance level. Nonetheless, depending on the desired application, higher accuracies can be easily reached by grouping together, for the same type of behavior, the different laterality of execution (right/left). Additionally, by moving the decoding algorithm further back in time, we observe the steepest slope in the accuracy profile including neuronal activity around 200-600ms before the event of alignment, suggesting that this time interval holds great potential for predicting upcoming behavioral events. This study serves as a first step toward the definition of a simple yet effective decoding algorithm for predicting spontaneous movements from the premotor cortex. Future steps include testing of a similar classifier for real-time trials, and direct comparisons with unnatural and controlled clinical settings.

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Poster

PSTR288: Motor Control of Free, Imagined, and Augmented Actions

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Program #/Poster #: PSTR288.09/I14

Topic: E.05. Brain-Machine Interface

Title: Contribution of cortical and striatal circuits to motor control throughout naturalistic movement

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Abstract: Naturalistic behaviors involve many movement components, from precise targeted reaches to innate motor sequences such as grooming. One challenge the nervous system faces is the need to accurately carry out each of these movements while also being able to flexibly switch between them. Motor cortex and striatum have previously been implicated in the execution and selection of motor actions, but how their activity dynamics are organized across naturalistic movement is still poorly understood. Previous studies have focused on interrogating the function of these regions using either highly constrained, individual movements, or sampling a narrowly circumscribed set of movements in a simplistic open field paradigm. Whether these regions engage specific subpopulations or specific modes of coordinated activity across a wide range of behaviors, and how these activity patterns are related to muscle activity throughout the full behavioral space is unknown. To address this, we developed a novel paradigm that allows for the investigation of neural dynamics across behaviors that require agility and dexterity, such as climbing and walking across an irregular grid, and innate behaviors such as eating and grooming. We used UMAP projections of muscle activity to parcellate the behavioral space into distinct states. In order to interrogate the organization of neural dynamics across these behavioral states, we chronically implanted neuropixels probes to simultaneously record hundreds of neurons in the caudal forelimb area of the motor cortex and the striatum. We identified striatal and cortical neurons whose activity ranged from highly selective for individual behaviors to uniform across all behaviors, and found greater behavioral specificity within striatum compared to cortex on an individual neuron level. Conversely, using canonical correlation analysis, neural decoding, and EMG triggered averaging, we found greater encoding of muscle activity within cortex compared to striatum across all behaviors. On a population level, we discovered both distinct neural subspaces corresponding to separate sets of behaviors as well as highly overlapping subspaces among more similar behaviors in both regions. We propose a model whereby striatal neurons encoding action state inform separate pattern generating circuits, including the motor cortex, to transition between different dynamical regimes throughout naturalistic behavior.

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Poster

PSTR288: Motor Control of Free, Imagined, and Augmented Actions

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Topic: E.05. Brain-Machine Interface

Support: R21 NS135413-01

Title: Using adversarial networks to align neural populations during unconstrained motor behaviors

Authors: *F. RIZZOGLIO, X. MA, A. KENNEDY, L. E. MILLER;
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Abstract: Intracortical brain-computer interfaces (iBCIs) are increasingly being used to restore voluntary movement to paralyzed persons. The brain-to-behavior mapping (i.e., the decoder) is typically trained while the user observes and attempts to imitate a kinematic trajectory. However, these decoders require repeated calibrations over time due to neural recording instabilities. Furthermore, this "observation-based" decoding is not feasible for iBCIs meant to directly control force or muscle activity. Our recent work has centered on creating a stable iBCI decoder that can be calibrated using data from a user (e.g., a monkey), collected on a given day, and then used effectively over extended periods of time and even applied to data from another user. Key to our approach is the observation that the neural activity during movement can be reduced to a small number of "latent" signals, lying in a low-dimensional manifold within the neural state space. We have previously used Canonical Correlation Analysis (CCA) to align these latent signals over time. However, CCA requires the behaviors themselves to be first time-aligned, thereby limiting its applicability to stereotypic behaviors. To address this challenge, we have recently employed Generative Adversarial Networks (GANs) to align just the static point clouds of neural recordings without any knowledge of the latent signals. We have successfully applied one such GAN, (cycle-GAN) to typical stereotyped tasks, and achieved better alignment than with CCA. However, cycle-GAN did not perform well when aligning data from more natural movements: food retrieval, grooming, and locomotion in the monkey's home cage. Even worse, cycle-GAN failed to align data across different subjects, something CCA did accurately. We hypothesized that these failures occurred because cycle-GAN, by design, neglects the temporal dynamics of the neural firing rates, a feature fundamental to CCA. Here, we attempted to improve cycle-GAN performance by adding limited information about neural dynamics (e.g., a derivative) without imposing the need for complete time alignment. We also replaced the original feedforward architecture with recurrent neural networks. Unfortunately, none of the modifications significantly improved performance for alignment across time in laboratory settings, and only modestly improved the performance for unconstrained behaviors and across subjects. Our findings suggest that we may have reached a performance ceiling for alignment of neural data with GANs. We are now investigating alternative methods, including diffusion models, which have been shown to outperform GANs in computer vision applications.

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Poster

PSTR288: Motor Control of Free, Imagined, and Augmented Actions

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Program #/Poster #: PSTR288.11/I16

Topic: E.05. Brain-Machine Interface

Support: C14/18/100

Title: A brain-computer interface for online navigation in a virtual 3D environment based on local field potential activity of macaque motor and premotor cortex

Authors: *S. DE SCHRIJVER, O. SAUSSUS, P. JANSSEN;
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Abstract: We recently showed that macaque monkeys can use a brain-computer interface (BCI) based on spiking activity to navigate in a virtual reality (VR) 3D environment, moving a sphere in three dimensions towards a target while avoiding obstacles. However, it is known that the yield of chronically implanted multi-electrode arrays decreases over time which reduces the ability to control a BCI based on spiking activity. To address this, we investigated the potential of local field potential (LFP) activity to control of a BCI for navigation in VR in two macaques. Both monkeys were implanted with three 96-channel Utah arrays in primary motor cortex, dorsal premotor area F2, and ventral premotor area F5c. Liquid crystal shutter glasses and a 3D screen on which pairs of left- and right eye images alternated allowed the simulation of a virtual 3D environment with binocular disparity. In the game development platform Unity, we designed two navigation tasks in which a sphere moved towards one of five equidistant targets. In the Center-out task, the sphere moved on a plane from a predefined starting point, while in the Continuous navigation task the camera view rotated and moved along with the sphere. In each experiment, the monkey first had to passively observe the task in which the sphere movements were driven by Unity. Secondly, the log power of the 100-200Hz component of the LFP (high-gamma) activity combined with the velocities of the sphere during the task were used to train a decoder based on the Preferential Subspace Identification (PSID) model. Third, every 50ms the model made online predictions about the velocities of the sphere based on the high-gamma LFP activity of the monkey controlling the movement of the sphere. In the third phase of the Continuous navigation task, we replaced the sphere by a monkey avatar and the plane by a forest environment to simulate a more realistic environment. Offline analysis of the latent variables trajectories indicated that accurate decoding with the LFP activity could be possible in both tasks. The latent trajectory for each direction of movement is composed of the values of the three latent variables that explain the most variance in the data. At each time point in the trial, this value was averaged over all trials of the training phase. In five online pilot sessions using the Continuous navigation task, a decoder trained with LFP activity was able to perform above the estimated chance level (equal to 28%) with an average success rate of 63%. Thus, a decoder trained with high-gamma LFP activity from motor and premotor cortex allows accurate online control of a sphere in VR, which has important implications for the clinical application of BMIs for a long period of time.

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Program #/Poster #: PSTR288.12/I17

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T&C Chen BMI Center
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Title: Reference frame transformations in human PPC during navigation

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Abstract: Navigation is a complex behavior that requires the transformation of spatial information from an allocentric map to an egocentric motor plan and execution. The hippocampus and surrounding areas have been associated with the formation of allocentric maps. However, it is still unclear how allocentric and egocentric information interact in the brain during navigation. The posterior parietal cortex (PPC) is in a unique position to integrate signals of complex behaviors because it is considered part of the dorsal visual pathway and has been implicated in spatial information processing, control of movements, and decision-making. Furthermore, lesions in PPC have been associated with alterations in the transformation of spatial information from allocentric to egocentric reference frames. To study the role of PPC and motor cortex (MC) during a goal-directed navigation task, we recorded single-neuron activity from four NeuroPort Arrays implanted in the cortex of one human tetraplegic participant involved in a brain-machine interface clinical trial. Two of the arrays were in motor cortex, close to the hand knob, and two were in the PPC, in the superior parietal lobule (SPL) and supramarginal gyrus (SMG). The participant performed a navigation task that started with a bird's-eye view of a map of a maze showing an initial location and orientation, and a destination. The participant had five seconds to plan his trajectory. This phase was followed by a navigation phase, in which the participant had to use attempted finger movements to navigate a virtual environment in a first-person view to go from the initial position to the target location previously indicated. Using linear discriminant analysis (LDA) based classifiers on the firing rates of the neural populations of each array, we found that SPL neurons encoded the transformation of spatial information from an allocentric reference frame into an egocentric plan during the map phase. Specifically, SPL neurons encoded both the initial and final positions early during the map phase and the actual

turns required to navigate between them later in this phase. In contrast, SMG and MC neurons showed cross-validated accuracies close to the chance level of the initial and final positions and the planned trajectory across the map phase. Furthermore, all recorded brain areas were involved in egocentric motor execution during the first-person navigation phase. These results suggest that SPL neural populations have access to allocentric information and a role in the transformation of spatial information from allocentric to egocentric motor plans during navigation planning.

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Poster

PSTR288: Motor Control of Free, Imagined, and Augmented Actions

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Topic: E.05. Brain-Machine Interface

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Title: Functional ultrasound-based decoding of action plan decision variables from posterior parietal cortex

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Abstract: Brain-machine interfaces (BMIs) can radically improve the quality of life of people living with chronic paralysis by restoring their motor function. BMIs have typically used invasive electrical recordings of brain activity. Recently, we have begun investigating a new technology for BMIs based on functional ultrasound imaging (fUS). fUS indirectly images neural activity by capturing hemodynamic signals at a high spatiotemporal resolution (100µm; 100ms). It is minimally invasive because the probe sits on the dura outside of the brain, which may confer greater long-term stability and higher safety for chronic use. Additionally, in contrast to electrode recordings that only offer a local signal around each contact point, fUS imaging offers a wide and deep field of view and is a promising method for studying the distribution of neural activity across networks at the mesoscopic scale.In a recent study, our group demonstrated online decoding of direction in a center-out task through fUS imaging of posterior parietal cortex (PPC) in a macaque model. This work established the feasibility of a fUS-based motor BMI. In this study, we work towards increasingly unconstrained motor control by investigating additional aspects of motor plans in a saccade-to-target task. We found that movement direction, amplitude,

and origin could be decoded from the same fUS imaging plane. Additionally, we could decode updated target locations when planned saccades changed. This suggests that the fUS signal in PPC can serve as the basis for continuous control of a motor BMI. While most BMI research focuses on motor restoration, there is mounting interest in developing BMIs to aid patients afflicted with neuropsychiatric disorders (for instance anhedonia, the inability to experience pleasure). Unlike motor applications, these BMIs would monitor neural activity for information related to mood and cognitive state. Numerous electrophysiological experiments have shown that PPC activity contains value- and motivation-related activity. As a model of the cognitive state of motivation, we modified our task to include variable target desirability. Some targets would yield higher juice rewards than others, increasing the target value and the motivation of the animal on a trial-by-trial basis. We were able to decode this value variable and the aforementioned spatial variables simultaneously, and could even use decoded value to determine in real-time which of two targets an animal would select. This demonstration of cognitive information decoding with fUS imaging suggests that the range of fUS-BMI applications may be broadened to include the treatment of debilitating neuropsychiatric disorders.

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Poster

PSTR288: Motor Control of Free, Imagined, and Augmented Actions

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Title: Classifying at-home movement states with cortical-pallidal neural activity in Parkinson's disease

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Abstract: A proposed improvement to deep brain stimulation (DBS) therapy to treat advanced gait disorders in Parkinson’s disease (PD) is to modify stimulation from conventional to ‘gait-optimized’ lower frequency settings when patients walk. However, implementation of this adaptive DBS (aDBS) architecture is hindered by the lack of neural biomarkers of movement states in naturalistic environments. This project aims to classify walking vs. non-walking states using chronic at-home recordings from implanted electrodes in the pallidum and motor cortical

areas in human PD subjects. Local field potentials (LFPs) from the globus pallidus (GP) and electrocorticography (ECoG) from the premotor (PM) and primary motor (M1) cortices were recorded from two human subjects with PD (subject 1: 61M, subject 2: 66F) implanted unilaterally with a novel bi-directional neurostimulator (Summit RC+S, Medtronic). Wearable ankle accelerometers (Rover Health) were used to track at-home movement; accelerometry from Rover and RC+S devices was used to synchronize neural and kinematic signals. Spectral analysis of 10-second epochs of continuous walking and non-walking was performed. Linear discriminant analysis (LDA) models were trained to classify movement states using average power within all possible frequency bands from 1 to 50 Hz during each epoch. The most important frequency bands determined by random forests (RF) were used to create 'system-constrained' LDA models meeting the specifications of the RC+S on-board classifier. Over 18 hours of data were analyzed (subject 1: 10.75 hours, subject 2: 7.7 hours). Spectral profiles of movement state differed between the two subjects. While one exhibited broadband increases in GP and PM power across multiple frequency ranges during walking periods, the second subject displayed more focused decreases in M1 beta band (13-30 Hz) power during walking. LDA models achieved areas under the curve (AUC) greater than 0.80 in both subjects when trained with all frequency bands from 1 to 50 Hz. 'System-constrained' classifiers performed with AUC greater than 0.70 for both individuals. These results support the hypothesis that oscillations from the motor cortex and basal ganglia are modulated by movement state. Our discoveries offer a pipeline for identifying patient-specific movement state biomarkers using long-term naturalistic recordings of neural and kinematic activity. These neural signatures will enable aDBS algorithms to switch from conventional clinical stimulation during non-walking to 'gait-optimized' parameters during walking to address both appendicular and gait-related symptoms of PD.

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Poster

PSTR288: Motor Control of Free, Imagined, and Augmented Actions

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T&C Chen BMI Center
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Title: Semantic processing in human PPC of stimuli related to subjects, verbs, objects and sentences

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Abstract: A fundamental element of language is semantic processing, which involves the perceptual representation of sensory stimuli as words, combinations of words, and sentences. Functional imaging studies have suggested that several brain areas across the human cortex are involved in the processing of semantic representations. Moreover, using different paradigms, some authors have found activation of regions in the posterior parietal cortex related to the semantic categories of actions and objects. However, it is still largely unknown how different semantic categories are encoded by populations of single neurons in the human brain. To study this, we recorded single-unit activity from the posterior parietal cortex (PPC) and motor cortex (MC) of one tetraplegic participant implanted with four NeuroPort Arrays as part of a brain-machine interface clinical trial. The participant had two arrays implanted in PPC, in the supramarginal gyrus (SMG) and the superior parietal lobule (SPL), and two arrays implanted in MC, located close to the hand knob. In this study, the participant was asked to look at a picture or listen to a brief description of a stimulus then after a go signal say out loud a word or sentence describing the stimulus. Some of the stimuli were representations of single subjects, actions, or objects, while others included complex representations of a subject performing an action on an object. Using linear discriminant analysis on the neural population activity of each area, we found a differential representation between subjects, objects, and action categories in SMG and SPL, while MC did not show any activity related to semantic processing. Additionally, both PPC areas exhibited a common neural substrate for visual and auditory sensory modalities of semantic representations. Finally, complete sentences formed by a subject followed by a verb and direct object had a compositional neural code comprised of the linear summation of the individual sentence elements. Our results suggest that SMG and SPL form part of a semantic processing hub, that represents compositional semantic information of sentences formed by actions, objects, and subjects in a shared neural substrate independent of the original stimulus modality.

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Poster

PSTR288: Motor Control of Free, Imagined, and Augmented Actions

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Topic: E.05. Brain-Machine Interface

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Title: Common practice in EEG emotion recognition feature selection causes overestimates of performance

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Abstract: There is growing interest in using electroencephalogram (EEG) for emotion recognition. A critical step in this process is feature reduction, which aims to identify relevant features while discarding unneeded information. However, if feature selection is applied before cross validation, as is common in the literature, the result is overestimated accuracy. We conducted experiments using two datasets: the DEAP [1] and a dataset collected in our own laboratory. For our data, we used 3 types of stimuli (pictures, facial images, and music) collected from published sources (references on poster). Thirty participants rated 240 stimuli on a 5-point Likert scale on three emotional axes (valence, arousal, and dominance). We used magnitude squared coherence estimation for feature extraction, followed by t-tests for feature selection, and an artificial neural network for classification. Binary classification was performed for each emotional axis, thresholded at 3. We used 5-fold cross-validation in two methods: feature selection on the entire dataset prior to cross-validation (method 1) and feature selection only on the training set within cross validation (method 2). We compared the results using balanced accuracy, applying Bonferroni correction to set the significance level at $\alpha/6$ (2 datasets and 3 axes), where $\alpha=0.05$. In Table 1, method 1 shows higher mean balanced accuracies across all participants compared to method 2 for all axes in both datasets ($p < 0.008$), except for the valence axis of the DEAP dataset. The findings indicate that improper application of feature selection methods can result in substantially overestimated performance. **References:**1. Koelstra, S., et al. “DEAP: A Database for Emotion Analysis; Using Physiological Signals.” IEEE Transactions on Affective Computing, vol. 3, no. 1, Jan. 2012, pp. 18-31.

Table 1: Mean balanced accuracies for all 32 participants of both datasets

Method 1: Feature selection applied on whole dataset					
	DEAP		Own dataset		
			IAPS	POFA	Music
Valence	66.67*		63.72	64.44	65.02
Arousal	64.46		67.37	69.98	66.32
Dominance	63.73		66.54	71.26	67.03
Method 2: Feature selection applied only on training set					
Valence	59.83*		51.81	50.81	50.06
Arousal	53.93		50.52	53.76	55.37
Dominance	55.37		52.80	57.48	53.61

*The difference did not survive Bonferroni correction ($p< 0.0083$)

Disclosures: N. Khan: None. **D.E. Thompson:** None.

Poster

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West Virginia University Center for Foundational Neuroscience Research and Education

Title: Targeted muscle reinnervation after transfemoral amputation enables direct ankle and foot control

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Abstract: Transfemoral amputees require coordinated control of the knee and ankle to walk with ease and stability; however, the control of powered knee-ankle prostheses has not been developed. Since a common pain management intervention, targeted muscle reinnervation (TMR), offers also signal amplification, the transplanted nerves can be the interface targets for volitional multi-joint control. In lower limb TMR, common peroneal (CPN) and tibial (TN) nerves are redirected to denervated parts of the biceps femoris and semitendinosus muscles, which are mechanically disconnected but still present in the residual limb. Since CPN generates activity related to ankle flexion, and TN is its agonist controlling ankle extension and toe movements, we tested the spatiotemporal representation of these actions in transfemoral amputees following TMR. The representative muscles of intact and residual limbs were instrumented with surface electromyography (EMG). The activity from the innervation sites was recorded with a high-density EMG device (4x4 electrodes) placed over four target locations spanning medio-laterally across the posterior thigh. Participants repeated bilateral movements in a seated position focused on i) temporal separation of antagonistic ankle actions—*ankle flexion and extension*, which were expected to correlate with both CPN and TN activity; and ii) *toe flexion and ankle inversion* movements, which correspond to the most distal branch of TN. The spatiotemporal activity was clearly separated for all movements. We did not observe the spatial separation of TN activity due to proximal and distal nerve branches participating differently in ankle extension and toe flexion. However, all actions were decoded from the temporal separation around general expected reinnervation sites. A non-time series offline decoder was developed based on quadratic discriminant analysis. The decoder could classify between ankle flexion,

ankle extension and toe flexion with an 83% accuracy. Notably, the decoder achieved a classification accuracy of 100% for about 70% of movement period following the initial ambiguous period with low activations. Further improvements are expected with the time-series classification. These findings support the idea that the spatiotemporal activity of CPN and TN after reinnervation provides a reliable source for volitional control of multi-joint distal leg segment actions.

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Poster

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Title: Neural correlates of detection thresholds during intracortical microstimulation in humans

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Abstract: Intracortical microstimulation (ICMS) of the human somatosensory cortex evokes vivid and reliable tactile sensations that can last for many years. These artificial percepts can inform behavior to improve the control of brain-controlled bionic limbs and manipulating stimulation parameters can affect the perceptual features of the stimulation. These features, ranging from simple detection thresholds through to more complex ones, such as percept quality, are typically assessed using standard psychophysical measurement techniques that require the direct participation and interaction of the individual. This knowledge is required to design and calibrate stimulation encoding algorithms for each person. However, as both the number of people implanted with devices and the number of electrodes per person increases, it will become progressively more challenging, and likely even impossible, to calibrate and recalibrate these features using time consuming psychophysics.
Here, we sought to investigate whether neural activity, collected during an ICMS detection task in which amplitude was varied, was related in any way to the psychometrically determined

detection threshold. The participant was enrolled in a clinical trial of a sensorimotor brain computer interface (BCI) in which two Utah arrays were implanted into both the somatosensory and motor cortices. ICMS was delivered during a standard two-alternative forced choice detection threshold paradigm and the participant was asked which of two intervals contained the stimulus. During the task we stimulated and recorded neural data simultaneously. We used a deep neural network model to reduce or eliminate the stimulation artifacts that were generated by stimulation. First, we found that this artifact rejection scheme allowed us to measure spiking activity about 1 ms after the end of a stimulus pulse. Electrodes in both the motor and somatosensory cortex exhibited marked changes in neural activity around the psychometrically determined detection threshold, indicating specific changes in population activity that were related to perception. Second, low-dimensional latent factors extracted from the population neural activity across trials on many electrodes were informative about whether a sensation was evoked by stimulation. This suggests that detection thresholds can be determined directly from measures of neural activity, simplifying the calibration of ICMS encoding algorithms, and improving our ability to monitor detection thresholds automatically.

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Poster

PSTR288: Motor Control of Free, Imagined, and Augmented Actions

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Title: A roadmap for implanting microelectrode arrays to evoke tactile sensations through intracortical microstimulation

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Abstract: Individuals with tetraplegia consistently report restoration of hand and arm function as a top rehabilitative priority. To address this need, researchers are developing brain-computer interfaces (BCIs) that can bypass the injured spinal cord to enable arm and hand control. However, motor control alone is not sufficient to fully restore the ability to interact with our surroundings. Somatosensory feedback is an essential feature of how we use our bodies. Tactile feedback enables manipulation and exploration of objects and provides affective qualities that are unavailable in a purely motor BCI. Therefore, we aim to restore intuitive, tactile sensory feedback through intracortical microstimulation (ICMS) of somatosensory cortex. Somatosensory cortex is an ideal candidate for surgical targeting as it has a somatotopic spatial arrangement, which for the hand involves the little finger to the thumb, represented mediolaterally. This somatotopic arrangement can be characterized using non-invasive neuroimaging techniques: functional magnetic resonance imaging (fMRI) and magnetoencephalography (MEG). Several studies have demonstrated highly preserved finger representations in people who were deafferented through amputation or spinal-cord injury; the two groups of people most likely to benefit from BCIs to restore upper limb function. As part of a multisite clinical trial, we implanted intracortical microelectrode arrays in the post-central gyrus of five study participants (2 arrays in each) with chronic spinal cord injuries to provide ICMS-based tactile sensory feedback. Here, we present a roadmap, from planning to implantation to stimulation, describing how to successfully target and evoke sensations localized to specific digits. We used fMRI and MEG to identify the regions of somatosensory cortex that represent touch sensations for digits. Presurgical imaging analysis and planning of array locations were performed by a broad study team. Using this plan and a structured surgical workflow, we successfully identified implant locations such that ICMS evoked sensations in the digits in all five participants. We expect that this workflow can define a pathway to broader use of BCIs that provide intuitive, somatotopically-matched sensory feedback.

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Poster

PSTR289: BCI

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Title: Training of real-time robotic grasp decoding from neuronal population activity in macaque motor cortex (M1)

Authors: *H. HAMEED[1], A. AGUDELO-TORO[1,2], M. CONTROZZI[3], H. SCHERBERGER[1,2];
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Abstract: The primate cortex can be viewed as a closed-loop dynamical system with very high dimensionality. Extracting a sub-sample of this activity and using it for the decoding of any encoded intention is a challenging task by virtue of the size of the sample relative to the full population. The problem is amplified by the brain's plasticity, which renders any effort towards decoding ephemeral.
Here we employed a modified Kalman filter to decode the grip of a robotic hand from population spiking activity recorded from two 64 channel Utah arrays implanted in the hand area of primary motor cortex (area M1) of a macaque monkey. After initial training in a reach and grasp task and array implantation, the head-fixated monkey was trained in the closing and opening of a prosthetic hand, visible on a screen, to grasp an object that he was previously trained to grasp with his own hand. The task paradigm specified a start cue and fixed maximal times for closing, holding, and releasing of the object.
The monkey was initially observing the robot hand performing the required movement under the control of a control computer for 20 trials. After this, the decoder was trained on the neural population activity recorded from the electrode arrays during action observation. Then the monkey and the control computer shared control of the hand, such that its commanded position was a weighted average of the two estimates: brain control and computer control. With the progression of time, control was gradually transferred from the computer to the animal, eventually giving full control to the monkey.
Our results confirm that a modified Kalman Filter (see: Agudelo-Toro et al., bioRxiv 2023) is suitable to successfully translate cortical activity to motor commands of a robot hand in real time. We also demonstrate the gradual learning by observation and internalization of the task by the monkey using two metrics - the reduction in the transitory nature of the decoder and the speed of the transfer of control of the prosthesis to the monkey. In early experimental sessions, the monkey was slow to take over control and the decoder required retraining within the experiment session, whereas in later sessions decoder training was required only at the beginning of the session and transfer of control was rapid. Furthermore, decoder training involved not only the training of the decoder on the neural activity, but it also adapted brain activity on the decoder, and an equilibrium had to be maintained between the learning processes. This equilibrium was achieved faster and maintained more robustly as the monkey became more

proficient in the task, hence demonstrating the viability of this learning-by-observation paradigm.

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Poster

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Title: Advancements in Intuitive Human-Machine Interfaces Through Wrist-Based EMG Control

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Abstract: The long-term goal of this research is to develop a smartwatch that uses electromyography (EMG) from the wrist alongside other sensors to function as a human-computer interface. Myoelectric control has traditionally been used to control prostheses. As machine learning algorithms have improved, myoelectric control has been able to regress numerous degrees of freedom and classify dozens of gestures. Here, we highlight various innovations and applications around a wrist-worn EMG interface for human-computer interaction. First, we show that EMG measured from the wrist results in similar performance as EMG measured from the forearm. Regression RMSE of a Kalman filter during proportional hand and wrist gestures was 0.29 ± 0.05 for wrist EMG and 0.27 ± 0.02 for forearm EMG ($p = 0.50$, paired t-test). Classification accuracy of a k-NN algorithm across 17 different hand and wrist gestures was 89.5 ± 1.6% for wrist EMG compared to 88.6 ± 0.8% for forearm EMG ($p = 0.21$, paired t-test). Second, we show that, somewhat surprisingly, high-performance EMG regression and classification are possible in hemiparetic stroke patients with immobile hands (modified Ashworth scale > 3). EMG signal-to-noise ratio was significantly worse on the paretic wrist

relative to the paretic forearm, but EMG classification accuracy was significantly better for the paretic wrist than the paretic forearm (91 ± 12% at the wrist vs 56 ± 65% at the forearm; $p < 0.001$, paired t-test). Next, having demonstrated the viability of wrist EMG, we showcase a novel EMG smartwatch, with 16 EMG electrodes embedded within the wriststrap. The EMG smartwatch contains an integrated battery, EMG amplification and recording circuitry, Wi-Fi and Bluetooth communication, an inertial measurement unit, and an ambient light sensor, all within a 2-in2 footprint. To ensure the electrodes maintain good contact, we also developed a novel spring-loaded electrode that uses magnetic encoding to monitor contact with the skin and serve as a secondary control signal. Finally, we show various consumer and healthcare applications of the EMG smartwatch, including monitoring activities of daily living, and controlling virtual/augmented reality, prostheses, exoskeletons, and smart-home devices. Altogether, this work constitutes an important step towards the realization of EMG as a ubiquitous human-computer interface.

Disclosures: C.D. Olsen: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Named as Inventor on Patent. **J.D. Gubler:** None. **B. Orellana:** None. **J.K. Torson:** None. **T.S. Davis:** None. **E.K. Iversen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Named as Inventor on Patent. **J.A. George:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Named as Inventor on Patent.

Poster

PSTR289: BCI

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR289.03/I26

Topic: E.05. Brain-Machine Interface

Support: Weill Neurohub

Title: An ECoG BCI framework for shared control of a robotic arm via supervised autonomy

Authors: *S. SEKO, N. NATRAJ, H. YAN, Y. GRAHAM, R. MIAO, E. F. CHANG, K. GANGULY;
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Abstract: Brain-computer interfaces (BCIs) have the potential to enable individuals with paralysis to perform activities of daily life using an assistive robotic arm. Our recent work demonstrated that electrocorticography (ECoG) signals produce stable neural representations of imagined whole-body actions. This provided robust inputs for “plug and play” robotic control of reaching and grasping with demonstrated stable performance across a period 7 months (Natraj

2023). While long-term stable decoding is possible, daily tasks require high-dimensional control and dexterous manipulation, which is both challenging and cognitively demanding for BCI users. In this work, we propose a flexible framework to augment BCI control with supervised autonomous assistance. A right-handed tetraplegic participant was implanted with a 128-channel ECoG grid over left sensorimotor areas. The participant utilizes imagined actions, decoded from neural features (delta (0.5-4 Hz), beta (12-30 Hz), and high-gamma (70-150 Hz) power), to control the endpoint kinematics of a 6-Dof Kinova Gen2 robotic arm and gripper during a reaching task with multiple objects. A computer vision system with an RGB-depth camera identifies potential goals in the environment, estimating the user's intended goal with Bayesian inference. We considered two assistance paradigms: 1) blended control, in which the user input and assistance are continuously combined, and 2) supervised autonomy, in which the assistance completes automated sub-routines while the user provides feedback to the assistance when necessary. This latter approach uses the same BCI commands for brief discrete feedback, reducing the time in which the user must provide active input to the system, while remaining flexible to changes in user intention. Both methods allow for seamless transitions between unassisted and assisted control, demonstrated in the task which required multiple hand-offs. Our results found that the supervised autonomy framework resulted in increased task success rate (94% vs 43%) and reduced time to complete (18.4 s ± 14.8 vs 29.9 s ± 21.0) when compared to blended assistance. We further demonstrate the potential of this framework in a complex task which requires opening cabinet doors and drawers. This work highlights the potential for shared autonomy to enhance the performance and capabilities of robust BCI commands for high-dimensional control, enabling translation to complex real-world tasks.

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Poster

PSTR289: BCI

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Program #/Poster #: PSTR289.04/I27

Topic: E.05. Brain-Machine Interface

Support: NIH DP5-OD029571
Meta Reality Labs Award #2990450277899571
VA Award UU-2022-SAHAT-01

Title: Co-adaptive learning improves the accessibility of myoelectric interfaces for stroke patients in proportional control tasks

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Abstract: The long-term goal of this research is to develop dexterous, intuitive, and inclusive myoelectric control interfaces for extended reality (XR) to improve productivity and quality of life for all. Existing XR interfaces rely on recording motion through cameras or instrumented gloves. Yet, due to neuromuscular and musculoskeletal disabilities, millions of users with limited hand mobility cannot use these systems. Myoelectric interfaces rely on muscle electrical activity to generate commands from motor intent, even when a person's hand cannot move due to weakness or paresis.

Here, we explore a co-adaptive learning method that leverages a person's ability to adapt to a control interface and an algorithm's ability to adapt to personalized muscle activity. We recruited five stroke participants and five healthy participants to perform a simultaneous, proportional control task (i.e., target-touching task) of a two-degree-of-freedom virtual hand. Using a modified Kalman filter, we map surface EMG signals from the forearm to two gestures: 1) a power grasp requiring muscle coactivation and 2) a tripod pinch requiring selective, differential muscle activation. The participants completed the task under three conditions to evaluate: 1) the decoder performance due to increasing dataset size (i.e., machine learning), 2) the human performance over time using a static decoder (i.e., human learning), and 3) the performance of the human-machine system over time using an adaptive decoder (i.e., co-adaptive learning).

First, we show that human learning alone is insufficient for this task; all participants were unable to improve their performance over time using a static decoder trained with minimal training data. In contrast, machine performance significantly improved with additional training data for all participants. Notably, this machine learning was still possible even in the case of weak and spastic EMG. Consistent with prior work, co-adaptive learning outperformed machine learning and human learning in terms of the learning rate and final performance. Importantly, under co-adaptive learning, stroke patients had a similar learning rate and final performance to healthy controls.

These results show that stroke survivors can learn to selectively modulate their muscle activity despite limited or no mobility, highlighting the potential of EMG as an inclusive human-computer interface. Co-adaptive learning can enhance control and minimize patient data.

Disclosures: F.R. Mino: None. **C.J. Thomson:** None. **C.D. Olsen:** None. **J.A. George:** None.

Poster

PSTR289: BCI

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Program #/Poster #: PSTR289.05/I28

Topic: E.05. Brain-Machine Interface

Support: Weil Neurohub

Title: Utilizing Beta Burst Dynamics to Improve BCI Robotic Control

Authors: *R. MIAO[1], N. NATRAJ[2], Y. GRAHAM[1], H. YAN[3], S. SEKO[2], E. F. CHANG[4], K. GANGULY[5];
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Abstract: Brain-Computer Interfaces (BCIs) present a promising solution to restore reach-to-grasp functionality in human patients with severe motor disabilities by translating movement-related neural signals into control signals for external assistive devices. These devices, particularly robotic arms with a high degree of freedom (DoF), can significantly enhance the autonomy of users and improve their quality of life. Our recent work (Natraj et al., 2023) has demonstrated prospects for reliable, real-world control over a high DoF robotic arm. However, several challenges continue to hinder the practical application of high DoF assistive devices. Notably, user fatigue resulting from prolonged engagement and control can significantly degrade performance. Additionally, the cumbersome decoding framework underlying current robotic control systems results in slow operation, which does not yet meet the daily living needs of users. To address those challenges, we propose to track the user's internal state of active engagement and high-level intention decoding using neural signals to control the robot to go and stop. We found rhythmic fluctuations in the amplitude of beta (13-30 Hz) power during continuous robotic control, which not only relate to the task cues and movement onset, but also present during BCI task control. Our study involved two participants, both of whom suffered from a brainstem stroke and are currently tetraplegic. The first participant, a 39-year-old right-handed male, has a 128-channel ECoG grid implanted over the left sensorimotor cortex. The second participant, a 48-year-old female, has a 256-channel ECoG array implanted over her sensory and motor cortices. We conducted a spatial-temporal analysis of beta bursts. We then found that temporal beta bursts exhibit a mixed pattern. Specifically, global beta bursts maintained a condition-independent consistent pattern, whereas local bursts exhibited diverse condition-dependent patterns across different targets. In a robot stop task with known ground truth on participant engagement and disengagement, we found a significant phasic relationship involving beta signal phases with significant high phase-locking values (PLV) compared to null states. Our results thus highlight the potential of using beta burst as a biomarker for tracking users' internal states and implement fast precise robot control by identifying whether participant is engaged in control or not.

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Poster

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Program #/Poster #: PSTR289.06/Web Only

Topic: E.05. Brain-Machine Interface

Support: National Post-Doctoral Fellowship, PDF/2020/002514, DST-SERB Government of India (GoI)

Title: Neuromuscular Fusion-based Hand 3D Trajectory Estimation Model

Authors: *A. BHONGADE[1], R. GUPTA[2], T. GANDHI[1];
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Abstract: Title: Neuromuscular Fusion-based Hand 3D Trajectory Estimation Model **Authors:** ***Amit Bhongade[2]**, Rohit Gupta[1], T. K. Gandhi[2]; [1]SRMIST, Tamil Nadu, India; [2]IIT Delhi, New Delhi, India. **Disclosures:** ***Amit Bhongade:** None, **Rohit Gupta**: None, **T. K. Gandhi:** None.**Support:** This work is supported by the National Post-Doctoral Fellowship, PDF/2020/002514, DST-SERB Government of India (GoI).**Abstract:** The integration of Brain-Computer Interface (BCI) technology into cyber-physical systems has led to significant advancements in control methodologies. However, the transition from discrete or model-based control methods to continuous control approaches remains crucial for achieving optimal and smooth control. This research focuses on the estimation of human hand trajectories in 3D space, leveraging multichannel Neuromuscular signals (EEG and EMG). In the current research, two models, NARX and CNN-LSTM, representing machine learning (ML) and deep learning (DL) paradigms, respectively, were developed and evaluated. The performance of these models was assessed using EEG and EMG signals separately and in combination (EEG+EMG). A genetic algorithm (GA)-based channel selection algorithm was employed for EEG channel selection. A total of 10 EEG channels were selected using the GA optimization algorithm, with a clear dominance of the motor cortex and visual cortex regions. Performance parameters, namely root mean square error (RMSE) and correlation coefficient (CC), were utilized to compare the efficacy of ML and DL models across different signal configurations. The results indicate that both ML and DL models demonstrated promising performance in estimating hand trajectories. When considering EEG, EMG, and their fusion, the models exhibited varying levels of accuracy, with the fusion of EEG+EMG signals generally outperforming individual modalities. Moreover, the GA-based channel selection algorithm contributed to enhancing model performance, particularly evident in the reduction of RMSE values. For the ML model, the average RMSE values ranged from 0.304 to 0.754, while the DL model exhibited RMSE values ranging from 0.219 to 0.724. Similarly, the average CC values ranged from 0.232 to 0.660 for the ML model and from 0.386 to 0.814 for the DL model across different signal configurations. These findings highlight the potential of integrating multichannel EEG and EMG signals with advanced learning models for real-time hand trajectory estimation. The utilization of GA-based channel selection further enhances the accuracy and robustness of the predictive models, thereby contributing to the advancement of BCI technology in control applications.

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Poster

PSTR289: BCI

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Program #/Poster #: PSTR289.07/I29

Topic: E.05. Brain-Machine Interface

Support: Feinstein Institutes for Medical Research

Title: Enabling precision grasping and tactile feedback in tetraplegia using a bidirectional brain-computer interface and a low-profile active orthosis

Authors: *Z. ELIAS[1], A. JANGAM[2], S. K. WANDELT[3], A. NEUWIRTH[4], C. MAFFEI[5], E. IBROCI[6], S. CHANDRASEKARAN[3], I. ROSENTHAL[3], J.-W. KIM[7], J. XU[8], M. F. GLASSER[9], D. GRIFFIN[10], S. BICKEL[11], A. B. STEIN[12], A. D. MEHTA[13], C. BOUTON[3]; [1]Feinstein Inst. for Med. Res., Great Neck, NY; [2]Brain Computer Interface, Feinstein Inst. for Med. Res., Manhasset, NY; [3]Feinstein Inst. for Med. Res., Manhasset, NY; [4]3D Design and Innovation, Northwell Hlth., Manhasset, NY; [5]Physical Med. and Rehabil., Feinstein Inst. for Med. Res., East Meadow, NY; [6]Northwell, Manhasset, NY; [7]Baylor Col. of Med., Houston, TX; [8]Radiology, Baylor Col. of Med., Houston, TX; [9]Dept. of Anat. & Neurobio., Washington Univ. Sch. of Med., Saint Louis, MO; [10]Northwell Hlth., East Meadow, NM; [11]Neurosurg. - Neurol., Feinstein Inst., Manhasset, NY; [12]Physical Med. & Rehabil., Northwell Hlth., Manhasset, NY; [13]Neurosurg., Hofstra Northwell Sch. of Med., Syosset, NY

Abstract: Approximately 18,000 individuals sustain a spinal cord injury (SCI) in the United States annually with a significant fraction involving upper limb impairment disrupting important activities of daily living. Brain-computer interface (BCI) technology allows for direct neural decoding and stimulation via implanted microelectrode arrays. BCIs can form a neural bypass, allowing intention-driven motion activation and functional movement in tetraplegia. However, research into restoring movement and sensation simultaneously in the human hand by establishing a bidirectional neural bypass (BNB) between the brain and the body has been largely unexplored. Assistive devices like active orthoses and exoskeletons have been used to assist in motor function for individuals with physical impairments; despite this, the use of a BNB to control an active orthosis with tactile sensation feedback has yet to be established. To restore grasp functionality, a custom 3D-printed low-profile orthosis with an embedded servo-driven tendon-like system was tested with a participant with a complete C4/C5 SCI. The development of the active orthosis was motivated by the participant's lower motor neuron dysfunction which has left their hand flexors nonresponsive to muscle stimulation. The orthosis holds the thumb in an open grasp position, provides wrist stability, and extends to the middle of the forearm where the servo-tendon system is located. This system facilitated controlled multi-joint finger flexion

with a stabilized thumb. The BNB employed a LSTM-based regression machine to decode motor intentions and a closed-loop algorithm, inspired by the interneuronal architecture of the human spinal cord, to modulate the intensity of the participant's grasp. Using this system, the correlation between the participant's intended grasp force level and the decoded output exceeded 80% in real time. Additionally, a tactile force sensor was embedded into the orthosis, recording the applied grasping force. The participant received primary somatosensory cortex (S1) stimulation as feedback, correlating the stimulation profile with the tactile force exerted. The participant successfully managed to grasp, lift, and release objects solely using the BNB system. The BNB system enabled the participant to accurately adjust their grasp strength, indicating that the integration of S1 stimulation with motor decoding using an active orthosis, can expand participant functional abilities, thereby increasing independence and quality of life.

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Poster

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Program #/Poster #: PSTR289.08/I30

Topic: E.05. Brain-Machine Interface

Support: NRF 2022M3E5E9016884
NRF 2022R1A6A1A03063039

Title: A compact, power-efficient bidirectional neural microsystem for brain-machine interface in non-human primates

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Abstract: Here we introduce a cutting-edge bidirectional neural interfacing system that integrates both neural signal recording and stimulation functionalities, promising significant advancements in the field of brain-machine interfaces (BMIs). To address the challenges of power efficiency and miniaturization, which are crucial for long-term implant viability, our system is fabricated using standard 180-nm CMOS technology. It includes neural interface microsystems (NIMs) that accurately measure neural signals across the entire ECoG frequency range of 0.1-300.0 Hz, while maintaining minimal power consumption of less than 0.4 mW. The design features a compact and implantable unit comprising recording and stimulation modules,

and a 16-MHz radiofrequency link for data and power telemetry. This enables robust interaction with an external unit through an inductively coupled coil, ensuring reliable communication and power transfer. The system's ability to detect sine waves with an amplitude of 4.8 uV and a bandwidth of 5-200 Hz from electrocorticographic BMIs demonstrates its sensitivity and precision. Furthermore, the stimulation component of the system can evoke neural signals effectively, operating at a low supply voltage of below 1.2 V and a power dissipation of only 1 uW per channel. The feedback system for stimulation, supplied at 3.3 V, consumes 234 uW and employs a square current pulse, which is modulated based on varying parameters set by the 1-MHz operational clock, with a maximum output of 310 uA to the tissue-electrode interface. The integrated system supports 128 signal recording channels and 32 stimulation channels, all within a compact chip area of 4.9 × 3.9 mm2. This scalability and integration capacity make it an ideal candidate for advanced BMI applications requiring high functionality and reliability in small, energy-efficient packages. Our system not only pushes the boundaries of what is technically feasible in neural interfacing but also sets new standards for the practical deployment of BMIs in medical and technological applications.

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Poster

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Topic: E.05. Brain-Machine Interface

Support: 1 DP2 HD087955

Title: High performance brain computer interface control of a virtual human hand using electrocorticography

Authors: *N. NATRAJ[1], S. SEKO[2], E. F. CHANG[3], K. GANGULY[4];
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Abstract: Enabling high performance neuroprosthetic control for reaching and grasping is a high priority for individuals with paralyses. Of particular importance is the dexterous control of the hand. Achieving reliable hand control has numerous translational benefits, from grasping and object manipulation to precise control of keyboard interfaces. While recent studies have shown the successful control of groups of fingers (Willsey et. al 2022, 2024), the feasibility of controlling all individual fingers, wrist and prehension postures remains unclear. A major issue is the fact that although the hand is high-dimensional, biomechanical and neuronal coupling of joints result in hand control being intrinsically lower-dimensional (Natraj et. al, 2022). This may

result in moment to moment decoding errors in a real time brain computer interface (BCI), e.g., due to synergistic coupling between the middle and index fingers during individuation and grasping. The goal of our study was to address this issue and enable high performance BCI hand control. The participant in the study was a tetraplegic and anarthric patient (B3) implanted with a 253 channel electrocorticographic (ECoG) grid over contralateral sensorimotor cortex. We evaluated BCI control of a virtual hand across a repertoire of 12 actions: flexion and extension of all individual fingers, three complex grasps (pinch, precision and power), and four wrist movements (flexion and extension, adduction and abduction). Neural features in three sensorimotor frequencies (delta 0.5-4Hz, beta, 13-30Hz and high gamma 70-150Hz) were extracted and a real-time decoder discerned movement-type based on activity across the grid in the three features. We leveraged the representational stability of ECoG that allowed the user to learn to flexibly regulate movements' statistics (e.g., neural variance) without somatotopic changes, during feedback driven real-time closed loop control (Natraj et. al., 2023). Thus, although daily open-loop representations exhibited neuronal coupling between synergistic movements, practice with the BCI using feedback (over 7 sessions) selectively altered representations to be significantly more discernible. This was especially prominent with batch-updates to the decoder, and suggestive of a BCI-specific shift in representations that overcame open-loop neuronal coupling. This process culminated in a real-time decoding accuracy of 93.75%, exceeding similar spike-based BCI studies of individual hand movements (Guan et. al, 2023). Our results here provide evidence for a framework using ECoG that allows for high performance BCI control of the human hand.

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Poster

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NIMDS R01NS105132

Title: A Multi-Camera Setup for Real-Time Hand Markerless Tracking during Function Electrical Stimulation

Authors: *J. JOSEPH[1], A. WARD[2], M. MENDER[3], M. KELBERMAN[3], J. LAM[2], T. A. KUNG[4], N. GANESH KUMAR[4], C. A. CHESTEK[3], A. DRAELOS[3];

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Abstract: Approximately 1% of the US population experiences limb paralysis which could be treated via the restoration of hand function to improve quality of life (Armour, 2016). Functional electrical stimulation (FES) uses embedded electrodes to stimulate muscles to evoke hand movements. Brain machine interface (BMI) control is one paradigm that has used FES to restore continuous non-human primate (NHP) controlled movements during temporary paralysis (Nason, 2023). However, naturalistic movement requires fine dexterous control of 27 degrees of freedom (DoFs), which current BMI-FES systems have not yet reached. To accurately map FES stimulation patterns to precise movements, robust quantification of hand movement is essential. However, current hand tracking methods are coarse or limited to a small number of DoFs and often involve physical constraints through the use of e.g. motion-capture gloves, bend sensors, or other manipulanda. To address this, we constructed a custom multi-camera rig within our existing setup for conducting FES experiments with NHPs. We placed three cameras to capture multiple angles of FES-evoked movements to better observe individuated finger and wrist movements. Estimated joint positions were extracted using a trained DeepLabCut (DLC) model, a markerless tracking software. We developed a processing pipeline that leveraged uncertainty estimates to interpolate and refine these measurements. Our preliminary analysis of finger movement during a muscle fatigue FES experiment showed a decreased range of motion as a function of time. Finally, we benchmarked our pipeline and demonstrated greater than real-time processing speeds of the captured video. With real-time feedback from these measurements in the future, we aim to iteratively refine FES patterns throughout an experiment to improve accuracy and control of dexterous finger movements.

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Poster

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Title: Sparse deep learning enables low-power, implantable neural interfaces

Authors: *J. T. COSTELLO[1], H. TEMMAR[2], L. CUBILLOS[3], M. MENDER[4], D. WALLACE[3], M. KELBERMAN[2], N. GANESH KUMAR[5], M. WILLSEY[6], T. A. KUNG[5], P. G. PATIL[7], C. A. CHESTEK[4];
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Abstract: Brain-machine interfaces (BMIs) have shown promise in restoring motor function and communication to people with sensorimotor impairments. Recently, the use of artificial neural network decoding algorithms and more recording channels has led to higher BMI performance but also higher power consumption. With the goal of fully implantable, untethered BMI, power consumption must be limited to prevent thermal tissue damage and enable long use without frequent battery charging. BMI power usage can be reduced by minimizing the number of computations performed by the decoder and turning off recording channels that are not needed for decoding, if performance can be maintained.
Here, we show how neural network "pruning" can significantly compress decoders and reduce the number of active channels for improved BMI power consumption. During neural network decoder training, pruning removes the lowest-magnitude weights of the decoder, resulting in a sparse set of network weights or selected channels. To test sparse decoders, one rhesus macaque monkey was implanted with Utah microelectrode arrays in motor cortex and chronic electromyography (EMG) electrodes in finger-related muscles. The monkey was trained to control a virtual hand using a manipulandum (offline trials) or using a decoder with either type of neural signal (online trials).
In offline analyses, recurrent neural network decoders of brain and EMG to finger movement could be compressed by 180x and 9x to require only 6800 or 8500 parameters respectively, while maintaining less than 5% increase in MSE compared to the optimal non-sparse decoder (averaged across 10 brain datasets and 5 EMG datasets). With significant compression, these sparse networks have the same order of magnitude number of parameters as a linear decoder and may be able to run on low-cost embedded microcontrollers. In online trials, sparse neural network decoders showed no significant loss of performance, completing trials 8% faster on average using a sparse decoder compared to a non-sparse decoder (5 comparison sessions).
Then, using pruning with a channel mask to select the most important subset of neural channels, we found that only 10 / 96 (Utah arrays, 10 datasets) or 100 / 1000 (simulated channels, 5 datasets) channels were needed to maintain near-optimal decoding accuracy. Using channel selection with simulated datasets of varied channel counts, we find that BMIs using a subset of a larger number of channels may use fewer active channels and less power than BMIs with few channels, for a given performance level.
These results suggest a path toward power efficient, implantable BMIs with flexibility in running the decoder on or off the implant.

Disclosures: J.T. Costello: None. **H. Temmar:** None. **L. Cubillos:** None. **M. Mender:** None. **D. Wallace:** None. **M. Kelberman:** None. **N. Ganesh Kumar:** None. **M. Willsey:** None. **T.A. Kung:** None. **P.G. Patil:** None. **C.A. Chestek:** None.

Poster

PSTR289: BCI

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR289.12/I34

Topic: E.05. Brain-Machine Interface

Support: NSF-NCS 1926576
AWD022672
NIH/NIGMS T32GM141746
R01NS105132

Title: Non-human primate motor point targeted functional electrical stimulation for highly selective movements

Authors: *M. M. KELBERMAN[1], A. WARD[1], M. MENDER[3], J. JOSEPH[1], J. LAM[2], N. GANESH KUMAR[7], T. A. KUNG[4], Y. SAADEH[5], C. A. CHESTEK[6];
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Abstract: Recent advancements in brain-machine interfaces (BMIs) have made them a valuable control system to restore independence to people with spinal cord injuries. Functional electrical stimulation (FES) has shown promise to restore hand function, which is a well-known priority for people with paralysis. In BMI-FES, commands decoded from brain signals modulate stimulation to create desired movements by reanimating the muscles. Despite these advancements, limitations in electrode number and placement also limit performance. Current systems cannot achieve finely graded, individuated finger control that mimics natural hand movement. To achieve additional movements that are consistent and selective, there must be improvements in the placement of FES leads.

To this end, we implanted 16 bipolar leads in the forearm of a rhesus macaque, targeting the points where the distal branches of the nerves enter the muscle belly (motor points) that control hand movement. This unique implantation strategy seeks to target more specifically and recruit muscle fibers in their natural order. To test FES efficacy in an able-bodied animal, an ultrasound-guided nerve block of the radial, median, and ulnar nerves in the forearm was performed with 1-2% lidocaine with epinephrine, temporarily paralyzing the hand.

At 8 months post-implantation, a range of stimulation (5 mA and 5-100 us PW) was used to characterize muscle response of these leads, which spanned 7 muscles. All 16 leads were able to

evoke the expected hand movements based on intraoperative stimulation and implant location, though selectivity varied.
Stimulation on 5 out of 16 leads (all wrist flexors and extensors) was selective to only the expected joint throughout the entire range. A total of 5 other leads were able to selectively activate only the expected finger movement but would recruit movements on other fingers and/or the wrist by the end of the range. This is anatomically intuitive because the finger muscle tendons cross the wrist to enter the hand, which will tend to cause a movement in the wrist when activated. These movements were selective for an average of 7 degrees and maximally moved an average of 19 degrees for less selective movements. Another 3/16 evoked movements similar to what was expected (plus or minus 1-2 joints) while the last 3/16 exhibited non-specific behavior. Selective movements were two or three fingers rather than single fingers, including index-middle, ring-small, and middle-ring-small movements.
Overall, motor point targeting allowed us to quickly implant a large number of leads in the forearm and created a degree of specificity that may enable more precise movements by stimulating multiple electrodes.

Disclosures: M.M. Kelberman: None. **A. Ward:** None. **M. Mender:** None. **J. Joseph:** None. **J. Lam:** None. **N. Ganesh Kumar:** None. **T.A. Kung:** None. **Y. Saadeh:** None. **C.A. Chestek:** None.

Poster

PSTR289: BCI

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Topic: E.05. Brain-Machine Interface

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Agencia Nacional de Investigacion y Desarrollo (ANID) of Chile
Dan and Betty Kahn Foundation Grant 2029755
Michigan Robotics Institute

Title: Presenting a large non-human primate cortical dataset during individuated finger movements spanning years for testing neural stabilization methods

Authors: *H. TEMMAR[1], Y. WANG[2], L. CUBILLOS[3], M. MENDER[1], J. COSTELLO[4], P. G. PATIL[5], A. DRAELOS[1], C. A. CHESTEK[1];
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Abstract: Brain-machine interfaces (BMI) aim to restore behavioral function to people living with disabilities by decoding neural signals into behavioral commands. Recently, studies have demonstrated high-performance BMI able to decode speech and multi degree-of-freedom (DOF) movements in real-time scenarios (Willett et al. 2023, Costello et al. 2023). However, the need for frequent decoder retraining due to instability of neural signals over time continues to limit translation to real-world use (Downey et al. 2018). While many studies have proposed promising stabilization methods (ex. Ma et al. 2023), they are often tested on different tasks, hindering comparisons. To help resolve this, we present a dataset containing neural and behavioral data spanning almost 4 years, to be used as a benchmark to compare various stabilization methods over time. One rhesus macaque (Monkey N) was implanted with Utah arrays in the right precentral gyrus and trained to perform a trial-based two-DOF finger movement task with the left hand. Our initial dataset contains multiple extracted neural features and kinematics from 400+ sessions of Monkey N performing center-out and random target versions of the task, spanning 3.78 years. Each day contains at minimum 400 trials. As a preliminary analysis into decoder instability over time, on each day, the first 300 trials were used as training data for linear (ridge regression) and artificial neural network (ANN, tcFNN by Willsey et al. 2022) decoders, using spiking-band power (Nason et al. 2020) as the neural feature. Each decoder was then used to predict movements on holdout trials from all sessions within 100 days of the training session. Decoder performance declined most on the first day before and after decoder training, with average (across all sessions) Pearson correlation of decoder predictions to ground truth dropping 0.10 (linear) and 0.14 (ANN) across all training sessions. After 100 days, average correlations drop a total of 0.25 (linear) and 0.27 (ANN). The drop in correlation appears to follow a single order exponential decay, but further investigation is needed. We also observed that decoders performed worse when tested forward in time than backwards, suggesting a potential loss in signal quality over time. We intend to refine this dataset and release it to the public for open use as a valuable resource to the BMI field. as well as to use the dataset to compare existing and novel methods for decoder stabilization over time.

Disclosures: H. Temmar: None. **Y. Wang:** None. **L. Cubillos:** None. **M. Mender:** None. **J. Costello:** None. **P.G. Patil:** None. **A. Draelos:** None. **C.A. Chestek:** None.

Poster

PSTR289: BCI

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR289.14/I36

Topic: E.05. Brain-Machine Interface

Title: Investigating evolution of features in Brain Computer Interface experimentation for robustness

Authors: *T. VENOT[1], F. DE VICO FALLANI[2], M.-C. CORSI[2];
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Abstract: Motor imagery brain-machine interfaces are fronting a hurdle: many subjects struggle to control devices effectively. Performance hinges on the features used, typically power spectral density (PSD) in alpha or beta bands, modulated by different mental tasks such as motor imagery (MI) and rest. Variations in features stem from Event-Related Desynchronization in the sensorimotor cortex. Monitoring feature evolution within a session is crucial for maintaining consistent performance that follows users 'intent.
In this work, we had 15 subjects (8 F, 25 ± 1.5 yo) coming for 3 sessions where they controlled a robotic arm using MI for the gripper closing and an eye tracker for the position to reach[1]. During a session, they went through 8 runs of 10 MI and Rest trials. 3 runs were of calibration (no feedback) then 3 runs of control based on a LDA trained on features of the calibration and then 2 runs of control with the LDA trained on the previous control runs. We investigated MI and resting state beta band PSD in the left sensorimotor cortex (postcentral and precentral area) using source reconstruction at the group level with all sessions confounded to see if patterns rose between blocks. We explored the beneficial impact of feedback in BCI experiments and examined how specific features contributed to this effect. We also investigated whether calibration features are linked to online performance. We discovered that patterns of motor imagery (MI) and rest differed over runs. We then examined how the variability of MI-Rest PSD during calibration correlated with overall performance. We found a significant correlation ($p = 0.006$, $r = 0.402$), suggesting that initial differences in the ERD between conditions during calibration leads to better performance. However, despite MI being consistent across control blocks, rest showed block-specific behavior, indicating its intrinsic variability in the experimental design.
Here, we explored features' evolution used in a BCI experimentation, we could correlate their early profile to performances. From this, we can draw different conclusions, i) features 'variation forces to adapt the algorithm either by retraining it or by using adaptive classifier, ii) As MI features were stable through the control period, they could be selected from the start as they seem robust, oppositely, rest features could gain in being retrained. To go further, it might be necessary to investigate other features more robust throughout experimentation such as connectivity[2].
[1] T. Venot et al., « Intentional binding enhances hybrid BCI control » (2023) [2] J. Gonzalez-Astudillo et al., « Network-Based Brain-Computer Interfaces: Principles and Applications » (2021)

Disclosures: T. Venot: None. **F. De Vico Fallani:** None. **M. Corsi:** None.

Poster

PSTR289: BCI

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR289.15/I37

Topic: E.05. Brain-Machine Interface

Support: The Miami Project to Cure Paralysis
The Buoniconti Fund
Florida Department of Health

Title: Brain-controlled cervical spinal cord stimulation upper-limb neuromodulation

Authors: *R. FADLI[1], A. SASAKI[2], T. NOMURA[3], M. MILOSEVIC[2];
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Abstract: Cervical transcutaneous spinal cord stimulation (SCS) has emerged as a promising neuromodulation approach for upper limb function by activating sensorimotor networks. Recent advancements in brain-computer interface (BCI) technology enable more natural control of neuromodulation during activity-based training. We aimed to investigate the neuromodulation effectiveness of a single 30-min session BCI-SCS intervention compared to an equivalent Random-SCS (control) condition in a crossover study with twelve participants. During the BCI-SCS intervention, the BCI system was used to detect pinch motor imagery in real-time from a single scalp surface electrode. If the BCI system detected the motor imagery within 15 s, SCS was triggered for 5 s. Otherwise, SCS was not triggered, and the subsequent trial was initiated. The overall success rate was 84.6±4.1%. During the Random-SCS intervention, SCS was triggered for 5 s in a random interval. The SCS in both interventions was applied using a cathode over the C7-T1 vertebrae level and an anode over the iliac crests. The intensity of the stimulation was set to elicit post-activation depression of evoked responses in multiple upper limb muscles during 30 Hz stimulation using 1 ms biphasic pulses (range: 50-80 mA), indicating the recruitment of dorsal root sensory fibers. Neuromodulation assessments in the first dorsal interosseous muscle involved evaluating corticospinal excitability via motor-evoked potentials induced by single-pulse transcranial magnetic stimulation over the primary motor cortex before (Pre), immediately (Post 0), and 30 min (Post 30) after the intervention. Our results showed that corticospinal excitability was significantly facilitated after (Post 0 min) the BCI-SCS intervention only (p=.045), and post hoc testing indicates that the facilitation only persisted at the Post 0 min time point (p=.021). On the other hand, the Random-SCS intervention did not elicit any significant modulation (p=.121). These findings suggest that pre-synaptic activation via the cortical and corticospinal activity and the post-synaptic activation of sensory nerves using SCS may strengthen the connections within the corticospinal tract through Hebbian plasticity. Overall, we demonstrated the feasibility of BCI-SCS technology and its potential for neuromodulation to facilitate upper-limb corticospinal tract excitability.

Disclosures: R. Fadli: None. **A. Sasaki:** None. **T. Nomura:** None. **M. Milosevic:** None.

Poster

PSTR289: BCI

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR289.16/I38

Topic: E.05. Brain-Machine Interface

Support: United States Department of Defense
Bryon Riesch Paralysis Foundation
Florida Department of Health
The Miami Project to Cure Paralysis
Buoniconti Fund

Title: Effectiveness of non-invasive brain-controlled spinal stimulation for neuromotor recovery after incomplete SCI

Authors: A. SASAKI[1], R. FADLI[3], **Z. BOOGAART**[1], J. D. GUEST[2], *M. MILOSEVIC[4]; [2]Neurolog. Surgery/Miami Project to Cure Paralysis, [1]Univ. of Miami Miller Sch. of Med., Miami, FL; [4]The Miami Project to Cure Paralysis, [3]Univ. of Miami, Miami, FL

Abstract: Transcutaneous spinal cord stimulation (TSCS) has been utilized to recover upper- and lower-limb motor function after spinal cord injury (SCI). Brain-computer interface (BCI) systems can integrate movement intention with spinal circuit activation through TSCS to enhance neuromodulatory effectiveness. The study aimed to investigate the efficacy of a BCI-TSCS intervention on lower-limb motor function and neural activity in individuals with chronic incomplete SCI. Participants with SCI underwent two different interventions on separate days: (1) BCI-TSCS: TSCS was delivered for 5 sec to the lumbothoracic spinal cord upon detection of cortical activity during motor imagery using our BCI system; and (2) Random TSCS: 5 sec TSCS bursts were delivered at random intervals while participants remained at rest without performing any movement imagination. Each intervention lasted 30 min. TSCS was delivered at 30 Hz to activate dorsal root sensory fibers as confirmed through evoked response post-activation depression. In the BCI-TSCS condition, electroencephalography signals were processed in real-time to detect event-related desynchronization of cortical oscillatory activity during ankle dorsiflexion motor imagery. Before each intervention, TSCS location and intensities were adjusted by monitoring TSCS-evoked spinal reflexes. Stimulation was delivered between T12-L3 vertebral, where the most prominent responses were elicited in the tibialis anterior (TA) muscle. The intensity was adjusted at the motor threshold, defined as the minimum intensity required to induce a visible spinal reflex. Assessments were conducted before and after the interventions. Motor evoked potentials in the TA muscle, elicited through primary motor cortex transcranial magnetic stimulation were assessed to evaluate corticospinal excitability. A lower-extremity motor coordination test and the foot tapping test were evaluated as a measure of lower-limb motor function. Results showed that corticospinal excitability was facilitated immediately after the BCI-TSCS intervention, but not after the Random TSCS intervention. Kinematic profiles during lower-limb motor function tests were also affected immediately after the BCI-TSCS intervention. Thus, the BCI-TSCS intervention transiently enhanced corticospinal

excitability and motor function in individuals with chronic incomplete SCI. We propose that incorporating the descending drive using a BCI system could enhance the efficacy of TSCS in neuromotor recovery applications.

Disclosures: A. Sasaki: None. **R. Fadli:** None. **Z. Boogaart:** None. **J.D. Guest:** F. Consulting Fees (e.g., advisory boards); AbbVie Inc., NervGen Pharma. **M. Milosevic:** Other; International Functional Electrical Stimulation Society.

Poster

PSTR289: BCI

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR289.17/I39

Topic: E.05. Brain-Machine Interface

Support: NSF 2137255

Title: Toward a registry for implanted brain-computer interfaces for motor, sensory, and communication applications (2024 update)

Authors: *M. PATRICK KRUEGER[1], I. BURKHART[3], J. L. CONTRERAS-VIDAL[2]; [2]Electrical and Computer Engin., [1]Univ. of Houston, Houston, TX; [3]Ian Burkhart Fndn., Columbus, OH

Abstract: Implanted brain-computer interfaces (iBCI), systems that translate brain activity recorded intracranially into commands for virtual or physical machines to restore or rehabilitate motor or speech functions, are rapidly advancing. With 25 years of research into long-term electrode implantation to drive computer apps or mechanical devices, as of April 2024 there are no iBCI systems fully approved by the U.S. Food and Drug Administration, nor have these systems been demonstrated as a lifelong viable solution. However, there has been a rapid expansion in the field with at least 25% of participant implantations happening in the past two years, Synchron™ entering its third round of clinical trials, the entry of Neuralink™ into human clinical trials, along with a number of other electrodes poised to enter human clinical trials in the coming two years. This abstract updates our global report for implanted BCI systems (PSTR280.14, SfN2023) in terms of participant demographics, electrodes used, and iBCI research groups. Based on the new entries into the clinical trials and the need for ensuring equal access for all prospective demographic groups and to advance the implementation science for iBCI systems, it is imperative to standardize clinical trial reporting, performance metrics, and develop a registry for tracking the ongoing implantation. As this technology enters translation, a registry will consolidate knowledge of iBCI trials, enhance rigor and transparency, improve public perception, and facilitates the dissemination of accurate information across stakeholders.

Disclosures: M. Patrick Krueger: None. **I. Burkhart:** F. Consulting Fees (e.g., advisory boards); Blackrock Neurotech, US FDA. Other; BCI Pioneer Coalition. **J.L. Contreras-Vidal:** None.

Poster

PSTR289: BCI

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Program #/Poster #: PSTR289.18/I40

Topic: E.05. Brain-Machine Interface

Support: NIH Grant UH3-NS121565-01A1
NIH Grant U24-NS113637-02

Title: Characterization and demonstration of the evoked potential functionality of the CorTec Brain Interchange

Authors: *H. CHO[1], A. GKOGKIDIS[3], M. BUCHHEIT[3], S. C. CRAMER[4], J. G. OJEMANN[2], T. DENISON[5], J. A. HERRON[2];
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Abstract: With improvements in recording, communication, and stimulation technologies, in addition to evolving research needs, there are continuous efforts to develop devices to help progress novel, therapeutic neuromodulation research. As new devices are introduced, there is a need to verify these systems' functionalities to increase confidence in their abilities to assist researchers in future investigations. However, the tools to fully characterize these systems' abilities are limited. This is particularly true for the case of capturing evoked potentials, a biomarker well-used to characterize cortical connectivity. There are numerous factors that may contribute to why we may not fully realize an evoked potential response *in vivo*, and this ambiguity becomes a barrier when debugging investigational systems. Here, we demonstrate a benchtop evaluation platform to evaluate the CorTec Brain Interchange's ability to capture evoked potential activity. With this characterization, we can assess how the Brain Interchange can support future efforts in better understanding neuroplasticity mechanisms and develop the accompanying signal processing needed to extract signals of interest. We utilize the NeuroTest board and assemble a testing rig to deliver simulated evoked potential activity in response to stimulation delivered by the Brain Interchange. In this benchtop environment, we go through various stimulation parameter permutations to identify the ideal configurations, system limitations, and appropriate data processing prior to *in vivo* applications. Insight gained from saline benchtop testing guides our signal processing approach to extract meaningful neurophysiological evoked responses collected with the Brain Interchange *in vivo*. Our findings

demonstrate the utility of this evaluation platform and the Brain Interchange's abilities to collect evoked potentials. Additionally, we present a more comprehensive understanding of this investigational device's functionalities that could be utilized for future research efforts towards modulating plasticity and improving the efficacy of potential therapeutic interventions.

Disclosures: H. Cho: None. **A. Gkogkidis:** A. Employment/Salary (full or part-time):; CorTec GmbH. **M. Buchheit:** A. Employment/Salary (full or part-time):; CorTec GmbH. **S.C. Cramer:** 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.; University of California, Los Angeles. **J.G. Ojemann:** 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.; University of Washington. **T. Denison:** F. Consulting Fees (e.g., advisory boards); CorTec GmbH. **J.A. Herron:** 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.; University of Washington.

Poster

PSTR289: BCI

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR289.19/J1

Topic: E.05. Brain-Machine Interface

Support: NIH UH3NS120191

Title: Measuring motor intent - a comparative analysis of Stentrode and scalp EEG

Authors: *N. CHETTY[1], J. BENNETT[2], P. YOO[3], K. KACKER[4], N. Y. HAREL[5], D. LACOMIS[6], N. OPIE[7], J. L. COLLINGER[8], T. J. OXLEY[9], D. PUTRINO[10], D. J. WEBER[11]; [1]Carnegie Mellon Univ., Pittsburgh, PA; [2]Synchron, Inc, New York, NY; [3]Neurosci., Synchron, Brooklyn, NY; [4]Biomed. Engin. Dept., Carnegie Mellon Univ., Pittsburgh, PA; [5]Neurology; Rehabil. and Human Performance, James J. Peters VA Med. Ctr., Bronx, NY; [6]Univ. of Pittsburgh Med. Ctr., Pittsburgh, PA; [7]Dept. of Med., Vascular Bionics Lab., Melbourne, Australia; [8]Univ. of Pittsburgh, Pittsburgh, PA; [9]Synchron Inc., New York, NY; [10]Mount Sinai Hosp., New York, NY; [11]Mechanical Engin. and Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: The Stentrode is a novel endovascular brain-computer interface (BCI) that is implanted within the superior sagittal sinus to record bilaterally from the motor cortices. The device has enabled people with severe paralysis to gain computer control and digital

communication. The Stentrode is currently undergoing an early feasibility clinical trial in the United States and to date, six participants with severe paralysis have been implanted.In order for any BCI to be viable long-term, the signals need to be high quality and remain stable over time to enable high-accuracy decoding of user intent. Multiple factors contribute to the signal-to-noise ratio (SNR) including distance to neural source, attention, electrical contact and spacing, referencing scheme, physiological noise (such as ocular, muscular, cardiac, etc), feedback, and environmental noise. While the Stentrode sits closer to the cortex, in the absence of interceding bone, than scalp electroencephalography (EEG) electrodes and would presumably have higher signal quality, there has been no reported comparison of signal quality in humans. Here, we explore the quality of vascular ECoG and scalp EEG signals during volitional motor attempts in one participant with paralysis due to ALS. Before the implant, the participant underwent a scalp EEG with a standard, commercially available 64-channel cap. During the session, they were visually cued to attempt various motor tasks, such as repeated flexion and extension of the wrists and ankles. After the Stentrode implant, the participant completed the same tasks. Signal quality was assessed by analyzing motor event related synchronization/desynchronization during cued movement tasks. SNR was defined as 10*log((Attempted movement band power)/(Resting band power)). During attempted flexion of both ankles, the most modulated channel on the EEG cap, Cz, had a mean SNR in the beta band (13-30 Hz) of -2.1 (a 37% decrease in RMS amplitude during attempted movement), while the most modulated channel on the Stentrode had an SNR of -2.4. Of particular interest is the high-gamma band (70-200 Hz), which offers rich, focal information with high utility in BCIs. In this band, the scalp EEG and Stentrode had SNRs of 0.58 and 2.2, respectively. The high SNR in the high-gamma band for the Stentrode, which doesn't face the attenuating effects of the skull, is promising for successful, reliable decoding of user intention.A variety of gestures across channels, frequency bands, and referencing schemes will be presented for both the scalp EEG and Stentrode. The ongoing study will continue to evaluate the signal quality and stability in multiple participants across modalities.

Disclosures: N. Chetty: None. **J. Bennett:** A. Employment/Salary (full or part-time):; Synchron employee. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock options from Synchron. **P. Yoo:** A. Employment/Salary (full or part-time):; Synchron. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock options from Synchron, patents related to Stentrode. **K. Kacker:** None. **N.Y. Harel:** None. **D. Lacomis:** 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.; Cytokinetics data monitoring committee, Mitsubishi Tanabe grant funding. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); UptoDate royalties. **N. Opie:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock options from Synchron, patents related to Stentrode, Cofounder of Synchron. **J.L. Collinger:** None. **T.J. Oxley:** A. Employment/Salary (full or part-time):; Synchron employee. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock options from Synchron, patents related to Stentrode,

Co-founder of Synchron. **D. Putrino:** None. **D.J. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock options from NeuroOne, stock options from NeuronOff, stock options from Reach Neuro, stock options from Iota Biosciences, stock options from Bionic Power, cofonder of Reach Neuro.

Poster

PSTR289: BCI

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR289.20/J2

Topic: E.05. Brain-Machine Interface

Support: UH3NS120191

Title: Neuroimaging predictors of Stentrode endovascular signal quality

Authors: *H. R. SCHONE[1], P. YOO[2], C. HERBERS[2], C. MOON[3], D. LACOMIS[3], T. J. OXLEY[4], D. J. WEBER[5], J. L. COLLINGER[1];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Synchron, Brooklyn, NY; [3]Univ. of Pittsburgh Med. Ctr., Pittsburgh, PA; [4]Synchron Inc., New York, NY; [5]Mechanical Engin. and Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Brain-computer interfaces (BCIs) are at the cusp of a paradigm shift in rehabilitative medicine, with clinical trials now being conducted in the home with the goal of restoring computer access and communication to people with significant motor impairments. One such technology is a endovascular BCI device known as the *Stentrode* (Synchron, Inc.) that aims to capture neural motor signals by positioning the device within the superior sagittal sinus (SSS) above motor cortex. Over the last 5 years, 10 people with severe motor impairments have been implanted with the Stentrode. Considering the progressive nature of ALS and previous evidence demonstrating ALS-induced cortical atrophy of motor cortex, it remains unclear whether the Stentrode's proximity to the M1 cortical surface impacts the neural motor signal. Separately, it is unclear whether pre-implant fMRI activity is predictive of movement-related signal quality. Moving towards clinical translation, a critical evaluation of the neural and clinical factors impacting the strength of the motor signal recorded by the Stentrode is warranted. Specifically, we investigated whether Stentrode signal quality is impacted by the following factors: (1) neuroanatomy, (2) functional activity, (3) neurovasculature and (4) motor impairment. To test this, all participants underwent pre-implant functional and structural MRI scans and post-implant CT scans. Post-implantation, Stentrode signal quality was assessed while subjects attempted cued movements using either their preferred movement (ankle or hand) or the ankle only. During the pre-implant fMRI scans, all participants showed the ability to significantly activate sensorimotor cortices during attempted ankle movements. Following implantation, participants

varied in the distance of their Stentrode relative to the cortical surface, as well as device overlap with pre-implant functional activity and anatomically defined cortical motor regions, in particular primary motor cortex. Additional analyses evaluate the cortical thickness of M1 and S1 relative to healthy controls and the width of the SSS. Finally, we used linear mixed-effects models to estimate the statistical relationship between clinical and neural measures with Stentrode signal quality. Future work will also evaluate whether signal quality has an impact on the degree of BCI performance that can be achieved. Overall, this work provides a critical framework to guide future Stentrode implantations suggesting that targeting motor neuroanatomical landmarks is sufficient to enable recording of movement-related signals, despite variability in other potential predictors of success.

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Poster

PSTR289: BCI

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR289.21/J3

Topic: E.05. Brain-Machine Interface

Support: UH3NS120191

Title: A comparative analysis of spatial filtering techniques on a Stentrode

Authors: *A. K. FELDMAN[1], N. CHETTY[1], K. KACKER[1], J. BENNETT[2], P. E. YOO[2], N. Y. HAREL[4], D. LACOMIS[5], N. OPIE[3], J. L. COLLINGER[6], T. J. OXLEY[2], D. F. PUTRINO[7], D. J. WEBER[1];
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Abstract: The Stentrode is a minimally invasive brain-computer interface (BCI) that can enable computer access for people with paralysis. Implanted endovascularly in the superior sagittal

sinus, the Stentrode is able to record field potentials bilaterally from the motor and somatosensory cortices, as well as the supplementary motor area. Other, more invasive BCIs that require neurosurgery, such as a linear depth electrode or an electrocorticographic grid, typically have an electrode layout that spans 1 or 2 dimensions, respectively. The Stentrode, on the other hand, has a unique spatial geometry - the electrodes form a cylindrical pattern about a vector in the sagittal axis of the human brain. Such a distribution of contact placement may be leveraged to better identify, separate and localize signal and noise sources in endovascular electrocorticographic (eECoG) recordings.

The Stentrode is currently undergoing an Early Feasibility clinical trial in the United States on 6 participants with severe paralysis. Here, we present data from one participant with Amyotrophic Lateral Sclerosis, where we apply and compare several spatial filtering techniques to enhance the Stentrode's signal quality and spatial pattern discrimination. The participant was instructed to perform a motor training task without feedback, consisting of approximately 5±1s rest periods followed by performance of 5 attempted movements over a period of 5s. Attempted movements involved: left ankle, right ankle, both ankles, left hand, right hand, and both hands. We then applied several spatial filtering techniques, including Laplacian re-referencing, principal component analysis, independent component analysis (ICA), and spatio-spectral decomposition, to the resulting eECoG recordings. Signal quality of the spatially filtered time-series was evaluated over the attempted movement (signal) and rest (noise) intervals, within the standard human cortical frequency bands: Alpha (8-13 Hz), Beta (13-30 Hz), Low Gamma (30-60 Hz) and High Gamma (60-200 Hz), to determine the band power and signal to noise ratio. The spatial patterns that align with the strongest signals from each technique are then evaluated to estimate the location of neural signal sources. We find that band-limited ICA results in the strongest separability of electrocardiographic artifacts from the recorded signals, and that choice of referencing scheme alters the signal quality.

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royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock options from Synchron, Patent holder with Synchron, Cofounder of Synchron. **D.F. Putrino:** 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 grant funding (UH3NS120191). **D.J. Weber:** 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 grant funding (UH3NS120191). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock options from NeuroOne, Stock options from NeuronOff, Stock options from Iota Biosciences, Stock options from Bionic Power, Stock options from Reach Neuro, Cofounder of Reach Neuro.

Poster

PSTR289: BCI

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR289.22/J4

Topic: E.05. Brain-Machine Interface

Support: ERC grant 101001448

Title: A power-efficient two-step data compression and distributed computing system for intracortical brain-computer interfaces

Authors: *H.-P. LIAW[1], Y. HE[1,2], L. GANDHAM[3,1], P. RUSSO[1,4], M. GOURDOUPARIS[1,3], C. SHI[4,1], Y.-H. LIU[5,4,3];
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Abstract: Over the past several decades, the number of electrodes allowed for simultaneous recording has doubled approximately every seven years. Large data from high-density electrode arrays stress every part of the recording system, including data transfer, storage, processing, and demand high power. An energy-efficient, online data compression method is urgently needed for large-scale electrophysiology recording.
Spikes are the sparse information carrier in the neural network. The proposed method, which compressed filtered data around the frequency band of action potentials by >100X with minimum distortion, consists of three parts:
1) A compressive signal sampler compares the extracellular signal between different timestamps, i.e., temporal delta, and outputs the difference as 2-bit ternary pulse trains. The delta-modulation signal potentially relieves the computational load for the following steps.

2) A spiking neural network compresses the delta-modulated events by ~10X. The training method used knowledge distillation from an autoencoder to reduce the network size on the implant while maintaining the quality of the reconstructed signal.
3) An event-based serializer packs pulse trains into packets tailored for energy-efficient wireless transmission. Leveraging the sparsity of spikes and spatial redundancy of high-density electrodes, the patented method achieves a compression ratio of >11X while consuming <1% of energy compared to state-of-the-art appliances.
Energy efficiency is crucial for avoiding heat dispersion to surrounding tissue. Together with the tissue-coupled transdural data telemetry and ultrasound powering method developed in the group, our intracranial Brain-computer interface can be free-floating and easily scalable to record multiple brain areas.

Disclosures: H. Liaw: None.

Poster

PSTR289: BCI

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR289.23/J5

Topic: E.05. Brain-Machine Interface

Title: Report from a long-term study with the Brain Interchange in a healthy sheep model - surgical approach and histopathology outcomes

Authors: *M. SCHUETTLER, M. BUCHHEIT, **C. GKOGKIDIS**;
Cortec GmbH, Freiburg Im Breisgau, Germany

Abstract: Long-term preclinical testing of neural interfacing technology in biological test systems is a cornerstone to evaluate investigational medical devices prior to first-time human applications. Two key factors are feasibility and ease of surgical approach suitable to ensure stability of device placement and the impact of chronic electrode implantation on brain and surrounding tissues. The present good laboratory practice study was designed to address, amongst others, these two factors. Nine (9) female adult sheep were implanted with functional Brain Interchange (BIC) Systems (CorTec GmbH, Freiburg, Germany) fitted with two electrocorticographic (ECoG) electrode arrays (2x6 contacts, 2.4 mm contact diameter, 5 mm vertical and 10 mm horizontal pitch), implanted subdurally and bilaterally on fronto-parietal cortex. The BIC body was implanted in the dorsum and cables were routed along the neck using a surgical tube tool. Craniotomies were performed with a triagonal burr hole approach and subsequent opening of the dura mater for electrode placement. Animals were followed-up for 182 days with direct cortical electrical stimulation regularly applied to the left hemisphere only. In four animals the feasibility of electrode removal was assessed prior to brain extraction and fixation. In five animals electrode were left *in situ*. Brains were trimmed and sections were

embedded in either in resin or paraffin that were processed with various staining and immunohistochemistry methods. The ease of implantation was rated very good with electrode placement procedure durations of a few minutes. Post-implant X-ray imaging showed flat and parallel positioning of electrode arrays and the stability of positioning was confirmed in pre-explant imaging in 8 out of 9 animals. In one animal, electrode arrays were folder along the longitudinal axis. Despite, no adverse events or severe adverse histopathological findings were reported for any study animal. Histopathological examination revealed fibrotic capsules around the electrodes in all animals and overall minimal to moderate tissue reactivity, with slightly different findings in the electrode removal group. Severity scores were similar across all animals and between stimulated and non-stimulated tissue sections. The results demonstrate that the ECoG-based BIC system can be safely and effectively be implanted with routine surgical procedures on the sheep cortex with long-term array position stability and without considerable impact on the tissular integrity. These findings pave the way for future preclinical neuroscientific studies and support the BIC's suitability for chronic human medical application.

Disclosures: M. Schuettler: A. Employment/Salary (full or part-time):; CorTec GmbH. **M. Buchheit:** A. Employment/Salary (full or part-time):; CorTec GmbH. **C. Gkogkidis:** A. Employment/Salary (full or part-time):; CorTec GmbH.

Poster

PSTR289: BCI

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR289.24/J6

Topic: E.05. Brain-Machine Interface

Title: Report from a long-term study with the Brain Interchange in a healthy sheep model - ECoG-based recording and stimulation

Authors: *A. GKOGKIDIS[1], M. BUCHHEIT[1], H. CHO[2], J. A. HERRON[3], M. SCHUETTLER[1];
[1]CorTec GmbH, Freiburg, Germany; [2]Dept. of Electrical and Computer Engin., Univ. of Washington, Seattle, WA; [3]Dept. of Neurolog. Surgery, Univ. of Washington, Seattle, WA

Abstract: Preclinical functional testing of implantable neural interfaces in both benchtop and biological test systems is one principal requirement in preparation for first human and potentially medical therapeutical application. Today's future-oriented neural interface applications demand basic bi-directional functionality: neural sensing with a sufficient spatial sampling and number of channels and flexible configuration of electrical brain stimulation paradigms. The fully implantable Brain Interchange (BIC) System (CorTec GmbH, Freiburg, Germany) provides these functional requirements: 32-channel recording and adaptable stimulation configurations. To assess the capabilities in a long-term setting, the system was implanted for 182 days in nine (9)

adult female sheep. Animals were implanted subdurally and bilaterally on the fronto-parietal cortex with two electrocorticographic (ECoG) electrode arrays (2x6 contacts, 2.4 mm contact diameter, 5 mm vertical and 10 mm horizontal pitch) attached to the BIC. Neural signals were obtained (1 kS/s sampling) under general anesthesia, post-mortem and in awake behaving animals. In the latter, recording and stimulation sessions were conducted within a regular predefined session schedule. Each session included impedance measurements, several minutes of resting/idle state recordings and different stimulation paradigms (1 Hz and 50 Hz, bi-phasic asymmetric charge-balanced pulses, first phase 250 µs pulse width, stim. Amplitudes up to 5.4 mA). Signals were analyzed regarding general properties in the states they have been recorded in. In addition, long-term resting/idle state signal behavior was investigated by signal, noise and signal-to-noise ratio (SNR). The ECoG-based stimulation data were analyzed regarding long-term functionality and stimulation-induced brain activity changes. The results show different signal characteristics for awake, anesthetized, and post-mortem state. In the awake state, signals show consistent characteristics over time with a moderate decline in neural signal strength and noise but stable SNR pointing to a stable and low system noise. Stimulation data analysis revealed changing maximum applicable amplitudes, presumably due to increases in impedance, but without loss of stimulation functionality. The findings indicate biological origin of long-term signal property and impedance behavior possibly caused by fibrotic capsule build-up around the arrays. In summary, the results demonstrate both the importance of preclinical device testing and the readiness of the BIC system to provide the two key functionalities necessary for future-human medical applications.

Disclosures: A. Gkogkidis: A. Employment/Salary (full or part-time):; CorTec GmbH, Freiburg, Germany. **M. Buchheit:** A. Employment/Salary (full or part-time):; CorTec GmbH, Freiburg, Germany. **H. Cho:** None. **J.A. Herron:** None. **M. Schuettler:** A. Employment/Salary (full or part-time):; CorTec GmbH, Freiburg, Germany.

Poster

PSTR290: Afferent and Descending Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR290.01/J7

Topic: E.07. Rhythmic Motor Pattern Generation

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Title: Spinal population dynamics underlying mammalian locomotor pattern generation

Authors: *C. WASHINGTON[1], L. N. WIMALASENA[2], N. GOVINDARAJAN[1], P. MEKDARA[3], N. AU YONG[2,4,5], A. LEVINE[3], C. PANDARINATH[1,6];
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Abstract: The spinal cord mediates the sensorimotor transformations underlying many voluntary behaviors. Perhaps the best-studied example of these is locomotion, where spinal circuits coordinate appropriately lifting and placing the foot at each step. Despite the rich history of studying the spinal cord, little is known about how networks of diverse spinal neural populations collectively coordinate their activity to generate motor output. A significant challenge is the technical difficulty of recording spinal activity in a freely moving animal; thus, previous studies have often resorted to studying isolated spinal cord preparations or spinalized animals. Here, we analyzed rare population recordings in the lumbar spinal cord of an awake, vertebrae-fixed mouse walking on a wheel at its own volition. A Neuropixels probe was inserted along the dorsal-ventral axis of the right spinal hemicord, such that recorded neural activity spanned both sensory-related and motor-related laminae. We applied AutoLFADS, an unsupervised deep learning method to infer latent dynamics, to provide de-noised firing rate estimates for sorted single-unit activity. To validate whether AutoLFADS provided superior de-noising over standard firing rate estimation methods (e.g., Gaussian smoothing), we evaluated how well firing rate estimates from each approach could predict simultaneously recorded behavior using linear decoding. Ipsilateral hindlimb kinematics were extracted from videos via DeepLabCut. Decoding toe marker velocity from the AutoLFADS-inferred firing rates substantially improved performance over decoding from smoothed spikes (quantified via R^2: 0.81 vs 0.63). We next used the kinematics to identify bouts of stable locomotion and annotated individual step cycles into their respective stance and swing phases. Visualizing the corresponding spinal population trajectories during locomotion revealed a low-dimensional periodic orbit with one period per step cycle. Identifying segments of each cycle's trajectories associated with the stance and swing phases revealed a clear organization of the state space: transitions between locomotor phases occurred at consistent state space locations – despite natural variations in the stance duration across individual cycles – suggesting that regions of spinal population state space hold a tight correspondence with the produced behavior. This work demonstrates the utility of applying a state space approach to interpret spinal network function and may serve as a framework for interrogating other spinal cord-mediated behaviors in the future.

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Poster

PSTR290: Afferent and Descending Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR290.02/J8

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH R01 NS130799
NIH F31 NS132514

Title: Lumbar spinal shox2 interneurons receive monosynaptic input from the lateral paragigantocellular nucleus in the adult mouse

Authors: *S. SINGH[1,2], L. YAO[1,2], W. HUANG[1], D. V. WANG[1], K. J. DOUGHERTY[1,2]; [1]Neurobio. and Anat., Drexel Univ., Philadelphia, PA; [2]Marion Murray Spinal Cord Research Center, Drexel University, Philadelphia, PA

Abstract: In vertebrates, the circuits underlying the promotion of locomotion span the central nervous system. The direct connection between supraspinal and spinal locomotor-related neurons is comprised of reticulospinal neurons which are thought to contact and provide tonic drive to the rhythm-generating layer of the spinal locomotor central pattern generator. Previous studies in the rodent and cat have identified the locations of reticulospinal input to locomotor-related circuitry. In addition to directly targeting motor neurons and commissural interneurons, neurons in the medial intermediate zone have been shown to receive dense reticulospinal input. This is consistent with the location of Shox2 INs, a putative rhythm-generating population. To date, the connection between reticular neurons and a genetically-identified population of locomotor-related lumbar spinal interneurons (INs) has not been examined in adult mice. Here, we hypothesize that lumbar spinal Shox2 INs receive monosynaptic input from excitatory reticulospinal neurons originating in the lateral paragigantocellular nucleus (LPGi). In adult Shox2cre; R26-lsl-tdTomato mice, we delivered bilateral injections of AAV9-CamKII-eGFP into the ventral caudal medulla, targeting the LPGi. Labeling of LPGi glutamatergic neurons was confirmed with RNAscope for vGlut2 RNA. We then performed immunohistochemistry on lumbar spinal slices to examine $eGFP^+/vGlut2^+$ synaptic puncta in close proximity to Shox2 interneurons. We found that excitatory synaptic puncta from projections originating in the LPGi are in close apposition to lumbar spinal Shox2 INs. In order to confirm functional connections electrophysiologically, we injected AAV9-CamKIIa-ChR2-eYFP into LPGi bilaterally and performed whole-cell patch clamp recordings 6 weeks later in lumbar spinal slices targeting Shox2 interneurons during light stimulation. We show that Shox2 INs receive light-evoked excitatory postsynaptic currents which persist after bath application of TTX+4AP. Taken together, this suggests that lumbar spinal Shox2 INs receive monosynaptic excitatory input from the LPGi in the medulla. Further experiments assessing this direct connection could reveal the precise role it plays in the context of locomotion and inform our ability to restore it after injury or disease.

Disclosures: S. Singh: None. **L. Yao:** None. **W. Huang:** None. **D.V. Wang:** None. **K.J. Dougherty:** None.

Poster

PSTR290: Afferent and Descending Control

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSF DBI 2015317

Title: Neural Dynamics in Locomotion: Sequence Generator Network Control of Rat Hindlimbs

Authors: *C. JACKSON[1], W. NOURSE[2], R. D. QUINN[3];
[1]Case Western Reserve Univ., Lyndhurst, OH; [2]Electrical Engin., Case Western Reserve Univ., Cleveland, OH; [3]Mechanical Engin., Case Western Reserve Univ., Cleveland, OH

Abstract: Mammalian locomotion is a complex behavior arising from interaction between neural and biomechanical systems, driven by rhythmic activity originating in the spinal cord. Although it has been extensively studied, the structure of the circuits that produce this behavior remains unknown. One approach to modeling the rhythmic activity is with half-center models, in which there are alternating periods of flexion and extension to coordinate muscle activity. While this approach is sufficient for simple antagonistic muscle pairs, it can be difficult to expand the controller for more complex models with muscle synergies. This work introduces a method of modeling the activity in the spinal cord with a population of neurons exhibiting a continuous cycle of activity, rather than the push-pull of half-centers. Our study integrates computational modeling techniques with biomechanical simulations to control the muscle activity in a pair of rat hindlimbs. The neural controller is based on a rank two connection matrix that exhibits oscillatory behavior akin to a limit cycle. This sequence generator network (SGN) is then connected to a motor circuit comprised of Ia inhibitory neurons, motor neurons, Renshaw cells, and interneurons to incorporate muscle feedback. Through iterative tuning and optimization, we demonstrate the effectiveness of our SGN-based neural model in controlling the hindlimbs, resulting in joint trajectories comparable to those observed during rat locomotion.

Disclosures: C. Jackson: None. **W. Nourse:** None. **R.D. Quinn:** None.

Poster

PSTR290: Afferent and Descending Control

Location: MCP Hall A

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Program #/Poster #: PSTR290.04/J10

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH/NINDS R01NS110550

Title: Effects of spinal transection and treadmill speed on muscle synergies of the cat hindlimb during locomotion

Authors: *A. KLISHKO[1], C. HANSON[2], I. A. RYBAK[3], A. FRIGON[4], B. I. PRILUTSKY[5]; [1]Georgia Inst. of Technol., Atlanta, GA; [2]Biol. Sci., Georgia Inst. of Technol., Atlanta, GA; [3]Dept. of Neurobio. and Anat., Drexel Univ., Philadelphia, PA; [4]Dept. of Pharmacology-Physiology, Univ. de Sherbrooke, Sherbrooke, QC, Canada; [5]Biol. Sci., Georgia Inst. Technol., Atlanta, GA

Abstract: It was suggested that during locomotion, the nervous system controls multiple muscles by combining them in a smaller number of groups (muscle synergies) and activating each of them by a specific time-dependent activation pattern. For example, we found that during treadmill forward and backward walking in spinal-transected cats (Harnie et al., 2021) and during overground level and slope working in intact cats (Klishko et al., 2021), most of the variance of EMG activities from 12-15 muscles was accounted for by 5 synergies and their activation patterns. Analysis of muscle synergies during locomotion can help reveal the organization of spinal locomotor networks and if/how it changes in different locomotor conditions before and after spinal cord injury. The goal of this study was to investigate the effects of locomotor speed and spinal transection on the number and composition of muscle synergies and their time-dependent activity patterns in adult cats. We hypothesized that muscle synergies and their composition are mainly controlled at a spinal level. EMG activities of 15 hindlimb muscles were recorded bilaterally in 9 adult cats of either sex during tied-belt treadmill locomotion at speeds of 0.4, 0.7, and 1.0 m/s before and after spinal transection at low thoracic level. Muscle synergies were extracted from recorded EMGs by non-negative matrix factorization. Five extracted synergies for each of six experimental conditions (3 speeds x 2 spinal cord states) accounted for at least 90% of variance in muscle EMG activities. There were two flexor synergies (groups of mostly flexor muscles) activated during the swing phase, and three extensor synergies composed of mostly extensor muscles activated during the stance phase. In the intact state, the composition of muscle synergies and their activation patterns generally did not depend on locomotion speed except for a few muscles and a slight delay in the activation onset of synergies with increasing speeds. Although the general muscle compositions of the five (two flexor and three extensor) synergies after spinal transection were similar to intact locomotion at different speeds, there were significant discrepancies in muscle contributions to synergies. In the spinal state, activation patterns were generally less correlated between the speeds within synergies, and synergy activations started earlier. We conclude that after spinal transection, muscle contributions to the synergies and their time-dependent activation patterns become less regular, although the general organization of the synergies during locomotion is maintained.

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Poster

PSTR290: Afferent and Descending Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR290.05/J11

Topic: E.07. Rhythmic Motor Pattern Generation

Title: An experimental staircase to study the contribution of sensory feedback to motoneuronal drive during stair climbing in able bodied humans

Authors: A. ANDERSEN[1], P. ABEDI[1], A. J. STEVENSON[2], ***T. SINKJÆR**[1];
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Abstract: The involvement of sensory feedback during complex motor tasks like stair climbing is not yet known. We have developed an experimental staircase where one of the stair steps can be moved slightly up, down, further away, or rotated when the participant is about to step on it. In this way we may compare the behavioral and neural consequences of correct and incorrect prediction of changed shape and time of ground contact during an unconstrained natural stair walking task. In the present study, we present the experimental staircase and investigated the involvement of afferent feedback contributions to motoneuronal drive during stair climbing. Participants climbed a seven-step perturbation stair apparatus unrestrained. Either no perturbation took place (Study 1) or on random trials the fourth step was moved 2,5 cm downward at foot contact (Study 2), producing a “drop” of the step and causing an “unload” of the ankle joint. All data were recorded from the left leg. Participants were instrumented with bipolar surface electromyography (EMG) electrodes over the soleus (SOL) and tibialis anterior (TA) muscles of the left leg. Foot-strike was recorded using a force sensitive resistor placed under the sole of the left shoe. Left ankle angle was recorded using a surface mounted electrogoniometer. The changes in the SOL EMG at spinal and supraspinal latencies as a function of changed ankle joint velocity were analyzed. The spinal SOL EMG response at short latencies increased with the velocity of the ankle joint angle in Study 1. During “drop” perturbations of the stair step in Study 2, a distinct unload response was measured. We speculate that the changes in SOL EMG at spinal latencies reflects how sensory feedback on a moment-to-moment basis contributes to motoneuronal drive during complex motor tasks like stair climbing.

Disclosures: A. Andersen: None. **P. Abedi:** None. **A.J. Stevenson:** None. **T. Sinkjær:** None.

Poster

PSTR290: Afferent and Descending Control

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Janelia visitor project

Title: Activity maps of orofacial rhythms

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Abstract: Rhythmic orofacial behaviors involve overlapping muscles and must be coordinated. For example, we speak only on exhalations. Networks of premotor neurons in the brainstem (central pattern generators) autonomously coordinate rhythmic movements, including breathing, chewing, drinking, and swallowing. Understanding this neural coordination is fundamentally important for a host of survival behaviors.
We used Neuropixel probes to map rhythmic activity related to breathing and licking in the mouse brainstem as mice licked for water. Orofacial movements were tracked with high-speed videography, while breathing was recorded with an airflow meter. Across 340 penetrations in 31 animals, 19,000 units were recorded across the pons and medulla. Recordings were aligned to the Allen Mouse Brain Common Coordinate Framework.
Distinct spatial clusters of medulla neurons exhibited rhythmic activity phase locked to breathing and licking. Breath synchronized units were localized to the ventral intermediate reticular nucleus (IRN), the preBotzinger complex, and the nucleus ambiguus. Units synchronized to licking were broadly distributed across the IRN, the trigeminal nucleus, and the hypoglossal nucleus, which coincided with the regions containing the jaw and tongue pre-motor and motor neurons. Within the regions showing licking-related activity, we further mapped the putative licking oscillator using optogenetic activation of Phox2b-expressing neurons (Dempsey et al., 2021). Rhythmic licking and associated jaw movements were selectively evoked by a cluster of neurons located in the posterior IRN.
Breathing and licking were tightly coordinated at the level of behavior, where licking was phase-

locked to breathing and did not coincide with inspiration. This coordination was bidirectional: inspiration rate influenced the lick timing and vice versa. At the level of neural activity, breathing and licking units showed functional coupling with anticorrelated activity. This phase relationship between licking and breathing was faithfully preserved even when licking was artificially induced by activating orofacial motor cortex. When mice volitionally initiated licking, the breathing phase was pre-emptively adjusted 120ms prior to licking onset to accommodate this licking-breathing coordination, suggesting an additional role of descending volitional control.
These results reveal multiple logics that govern two orofacial rhythms and outline a roadmap for their neural circuit substrate.

Disclosures: H. Kaku: None. **L.D. Liu:** None. **A. Finkelstein:** None. **S.J. West:** None. **K. Svoboda:** None. **N. Li:** None.

Poster

PSTR290: Afferent and Descending Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR290.07/J13

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH HL163008
NIH HL155721
NIH HL131716

Title: Microinjection of gabazine into the PreBÖtzinger complex suppresses swallowing in the cat

Authors: *D. BOLSER[1], T. SHEN[2], I. POLIACEK[4], M. J. ROSE[2], Z. KOTMANOVA[5], M. N. MUSSELWHITE[3], L. MARTVON[5], J. A. HAYES[2], P. W. DAVENPORT[6], T. PITTS[7]; [2]Physiological Sci., [3]Neurosci., [1]Univ. of Florida, Gainesville, FL; [4]Jessenius Fac. of Med., Institue fo Med. Biophysics/Comenius Univ., Martin, Slovakia; [5]Med. Biophysics, Comenius Univ., Martin, Slovakia; [6]Physiological Sci., Univ. Florida, Gainesville, FL; [7]Speech Language and Hearing Sci., Univ. of Missouri, Columbia, Columbia, MO

Abstract: The swallow motor pattern generator consists of the dorsal swallow group (DSG) in the nucleus tractus solitarius. DSG is proposed to generate the swallow motor command and control the temporal aspects of the behavior. The ventral swallow group (VSG), located in the nucleus ambiguus/ventral respiratory column and surrounding reticular formation, is proposed to distribute the swallow command sequentially to the cranial and spinal motoneuron pools. The PreBÖtzinger complex (PreBOT) has a critical role in generating the breathing rhythm and may participate in the control of orofacial behaviors, including swallowing. We have previously

shown that microinjection of the nonspecific glutamate receptor antagonist, kynurenic acid, into the PreBOT had no effect on the number of swallows elicited by water injection into the pharynx; but did increase swallow duration and suppress the magnitudes of laryngeal elevator muscle electromyograms (EMGs). We hypothesized that microinjection of gabazine (GBZ), a GABA-B receptor antagonist, into the PreBot would suppress swallowing in response to water injected into the oropharynx. Upper airway muscle EMGs were recorded in anesthetized, spontaneously breathing cats (n=3). Swallowing was induced by injection of 3 cc of water into the oropharynx. Multi-barrel micropipettes were employed to inject artificial cerebrospinal fluid or GBZ (100 µM, 50 nL per injection) bilaterally into the PreBOT region. Microinjection sites were confirmed histologically by detection of fluorescent beads that were suspended in the microinjectate. Microinection of GBZ into the PreBOT significantly reduced swallow number by up approximately 50% within 20 minutes and 75% by 80 minutes post injection. By 140 minutes post injection, swallow number had recovered to within 32% of control. In one animal, swallowing was eliminated for 100 minutes. We conclude that GABA-B receptors in the PreBOT are important in the production of swallowing in the anesthetized cat. Further, these data support the concept that neurons in this area are involved in functionally important pathways with the DSG.

Disclosures: D. Bolser: None. **T. Shen:** None. **I. Poliacek:** None. **M.J. Rose:** None. **Z. Kotmanova:** None. **M.N. Musselwhite:** None. **L. Martvon:** None. **J.A. Hayes:** None. **P.W. Davenport:** None. **T. Pitts:** None.

Poster

PSTR290: Afferent and Descending Control

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR290.08/J14

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH HL155721
NIH HL163008
NLHB HL103415
VEGA Project 1/0072/16
VEGA Project 1/0253/15

Title: Kynurenic acid injected into the nucleus tractus solitarius stunts swallow production

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Abstract: Swallowing is a behavior that requires the well-formed coordination of a series of muscles to convey a bolus of food/liquid down the esophagus. These muscles are controlled by motoneurons in different areas of the hindbrain and spinal cord and are also the target of other brainstem circuits such as respiratory networks. We conducted experiments in anesthetized, spontaneously breathing cats to determine the effects of drugs microinjected into the nucleus tractus solitarius (NTS) on swallow, while recording electromyograms from pharyngeal, laryngeal, and respiratory-related muscles.
Kynurenic acid (KYNA) is an endogenously produced metabolite of the essential amino acid tryptophan, and is a non-specific ionotropic glutamate receptor antagonist that may also have effects through other receptor pathways that are not fully understood. We found that when KYNA was injected into the NTS bilaterally it inhibited the coherent production of swallow from pharyngeal water stimuli. After microinjection of KYNA, swallows from water stimuli were impeded for up to an hour or more but gradually recovered. These data suggest a relatively long-term action by KYNA beyond simple acute antagonism of ionotropic glutamate receptors that creates an "open circuit" between the afferent sensory feedback from pharyngeal water stimulation to elements of the swallow pattern generator.

Disclosures: J.A. Hayes: None. **I. Poliacek:** None. **M. Rose:** None. **T. Pitts:** None. **D.C. Bolser:** None.

Poster

PSTR290: Afferent and Descending Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR290.09/J15

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NINDS
Department of Veteran Affairs
NIDILRR grant 90SIMS0015

Title: A portable system to measure knee extensor spasticity after spinal cord injury

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Abstract: The pendulum test is a quantitative method used to assess knee extensor spasticity in humans with spinal cord injury (SCI). Yet, the clinical implementation of this method remains limited. The goal of our study was to develop an objective and portable system to assess knee extensor spasticity during the pendulum test using inertial measurement units (IMU). Spasticity

was quantified by measuring the first swing angle (FSA) using a 3-dimensional optical tracking system (with external markers over the iliotibial band, lateral knee epicondyle, and lateral malleolus) and two wireless IMUs (positioned over the iliotibial band and mid-part of the lower leg) as well as a clinical exam (Modified Ashworth Scale, MAS). Measurements were taken on separate days to assess test-retest reliability and device agreement in humans with and without SCI. We found no differences between FSA values obtained with the optical tracking system and the IMU-based system in control subjects and individuals with SCI. FSA values from the IMU-based system showed excellent agreement with the optical tracking system in individuals with SCI (ICC > 0.98) and good agreement in controls (ICC > 0.82), excellent test-retest reliability across days in SCI (ICC = 0.93) and good in controls (ICC = 0.87). Notably, FSA values measured by both systems showed a strong association with MAS scores (pho ~ 0.80) being decreased in individuals with SCI with higher MAS scores, reflecting the presence of spasticity. These findings suggest that our new portable IMU-based system provides a robust and flexible alternative to a camera-based optical tracking system to quantify knee extensor spasticity following SCI.

Disclosures: D. De Santis: None. **M.A. Perez:** None.

Poster

PSTR290: Afferent and Descending Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR290.10/J16

Topic: E.07. Rhythmic Motor Pattern Generation

Title: Understanding Ankle Clonus in Humans with Spinal Cord Injury

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Abstract: Ankle clonus is an involuntary motor behavior involving rhythmic muscle contractions commonly observed in humans with spinal cord injury (SCI). Although clonus is associated with symptoms of spasticity its neural mechanisms remain poorly understood. Impaired inhibitory control in combination with altered regulation of motoneuronal persistent inward currents have been shown to serve as the basis for prolonged involuntary motor output. Therefore, the purpose of this study was to examine the relationship between the cutaneous reflex (early component, LPR; late component, LLR) and the presence of clonus in individuals with SCI. We tested individuals with chronic SCI ($\geq$ 1 year a post-injury) with short/no clonus (n=7) and long-clonus (n=9). The cutaneous reflex was elicited by electrical stimulation of medial plantar nerve and electromyographic activity was measured in the tibialis anterior muscle. Ankle clonus was elicited in individuals with SCI by applying the drop test and manual stretch to

the ankle joint (short/no clonus=6.2±2.4 seconds duration; long-clonus=134.4±126.7 seconds duration). We found that the duration of the long-lasting part of the cutaneous reflex (referred to as the 'LLR') was prolonged (1.3±0.6 seconds) compared to individuals with no clonus or short duration of clonus (0.2±0.09 ms) in participants with chronic SCI. In contrast, we found that the duration of the early component of the cutaneous reflex (referred to as the 'LPR') was similar between individuals with short/no clonus (0.4± 0.01 seconds) and long-clonus (0.4± 0.02). Our results suggested that adaptations in neuromodulatory receptors on motoneurons contribute, at least to some extent, to the generation of prolonged ankle clonus in humans with SCI.

Disclosures: E. Curuk: None. **M.A. Perez:** None. **C. Heckman:** None.

Poster

PSTR290: Afferent and Descending Control

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR290.11/J17

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant HD101395

Title: Neural effects of transcutaneous spinal cord stimulation in humans with chronic spinal cord injury

Authors: *M. FARLEY[1], C. HECKMAN[2], M. A. PEREZ[3,4];
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Abstract: Transcutaneous spinal cord stimulation (tSCS) is a neurostimulation approach that has been reported to improve motor outcomes in individuals with spinal cord injury (SCI). Yet, the neural mechanisms contributing to the reported clinical improvements remain not well understood. The purpose of this study was to examine the effect of a single session of lumbar tSCS applied between the L1-L2 vertebral interspace (30Hz pulses with a 10kHz carrier frequency) on the soleus H-reflex amplitude in participants with chronic SCI. tSCS was delivered at 50% of the intensity needed to elicit a 50μV peak-to-peak posterior root reflex in the soleus muscle. Soleus H-reflexes were tested using ~50% (half H-reflex) of maximal H-reflex amplitude (H-max) in participants with complete or incomplete chronic SCI before and after 20 minutes of tSCS stimulation. H-reflex amplitude was normalized to the size of each participant's maximum motor response (M-max). Average peak-to-peak amplitudes for the soleus M-max and H-max were 6.46mV ± 3.24mV and 3.26mV ± 2.50mV respectively. The average H-max amplitude was 49% ± 22% of M-max. At baseline, average half H-reflex amplitude was 24.46% ± 10.82% of the M-max. We found that soon after tSCS, the half H-reflex amplitude was

suppressed (Average amplitude = 11.11% ± 11.1% of the M-max) and remained suppressed up to 30 minutes after tSCS (Average amplitude = 15.17% ± 13.11% of the M-max). These results suggest tSCS delivered at 30Hz with a 10kHz carrier frequency suppresses Ia afferent input to motoneurons in participants with chronic SCI. The neural mechanisms contributing to this effect are under investigation.

Disclosures: M. Farley: None. **C. Heckman:** None. **M.A. Perez:** None.

Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.01/J18

Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant P41 EB018783 (Wolpaw)
NYS SCiRB C37714GG (Gupta)
NYS SCiRB C38338GG (Wolpaw)
Stratton Veterans Affairs Medical Center

Title: Soleus H-reflex size versus stimulation rate in the presence pf background muscle activity: A methodological study

Authors: *J. A. BRANGACCIO[1], D. GUPTA[2], J. HILL[3], H. MOJTABAVI[4], R. L. HARDESTY, Jr.[5], J. S. CARP[7], T. M. VAUGHAN[8], J. J. NORTON[10], D. E. GEMOETS[9], M. A. PEREZ[11], J. R. WOLPAW[6];
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Abstract: Objective: Hoffmann reflex (H-reflex) operant conditioning is a successful treatment intervention of growing interest and importance in the rehabilitation of individuals with neurological injury (doi:10.3389/fnint.2014.00025). During conditioning sessions, H-reflexes are typically elicited at low frequency (~0.2 Hz) to avoid the rate-dependent depression that occurs at faster rates in the absence of background electromyographic activity (EMG). The resulting long session duration may be demanding for participants. This study examined the impact on H-

reflex size when stimulating at faster rates. We used the protocol described in Thompson et al. (doi:10.1523/JNEUROSCI.3968-12.2013), in which a stable low level of background EMG activity is present during H-reflex elicitation.
Methods: Fifteen healthy participants (7 females, 8 males, aged 51±19SD years) maintained a low level of background soleus EMG activity (5-18mV, ~1-3% of the maximum direct muscle response (M-wave)) while standing. Soleus H-reflex and M-wave recruitment curves were obtained at frequencies of 0.2, 1, and 2 Hz. Maximum M-wave size (Mmax), maximum H wave size (Hmax), and M and H stimulation thresholds (Mthresh and Hthresh) were calculated from the curves. Then, 75 H-reflex control trials were collected for each stimulation frequency at the stimulation level that produced a target M-wave size (~10-20% of Mmax). This sequence was completed twice for each interval in random order for each participant.
Results: Linear mixed effects models were used to compare the effects of protocol (i.e.frequency) and session (i.e. run) per participant. Recruitment curves elicited at the three stimulation frequencies were similar, with no significant differences in Mmax, Hmax, Mthresh, or Hthresh ($p>0.3$). In the control trials, Hmax (% of Mmax) was slightly but significantly lower at 1 Hz and 2Hz than at 0.2 Hz (-12±4SE ($p=0.02$) and -10±6SE ($p=0.04$), respectively, as % change).
Discussion: Rate-dependent H-reflex depression is known to be reduced by background activation of the muscle. The present results show that H-reflex conditioning protocols like those used in our lab can stimulate at frequencies as fast as 2 Hz with only minimal reduction in H-reflex size compared to our standard 0.2 Hz. The ability to present the H-reflex stimuli more quickly may facilitate faster and possibly more effective H-reflex conditioning that is also less demanding for the participant.

Disclosures: J.A. Brangaccio: None. **D. Gupta:** None. **J. Hill:** None. **H. Mojtabavi:** None. **R.L. Hardesty:** None. **J.S. Carp:** None. **T.M. Vaughan:** None. **J.J. Norton:** None. **D.E. Gemoets:** None. **M.A. Perez:** None. **J.R. Wolpaw:** None.

Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.02/J19

Topic: E.09. Motor Neurons and Muscle

Support: NIH NINDS U44NS114420

Title: Assessing motor unit activity during the evoked H-reflex in healthy individuals

Authors: *E. MEYERS[1], N. TACCA[2], M. DARROW[3], D. FRIEDENBERG[4], M. L. MCKINNON[5], J. J. NORTON[6], J. A. BRANGACCIO[7], J. HILL[8], J. S. CARP[9];
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Abstract: Spasticity is an extremely debilitating condition caused by increased excitability in stretch reflex pathways, and commonly occurs in neurological disorders such as stroke, cerebral palsy, spinal cord injury, and multiple sclerosis. Current treatments, such as Botox injections and baclofen, only offer temporary relief, have serious side effects, and aim to manage the symptoms rather than treating the underlying cause. To help address this significant unmet clinical need, our group has developed a method to reduce the size of the H-reflex (the electrical analogue of the stretch reflex) by operant conditioning (HROC), which has been demonstrated to induce wider beneficial plasticity and reduce hyperreflexia in patients with spasticity. Our previous work has shown that these effects are mediated largely through the corticospinal tract (CST). Techniques to examine corticospinal signaling in real-time during HROC may offer new insights into the effectiveness of the treatment and provide an avenue for optimizing HROC. High-density electromyography (HD-EMG) has recently emerged as a technique to examine activity within a muscle with high spatial resolution, and, when combined with blind source separation (BSS), can detect single motor unit activity, which reflects the activity of spinal cord motoneurons. Analysis of motor unit data has demonstrated causal relationships with beta oscillations in motor cortex, thus providing indirect measures of CST signaling. In this study, we collected data using a HD-EMG grid (BioCircuit) over the soleus muscle of the leg while eliciting the H-reflex in 8 healthy volunteers. HD-EMG signals were decomposed into motor unit activity using a convolutive blind source separation model (Formento, 2021). We demonstrate that motor unit firings synchronize with the activation of the direct muscle (M wave) and H-reflex responses, with consistent activation patterns across all participants. These results provide an early feasibility demonstration of reliably extracting motor unit firings during the M wave and the H-reflex, despite the expected high degree of synchronization of motor unit firing. Future work will examine the utility of motor units in the context of H-reflex conditioning, including using motor units as a tool to measure M wave and H-reflex responses, investigate the mechanisms of HROC, and serve as a potential biomarker for predicting responsiveness to conditioning.

Disclosures: E. Meyers: None. **N. Tacca:** None. **M. Darrow:** None. **D. Friedenberg:** None. **M.L. McKinnon:** A. Employment/Salary (full or part-time):; BioCircuit. **J.J. Norton:** None. **J.A. Brangaccio:** None. **J. Hill:** None. **J.S. Carp:** None.

Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.03/J21

Topic: E.09. Motor Neurons and Muscle

Support: Meta Platforms, INC

Title: Closed-loop Control of Motor Unit Activity After Tetraplegia for Human-Computer Interactions

Authors: *D. L. DESPRADEL[1], L. BORDA[1], N. VERMA[1], P. YADAV[1], J. D. SHANAHAN[2], N. J. MARSHALL[3], E. FORMENTO[3], M. BRÄCKLEIN[3], J. YE[3], D. MORRISON[3], P. WALKINGTON[3], N. MAHESWARANATHAN[3], C. WARRINER[3], R. RAJALINGHAM[3], D. SUSSILLO[3], S. NAUFEL[3], D. A. GUTNISKY[3], J. L. COLLINGER[4], D. J. WEBER[5];
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Abstract: A growing number of studies have demonstrated that most people with motor complete spinal cord injuries (SCIs) retain the ability to activate motor units below the level of injury, offering the potential to use myoelectric controllers for computer-based tasks. Building upon this knowledge, we present a novel wearable neuromotor interface driven by surface electromyography (sEMG). This innovative wristband device, designed for seamless plug-and-play functionality, adeptly captures myoelectric activity from the forearm. By translating intentional neuromotor commands into computer inputs, this interface introduces a new medium for human-computer interaction (HCI), particularly benefiting individuals with SCIs. Our study demonstrates real-time detection and decoding of motor units (MUs) in three individuals with tetraplegia (AIS A or B), enabling closed-loop control of computer-based tasks via a non-invasive neuromotor interface worn on the forearm. Our methodology involved detecting individual MUs as participants attempted gestures with their dominant hand while recording sEMG activity. Despite the absence of visible movement, distinct patterns of MU activity corresponding to attempted digit and wrist movements were identified. Through rest and tonic activation cycles, participants calibrated a spike sorting model, which recorded and sorted MU spiking data. These MU activities were then mapped to different degrees of freedom (DOF), enabling control over cursors/characters in training tasks and multi-DOF games. We then analyzed participants' control strategies and performance across sessions, ensuring a minimum of two MUs controlled per session, each associated with a different movement. Performance metrics included speed, task completion time, achievement of objectives (e.g., reward collection, obstacle avoidance), and task completion consistency within designated time frames. Furthermore, we assessed participants' ability to control a generalized model — a neural network classifier trained on data from 300 nonclinical participants, optimized for minimal movement — to predict five movement classes from sEMG signals. Remarkably, participants could accurately control one output of the generalized model despite their injury. In summary, these findings highlight the potential of MU firing as control inputs for computer tasks, enabling individuals with tetraplegia to participate in gaming and social interactions. This device's wearability and

ease of use offer an innovative HCI solution. By leveraging residual MU and sEMG activity, this innovation promises to enhance the quality of life for those with SCI.

Disclosures: D.L. Despradel: None. **L. Borda:** None. **N. Verma:** None. **P. Yadav:** None. **J.D. Shanahan:** None. **N.J. Marshall:** None. **E. Formento:** None. **M. Bräcklein:** None. **J. Ye:** None. **D. Morrison:** None. **P. Walkington:** None. **N. Maheswaranathan:** None. **C. Warriner:** None. **R. Rajalingham:** None. **D. Sussillo:** None. **S. Naufel:** None. **D.A. Gutnisky:** None. **J.L. Collinger:** None. **D.J. 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, and Bionic Power; cofounder of Reach Neuro.

Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.04/J22

Topic: E.09. Motor Neurons and Muscle

Support: Meta

Title: Motor Unit Yield and Location Using High-Density Surface Electromyography from the Forearm of Persons with Tetraplegia

Authors: *M. MURPHY[1,2], D. DESPRADEL[3], L. BORDA[3], N. VERMA[3], P. YADAV[3], J. A. BEAUCHAMP[4], J. SHANAHAN[3], J. L. COLLINGER[5,2], D. WEBER[3];
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Abstract: Many individuals with tetraplegia due to spinal cord injury (SCI) retain the ability to modulate motor unit action potentials (MUAPs) in the upper extremity, presenting a promising avenue for new or augmented assistive interfaces. This study utilized high-density surface electromyography (HDsEMG) to map the location and density of upper extremity MUAPs in persons with mid-cervical SCI. We covered the right proximal and distal forearm extensors and flexors using four 8x8 arrays of HDsEMG electrodes having 8.75mm pitch (TMSi SAGA64+). Participants performed an array of unimanual attempted gestures, which were demonstrated and subsequently cued by a 3D hand animation. An optical motion capture camera system (Vicon) was used to track the movement of the right digits and wrist, which was negligible for most joints. During intended gestures, EMG signals were clearly modulated without visible overt hand movements. EMG signals for each selected gesture were amplitude normalized using the average EMG root-mean-square (RMS) power computed over an epoch during which the participants were instructed to exert maximum effort in producing the gesture without moving. The EMG

RMS envelope on the same electrodes was subsequently used to control a unidimensional ramp-to-step target task with online visual feedback of the intended gesture strength for 10%, 30%, and 50% of the calibrated RMS level.
Motor unit decomposition using the convolutional kernel compensation (CKC) technique yielded up to 13 distinct MUAPs (pulse-to-noise range: 20.3 - 31.3 dB) across the distal and proximal extensor and flexor grids. MUAPs were primarily localized to the proximal arrays, and were most-prevalent during the 50% ramp conditions. During ramp trials, MUAPs predominantly followed the expected recruitment profile from low-to-high threshold units, but there were often units that deviated from the expected recruitment-decruitment profile. These atypical MUAPs were likely the result of patient-reported spasms, which were not evident in the kinematics of the digit and hand markers. In the 50% ramp and maximum effort calibration trials, a subgroup of MUAPs that continued firing with a phase lag relative to the cessation of gesture intent may have been related to the activation of persistent inward currents (PICs), as could be expected following sustained motor recruitment over a period of several seconds. These findings indicate that despite limited or non-existent ability to move the distal extremities, myoelectric wearable sensors placed appropriately on the forearm of persons with cervical spinal injury can still detect the subtle signals related to motor recruitment.

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Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.05/J23

Topic: E.09. Motor Neurons and Muscle

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NIH Grant UL1TR00235
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McDonnell Center for Systems Neuroscience at Washington University in St. Louis
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Title: Heterogeneity of pathophysiological voluntary motor commands in patients with multiple sclerosis

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Abstract: Multiple sclerosis (MS) is a demyelinating, neurodegenerative disease within the central nervous system. Damage can occur throughout the central nervous system and can therefore affect voluntary motor commands at any stage of processing. Voluntary motor commands originate cortically and are shaped at all levels of the neuraxis, culminating as three types of inputs to spinal motoneurons: excitatory, inhibitory, and neuromodulatory. These components must be appropriately balanced for skilled motor output. MS is heterogeneous in terms of lesion locations, clinical symptoms, and disease course, which impedes systematic research of neurophysiological correlates of motor dysfunction. Our ongoing study is characterizing excitatory, inhibitory, and neuromodulatory components of the voluntary motor command to gain insight into the heterogeneous neural mechanisms of motor deficits in MS. To do so, we use high-density surface EMG decomposition and a novel paradigm for reverse engineering of motor unit population discharge that provides information on the distribution of excitatory input across the motoneuron pool, the pattern of inhibitory input relative to excitatory input, and the level of neuromodulatory input. We have tested 30 ambulatory patients with MS with a range of disability levels, from none to severe. For some of the reverse engineering parameters, such as delta-F and maximal firing rate, there were group mean decreases within the MS group compared with age- and sex-matched controls that were generally consistent among participants. For other parameters, such as acceleration slope and self-sustained firing duration, there were no group mean differences but values in the MS group were more variable than the control group, with a range extending higher and lower than control values. Given that the reverse engineering parameters are sensitive to more than one type of motor command component, we also employed principal component analysis as an unsupervised machine learning method to explore how the groups differed in terms of linear combinations of the variables. The principal component that best separated the groups was consistent with the pattern of inhibitory input, ranging from proportional to excitatory input to reciprocal to excitatory input. Additionally, the MS group was more variable than the control group for all principal components. Examination of individual participant principal component values may yield insight into the specific type of pathophysiology in their motor commands, informing the development and selection of personalized, mechanistically-targeted neuroplasticity-inducing rehabilitation interventions.

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Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.06/J24

Topic: E.09. Motor Neurons and Muscle

Support: NINDS Grant F31NS130767

Title: A multi-site analysis of motor unit discharge patterns in females across the menstrual cycle

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Abstract: Studies on the neural control of movement have historically excluded females, leading to a reasonable understanding in males but significant gaps in our understanding of female motor physiology. Studies that include females are often underpowered or do not examine across the menstrual cycle. Given the sex-related differences in neurological disease and human performance, there is a great need to study females. Emerging evidence has revealed sex-related differences in motor unit (MU) discharge patterns and neuromuscular fatigue across the menstrual cycle, but the mechanism behind these differences is unknown. One possibility is altered motoneuronal persistent inward currents (PICs) across the menstrual cycle, which are higher in females. PICs are facilitated by serotonin and norepinephrine from the brainstem nuclei and are responsible for amplification and prolongation of MU discharge. Estradiol and progesterone, two hormones that fluctuate across the menstrual cycle, have known effects on monoamines, and consequently may affect PIC magnitude and MU discharge in females. The purpose of our multi-site research study is to quantify discharge properties in females across the menstrual cycle. At four universities, we analyzed MU discharge properties and hormone levels at the early follicular, late follicular, and late luteal phases. At each session blood samples were taken, and high-density surface electromyographic arrays recorded muscle activity from the tibialis anterior during isometric contractions. Plasma levels of estradiol and progesterone were quantified, and blind source separation algorithms were used to identify MU spike times. PIC magnitudes were estimated by quantifying MU discharge rate hysteresis (ΔF) and the nonlinearity of firing with respect to torque (brace height). Preliminary data indicate that in the luteal phase, when estradiol and progesterone levels are elevated, ΔF is higher (5.84 ± 0.35 pps) compared to the early (5.45 ± 0.34 pps) and late (5.37 ± 0.35 pps; $\chi 2 = 8.14$, $p = 0.017$) follicular phases. Brace height was higher in the early follicular (0.422 ± 0.015) and luteal (0.45 ± 0.016) phases compared to late follicular phase (0.38 ± 0.016; $\chi 2 = 22.95$, $p < 0.001$), when only estradiol is elevated. These findings suggest that increased levels of sex hormones across the

cycle impact estimates of PICs and may increase descending monoaminergic drive to MUs, alter patterns of inhibition, and/or have direct effects on intrinsic motoneuron properties. These novel findings underscore the necessity of studies on female participants both to ensure a comprehensive understanding of motor physiology and to strive towards scientific equity.

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Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.07/J25

Topic: E.09. Motor Neurons and Muscle

Support: R01HD039343
R01NS098509

Title: Monoaminergic contributions to post hemiparetic stroke motor impairments

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Abstract: A stroke-induced loss of corticospinal tracts forces a greater reliance on weak and indirect motor pathways from the contralesional hemisphere. Furthermore, damage to cortico-reticular tracts from the lesioned hemisphere perturbs the balanced control of reticulospinal projections, potentially leading to a dysregulation of monoaminergic output to the spinal cord. Monoamines (e.g., norepinephrine) are potent drivers of motoneuron excitability, and modulate dendritic persistent inwards currents (PICs) in spinal motoneurons to amplify excitatory synaptic inputs. Consequently, in chronic stroke, monoaminergic dysregulation may yield a pathological deviation to motoneuron excitability that exacerbates motor impairments. Specifically, greater monoamines increase spinal motoneuron excitability and, therefore, could yield hyperexcitable motoneurons that contribute to hypertonicity/spasticity while also amplifying weak and indirect

motor pathways that generate flexion synergy expression.
To support this framework, we carried out two studies on individuals with chronic hemiparetic stroke. In the first study, we used high-density surface EMG and convolutive blind source separation to decompose biceps brachii motor units during both a volitional elbow flexion contraction and a non-volitional contraction generated during flexion synergy expression. We then used outcome metrics developed from realistic models of spinal motoneurons to reverse engineer the motor commands employed during these two distinct contractions. Across eleven individuals, we found significant changes in outcome metrics during the flexion synergy task, indicative of greater monoaminergic drive (increase in brace height by 7.92 %rTri, 95% CI: [3.74 12.1], d = 0.46) and an increase in inhibitory inputs to motoneurons (decrease in attenuation slope by 0.370 pps/%MVT, 95% CI: [0.191 0.549], d = 0.50). Together these changes indicate a shift in the descending motor commands employed in chronic stroke, suggesting greater monoaminergic output and use of indirect motor pathways that drive both excitatory and inhibitory inputs to motoneurons (e.g., reticulospinal). In a second pilot study, we modulated monoaminergic output with Tizanidine (alpha-2 agonist) to confirm its role in the changes in motor unit characteristics observed in the first study. As expected, preliminary analysis indicates a reversal in the motor unit outcome metrics quantified in the first study. Collectively, these data suggest that a shift in descending motor strategy (i.e., greater monoaminergic output and reliance on indirect motor pathways) may drive motor impairments in chronic stroke.

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Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.08/J26

Topic: E.09. Motor Neurons and Muscle

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NIH R01NS125863
NIH T32HD007434
McDonnell Center for Systems
Neuroscience at Washington University in St. Louis
Foundation for Barnes-Jewish Hospital

Title: Latent variables underlying motor unit firing patterns in a diverse set of muscles

Authors: *D. FREE[1], T. REECE[2], S. SIMON[3], G. E. PEARCEY[5], J. A. BEAUCHAMP[6], S. T. JENZ[7], M. FAJARDO[8], C. HECKMAN[9], K. LOHSE[3], L. M. MCPHERSON[4];
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Abstract: The motor unit, consisting of a single α-motoneuron and the muscle fibers it innervates, is a neuromechanical transducer that transforms neural inputs from afferent, spinal, and descending sources into motoneuron discharge patterns and resulting muscle forces. The neural inputs that converge on the motoneuron constitute the motor command and are classified into three types: excitatory, inhibitory, and neuromodulatory. Motoneurons have complex and malleable input/output functions that depend on the mixture of excitatory, inhibitory, and neuromodulatory input. Recently, a reverse engineering paradigm was developed to identify temporal features of motoneuron discharge that can estimate aspects of excitatory, inhibitory, and neuromodulatory input. However, the common parameters used are sensitive to more than one type of input, which limits the ability to draw unique physiological interpretations from findings. The first purpose of the study was to determine whether there are linear combinations of the motor unit discharge parameters that reflect latent factors common to some or all of the parameters (exploratory factor analysis on motor unit level data). The rationale is that any identified latent factors may better reflect excitatory, inhibitory, and neuromodulatory inputs than individual variables alone. Previous work has shown that muscles with different structures and function have different parameter values on the individual parameters. Thus, the second purpose of the study was to determine whether linear combinations of the motor unit discharge parameters could distinguish between motor units of different muscles better than individual parameters (principal component analysis on participant means). We present our findings from the above analyses conducted on motor unit discharge parameters calculated from populations of motor units measured from the tibialis anterior, soleus, deltoid, biceps, triceps, finger flexors, finger extensors, and first dorsal interosseus using high-density surface EMG decomposition.

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Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.09/J27

Topic: E.09. Motor Neurons and Muscle

Title: Characterization of ankle musculature motor unit discharge patterns in humans with incomplete spinal cord injury

Authors: *A. BENEDETTO[1,2,3], S. T. JENZ[1], M. FARLEY[1,3,2], J. A. BEAUCHAMP[4,5], C. HECKMAN[1,6], M. A. PEREZ[7,5,3], G. E. PEARCEY[8,5];
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Abstract: Motor commands are comprised of excitatory, inhibitory, and neuromodulatory components, and disruptions in descending tracts caused by spinal cord injury (SCI) affects all three of these components. The aim of this study was to determine if these disruptions alter motor unit discharge patterns in the ankle musculature of individuals with an incomplete spinal cord injury. Experiments were performed on seven people (1 female), aged 21-50 years old (35.7 ± 10 yrs.) with chronic, motor incomplete SCI at the cervical or thoracic level. Participants were seated with their foot secured to a force transducer and high-density surface electromyographic arrays were placed over the tibialis anterior (TA) and triceps surae muscles. Dorsiflexion and plantar flexion maximal voluntary isometric contractions (MViC) were used to normalize subsequent contractions, after which participants performed submaximal isometric triangular and trapezoidal ramps between 20 - 70% of MViC. Blind source separation was used to identify motor unit spike times and persistent inward currents (PICs) were estimated using the paired-MU analysis technique, which quantifies discharge rate hysteresis (delta frequency; ΔF). Data from seven non-injured (NI) subjects (mean age 28.9 ± 6 yrs., 1 female) from a previous study was used as control data for comparison. Preliminary analysis of TA motor units (SCI n = 274; NI n = 452) decomposed during 30% MViC triangular ramps revealed that peak firing rate was 23.6% lower in the SCI group (12.3 ± 0.85 pps) compared to the NI group (16.1 ± 0.69 pps). Firing rate modulation across the contraction was 24.8% lower in the SCI group (8.26 ± 0.61 pps) compared to the NI group (10.9 ± 0.57 pps). Discharge rate hysteresis (ΔF) was normalized to account for this difference in rate modulation and was found to be 10.4% higher in the SCI group (0.76 ± 0.04 pps, n = 142) compared to the NI group (0.69 ± 0.04 pps, n = 337). These results suggest a potential role for increased PICs to compensate for the loss of firing rate modulation and help restore the ability to produce functional movements after SCI. Participant recruitment and analysis is ongoing and will be presented in person.

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Poster

PSTR291: Human Motor Units and Physiologic Recordings

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Topic: E.09. Motor Neurons and Muscle

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NIH Grant R01NS125863
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McDonnell Center for Systems Neuroscience at Washington University in St. Louis
Foundation for Barnes-Jewish Hospital

Title: Longitudinal assessment of voluntary motor commands to spinal motoneurons in patients with multiple sclerosis

Authors: *S. SIMON[1], D. FREE[1], T. REECE[1], K. LOHSE[1], A. H. CROSS[2], L. M. MCPHERSON[1];
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Abstract: Multiple sclerosis (MS) results in demyelination and axonal degeneration within the central nervous system (CNS) that can degrade neural control of nearly all major physiological systems. MS is often characterized by episodic relapses of new or worsening neurological symptoms driven by focal inflammatory demyelination. However, 72% of patients with MS experience a gradual, progressive advancement of clinical symptoms associated with "smoldering" neurodegenerative processes that occur diffusely throughout the brain and spinal cord. Both relapse-associated and progressive symptoms lead to accumulating disability over time, often due to motor symptoms such as weakness and impaired gait and balance. Detection and prevention of relapses has been a major focus of MS research for several decades. However, the progressive component of MS is less well understood. Both relapse-associated and progressive symptoms contribute to the accumulation of disability over time, often due to motor symptoms such as weakness and impaired gait and balance. In a recent cross-sectional study with patients with MS, we characterized pathologic changes in voluntary motor commands at the level of spinal motoneurons. Using motor unit population discharge decomposed from high-density surface EMG from the tibialis anterior and soleus muscles, we implemented a reverse engineering paradigm to characterize excitatory, inhibitory, and neuromodulatory components of the voluntary motor command. Here, we present data from 1 year follow up visits from the same participants. We assessed how our measures changed in MS participants after a year compared with age- and sex-matched control participants without MS.

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Poster

PSTR291: Human Motor Units and Physiologic Recordings

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Topic: E.09. Motor Neurons and Muscle

Support: NSERC Discovery Grant 2023-05862
NIH Grant R01NS098509-01
NIH Grant R01NS098509-05

Title: Diverse motor unit firing patterns reflect divergent motor command structure across human arm muscles

Authors: *G. PEARCEY[1,2], J. A. BEAUCHAMP[4], S. T. JENZ[2], M. FAJARDO[2], F. NEGRO[5], J. P. DEWALD[3], L. M. MCPHERSON[6], C. HECKMAN[2];
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Abstract: Human muscles are diverse in terms of both their structure and function, ranging from large, proximal muscles for stabilization to small, distal muscles for dextrous movements. Each muscle is controlled by recruitment and rate modulation of its population of motor units, whose firing patterns are generated by processing of excitatory, inhibitory, and neuromodulatory inputs (i.e., motor commands) by the input-output properties of motoneurons. Therefore, if either motor commands or motoneurons are specialized for control of muscles with different structures and functions, these specializations should manifest as firing pattern differences across muscles. Neuromodulatory inputs facilitate persistent inward currents (PICs) that induce a high degree of excitability in motoneurons. While strong PICs seem appropriate for proximal muscles, they may impede fine control in distal muscles. Therefore, we hypothesized the existence of a heretofore unknown motor command gradient along the arm, with neuromodulatory effects on PICs being strongest proximally and weakest distally, and tested this hypothesis by comparing motor unit firing patterns from 6 different muscles along the arm (deltoids, triceps brachii, biceps brachii, finger extensors, finger flexors, first dorsal interosseous [FDI]) in 15 neurologically intact participants. High-density surface electromyographic signals were decomposed via blind source separation to identify firing patterns of several motor units during 10 second linear increases to 20% of maximum, followed by a 10 second linear relaxation to rest for each muscle group. Our results generally support our hypothesis. Delta F, which quantifies firing rate hysteresis induced by PICs, was greatest in the deltoids (6.44 [5.94; 6.94] pps) and smallest in the first dorsal interosseous (4.06 [3.65; 4.46] pps). Brace height, which quantifies non-linearity in the ascending discharge rate modulation and is proportional to neuromodulatory input, was also greatest in the deltoids (44.5 [42.7; 46.3] %) and smallest in the FDI (40.7 [39.2; 42.1] %). Post-acceleration firing rate modulation, which is inversely related to depolarization induced by PICs, was greatest in the FDI (-0.01 [-0.06; 0.05] pps/% effort) and smallest in the deltoids (-0.18 [-0.241; -0.123] pps/% effort). These findings suggest the existence of a divergent structural arrangement in the descending commands to motoneuron pools with diverse functional roles.

This gradient aligns with the classical studies showing strong brainstem (e.g., reticulospinal) input proximally versus strong projection of the corticospinal input distally.

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Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.12/J30

Topic: E.09. Motor Neurons and Muscle

Support: European Research Council Consolidator Grant INcEPTION (contract no. 101045605 to FN)

Title: Corticospinal transmission to spinal motor neuron output using repeated transcranial magnetic stimulation.

Authors: M. SANTOS[1], H. V. CABRAL[1], A. RIZZARDI[2], M. BENEDINI[3], M. DESMONS[1], E. POURREZA[1], A. PADOVANI[1], A. PILOTTO[1], ***F. NEGRO**[1];
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Abstract: Repetitive transcranial magnetic stimulation (rTMS) has been extensively used to modulate cortical excitability in psychiatric and neurological disorders. However, the extent to which different rTMS frequencies are effectively transmitted to spinal motor neurons remains unexplored. Here, we investigated the corticospinal transmission of different rTMS-induced oscillations in the primary motor cortex (M1) to the neural drive to muscles. Eight participants performed 10% MVC isometric thumb flexions, while high-density surface electromyography (HDsEMG) was recorded from the thenar muscles. During the contractions, rTMS pulses were delivered on M1 contralaterally to the tested hand at 70% of the resting motor threshold. Stimulation frequencies of 5, 10, 20, 30, 50 Hz were randomly applied. HDsEMG signals were decomposed into motor unit (MU) spike trains using a convolutive blind source separation algorithm. Z-coherence between the stimulation trigger (i.e., input) and the cumulative spike trains (CSTs) of decomposed MUs (i.e., output) was calculated and normalized by the number of MUs for each participant. Moreover, in a subgroup analysis (n = 4), we matched MUs between pre and during rTMS to calculate within muscle z-coherence. For both analyses, we quantified the area under the curve at each stimulus frequency and subsequent harmonics up to 100 Hz (windows of 2 Hz around each frequency). We applied statistical parametric mapping (t-test) to assess statistical significance and verify whether the areas under the z-coherence values differed from 0. On average, the number of MUs on each frequency were 11 (5Hz), 10 (10Hz), 8 (20Hz),

9 (30Hz) and 8 (50Hz). Main results showed that at low-frequency rTMS (5 and 10 Hz), significant coupling between the input and output occurred only at harmonic frequencies >= 20 and 30 Hz, respectively (P<0.001 for both). Conversely, for rTMS at 20, 30, and 50 Hz, significant coupling was observed at the stimulus frequency and subsequent harmonics (P < 0.009). The results were confirmed in the subgroup analysis. These preliminary findings suggest that the transfer function of the corticospinal pathway to the alpha motoneuron output induced by rTMS has a marked bandpass behavior. In other words, high-frequency (> 20 Hz) cortical rTMS-induced oscillations appear to be more efficiently transmitted into the neural drive to the muscle. Therefore, this study provides insights into the corticospinal transmission of rTMS during isometric contractions and the effects of neuromodulation in pathological conditions. Additionally, it opens new perspectives on identifying robust neural biomarkers of corticospinal connectivity in humans.

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Poster

PSTR291: Human Motor Units and Physiologic Recordings

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.13/J31

Topic: E.09. Motor Neurons and Muscle

Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001

Title: Isometric handgrip contraction increases estimates of persistent inward currents in dose-dependent manner in tibialis anterior motoneurons

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Abstract: Spinal motoneurons can generate long-lasting depolarising persistent inward currents (PICs). PICs can accelerate, amplify, and prolong motoneuron firing output according to the level of serotonergic input onto the motoneuron. Remote muscle contractions can diffusely increase serotonergic input onto the spinal cord, facilitating persistent inward currents (PICs) in motoneurons innervating muscles unrelated to the task. In humans, a handgrip remote contraction at 40% of maximal force, held for 30 s, can increase estimates of PICs in the tibialis anterior. However, it is not yet known whether the remote contraction intensity level, the duration, or the relationship between intensity and duration (i.e., impulse) would theoretically

result in different levels of serotonergic release at the spinal cord, thus differently influencing tibialis anterior PIC responses. We investigated whether handgrip intensity, duration, and/or impulse would determine tibialis anterior estimates of PICs response. Electromyograms were recorded, from the tibialis anterior of 21 young adults (18-40 years, 6 women), using a 64-channel electrode matrix during dorsiflexion contractions before and after four handgrip conditions: i) 80%15s, high-intensity (80% of their maximal handgrip strength), short-duration (15 s) handgrip contraction; ii) 40%15s, low-intensity (40%) short-duration (15s); iii) 40%30s, low-intensity (40%) and long-duration (30 s); and iv) Control. Conditions 80%15s and 40%30s matched impulse. Electromyograms were then decomposed into individual motor unit spike trains and the PIC contribution to motoneuron firing was estimated as the delta frequency (ΔF) using the paired motor unit analysis. We also used a quasi-geometric approach to calculate the 'brace height' of the ascending phase of the motor unit firing rates (normalized as a % of maximal), to quantify the non-linearity caused by the effect of monoaminergic drive on motoneuron firing patterns. As a result, we found that ΔF increased similarly by 0.30 pps (95%CI 0.11-0.49) after 40%30s and by 0.20 pps (0.04-0.36) after 80%15s but remained unchanged after 40%15s and Control conditions. A similar behavior was observed on brace height, which increased by 2.39 (0.55-4.23) % after 40%30s and by 2.74 (1.14-4.34) % after 80%15s; and remained unchanged after 40%15s and Control conditions. Thus, the theoretical increases in serotonergic input onto the spinal cord following a remote handgrip contraction, and consequent increases in tibialis anterior neuromodulatory drive and estimates of PIC, are dependent on a certain level of impulse rather than muscle contraction intensity or duration alone.

Disclosures: L. Campos Ugliara: None. **L. Orssatto:** None. **A. Vieira:** None. **G. Trajano:** None.

Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.14/J32

Topic: E.09. Motor Neurons and Muscle

Support: Grant from the Novo Nordisk Foundation to Prof. Jakob Lorentzen
Novo Nordisk Foundation to prof. Ole Kiehn
Department of Neuroscience

Title: The calcium channel blocker, Nimodipine, inhibits spinal reflex pathways in humans

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Abstract: The study investigated the acute effect of Nimodipine on the transmission in human spinal reflex pathways (the H/M-ratio and the stretch reflex) that are involved in the development of spasticity. This served to locate the action site of Nimodipine in the human spinal cord, and to explore the prospect of Nimodipine's use in future antispasmodic treatment.
In a double-blinded cross-over study, we tested the transmission in spinal neural pathways in nineteen healthy subjects before and after Nimodipine (tablet 60mg) and Baclofen (tablet 50mg) by computer-controlled muscle stretches, and electrical stimulation of mixed and cutaneous nerves combined with EMG measurements. The size of the stretch reflex and H/M-ratio were statistically analysed using one-way RM ANOVA.
Nimodipine significantly reduced the H/M-ratio at 30, 60, and 90 minutes ($p < 0,0001$) and the stretch reflex ($p = 0,04$) after oral delivery. A comparable, but less pronounced effect, was seen by control Baclofen (H/M-ratio: $p=0.0242$; stretch reflex: $p=0.017$). Interestingly, both the M-wave and stretch reflex, when evoked during voluntary activation of the Soleus muscle, were unchanged by both drugs. No severe adverse effects were reported by any of the participants when treated with Nimodipine.
This suggests that Nimodipine acutely reduces electrophysiological measures of spasticity in healthy individuals. The effect seems to be located at the spinal level, and voluntary activation compensates for the inhibition caused by the drug on the stretch reflex, highlighting its potential in future antispastic treatment.

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Poster

PSTR291: Human Motor Units and Physiologic Recordings

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Topic: E.09. Motor Neurons and Muscle

Support: European Research Council - Consolidator Grant, Agreement Number 101045605
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Title: Motor unit discharge and recruitment behaviour in human muscles: a systematic-review and meta-analysis

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Abstract: Since the seminal work of Sherrington, the motoneuron has drawn much interest as the final common pathway of the neuromuscular system. The study of motoneurons in humans has focused on the activity of the motor unit (MU) during various tasks. Increased interest and

technological advancements have led researchers to attempted to characterize MU behaviour throughout force gradation. However, because of the wide range of methods used across studies in relatively small sample sizes, it has been challenging to draw broad conclusions based on data from single studies. The primary aim of this systematic-review was to identify and summarize the findings of studies investigating MU discharge behaviour in various human muscles during isometric voluntary contractions. The secondary aim was to determine the influence of force output on MU discharge behaviour. Studies were searched from 1950 to 2024 in PubMed, Medline, and Web of Science databases. Included studies were primary surface or intramuscular electromyography (EMG) MU behaviour investigations using voluntary isometric contractions on baseline or control human subjects ≥18 years and who were free of neuromuscular impairment. Searches resulted in 14,759 papers identified, 8,931 remained after removing duplicates. 561 screened papers were identified for full text retrieval (33 not retrieved). Of the 528 remaining papers, after screening, 250 were included for data extraction. In addition, 11 were included through hand-searching. A data table was constructed from retrieved data and analysed to address the above research questions. Meta-means of MU discharge rate (MUDR) were calculated using multi-level models with random intercepts for study-level and were weighted using inverse-variance method. Subgroup analyses splitting the force outputs into HIGH (> 60%MVC) and LOW (≤ 20%MVC) were performed for the tibialis anterior (TA), vastus lateralis (VL), vastus medialis (VM), biceps brachii (BB), and first dorsal interosseous (FDI). There were significant increases in MUDR from the LOW to HIGH force output for all muscles (All $p < 0.001$; TA: 10.15 [9.66 10.64] pps; VL: 3.82 [3.37 4.27] pps; VM: 3.36 [2.74 3.99] pps; BB: 11 [9.81 12.18] pps; FDI: 8.92 [7.93 10.04] pps). Although all muscles MUDR increased from LOW to HIGH force output, the greatest differences were seen in the TA, BB and FDI compared to the VL and VM which may be related to differences in central and/or peripheral mechanisms. Overall, in the preliminary data, there is a significant increase in MUDR from LOW to HIGH force output suggesting the reliance on rate coding in the modulation of force. However, the magnitude of increase may be muscle dependant.

Disclosures: J. Inglis: None. **H.V. Cabral:** None. **C. Cosentino:** None. **A. bonardi:** None. **F. Negro:** None.

Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.16/J34

Topic: E.09. Motor Neurons and Muscle

Title: Differences in motor unit activity during movement depending on muscle structure

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Abstract: Muscles have a variety of characteristic structures, such as biceps, fusiform, and bipennate muscles. Each muscle plays a different role depending on its attachment position, such as muscle force, speed, and endurance. However, the relationship between these structural differences and motor unit (MU) activity in static and dynamic tasks has not been clarified. This study aimed to describe the differences in neuronal control in muscles with different structures based on motor unit activity and myofiber dynamics.Three healthy adult male subjects were used as subjects, and the target muscle was the medial head of the gastrocnemius muscle and tibialis anterior muscle (MGAS and TA). BIODEX, Ultrasounds, and HDEMG were used. The target torque was set at 10 and 25% MVC. The task was isokinetic contraction at each target torque. The joint motion was controlled using BIODEX so that the joint angular velocity was 5 and 10 degrees per second. MUs with a PNR of 28 dB or higher were included. In the analysis, the maximum pennation angle (PA), delta muscle fiber length (delta-L), Mean discharge rate (MDR), and discharge rate increase/decrease rate (Slope) during the task were calculated.In isometric contraction, TA delta-L tended to be longer in dorsiflexion than in plantar flexion, but MDR showed no similar trend, although there were significant differences in some joint angles. On the other hand, MGAS PA tended to be significantly smaller in dorsiflexion than in plantar flexion, and the MDR tended to be significantly smaller as well. In isokinetic contractions, the slope of TA tended to increase significantly with increasing velocity, but this was not the MGAS case.The MGAS is a bipinnate muscle and is continuously attached to the Achilles tendon. So, shortening of the muscle length increases the PA, but the muscle and the tendon also slackness, requiring more neuromuscular activity. However, as the muscle lengthens and speed increases, it is suggested that the tendon is used for passive and efficient neuromodulation. In isokinetic contractions, slope decreased significantly with increasing velocity, suggesting that changes in velocity do not affect neuromuscular activity regulation. On the other hand, TA is a fusiform muscle with no large tendon tissue, suggesting that the amount of neuromuscular activity required is constant regardless of the muscle length change. In isokinetic contractions, the neuromuscular activity is regulated in response to changes in velocity. Thus, neuromuscular control differs depending on muscle structure, and the presence or absence of tendon tissue may also alter the regulation of neuromuscular activity.

Disclosures: K. Nogi: None. **T. Kokubun:** None.

Poster

PSTR291: Human Motor Units and Physiologic Recordings

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Topic: E.09. Motor Neurons and Muscle

Support: NSERC discovery 2020-04157

Title: Velocity-dependent Motor Unit Firing Rates and Muscle Activation in Unconstrained Dynamic Contractions

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Abstract: Our current understanding of the neural control of movement is largely based on isometric or highly constrained dynamic contractions. To better understand the neural strategies underlying "real world" movement, we sought to record motor unit (MU) firing behavior during unconstrained movement (squat jumps) using decomposition EMG. Twenty participants (24.2±4.5 years, 9 females) performed 3 blocks of maximal squat jumps. Each block included 3 sets of 5 jumps. Sets were completed at 30%, 60%, or 90% of maximal velocity of an unloaded squat jump (V30, V60, V90 respectively) in a randomized order. Surface EMG was recorded from vastus medialis (VM) and vastus lateralis (VL) to permit EMG decomposition and analysis of motor unit discharge rate (MUDR). Bicep femoris (BF) and gluteus medius (GM) muscle activation was also recorded. Muscle activation was expressed as % maximal EMG amplitude (%maxEMG). Kinematic variables of the squat jump were recorded using a linear position transducer. Repeated-measures ANOVA was used to assess peak, average and initial MUDR, and muscle activity. During the concentric phase, peak VL MUDR increased as velocity decreased (V30: 24.09 ± 6.31Hz, V90: 21.51 ± 4.37Hz, $p<0.01$, $\eta_p^2=0.29$). However, peak VL MUDR was correlated to peak force ($r = 0.35$, $p<0.01$). Additionally, initial MUDR (first 4 spikes), increased as velocity increased (V30: 13.38 ± 4.68Hz, V90: 15.73 ± 3.63Hz, $p < 0.01$, $\eta_p^2 = 0.65$) and the recruitment interval decreased (V30: 0.38 ± 0.22s, V90: 0.15 ± 0.07s $p < 0.01$, $\eta_p^2 = 0.69$). Furthermore, there was an effect of velocity on overall muscle activation of both antagonist (BF) and synergist (GM) muscles, where BF (V30: 72 ± 28%maxEMG, V90: 56 ± 40%, $p < 0.01$, $\eta_p^2 = 0.27$) and GM (V30: 83 ± 40%maxEMG, V90: 72 ± 28%maxEMG, $p < 0.01$, $\eta_p^2 = 0.23$) activity decreased as movement velocity increased. This effect was also seen with direct coactivation (BF:VL, and GM:VL) of the BF (V30: 1.33 ± 0.78, V90: 1.02 ± 0.94% $p < 0.01$, $\eta_p^2 = 0.3$) and GM (V30: 1.43 ± 0.68, V90: 1.19 ± 0.45, $p < 0.01$, $\eta_p^2 = 0.32$) at peak VL activation. Nevertheless, since MUDR is correlated with force production, changes in velocity of a squat jump does not affect MUDR of vastii knee extensors when ANCOVA was performed to account for force ($p <0.05$, $\eta_p^2 = 0.09$). However, increasing velocity did cause increased initial MUDR and shortened the recruitment interval implying that velocity may play a role on early recruitment strategies. Furthermore, both overall activation and coactivation of an antagonist and synergist muscle decreased with increasing movement velocity which effects the velocity-dependent neural control of the unconstrained dynamic movement.

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Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.18/J36

Topic: E.09. Motor Neurons and Muscle

Title: A non-invasive, quantitative method for detection of muscle fatigue and neural mechanism of compensation during muscle fatigue

Authors: *B. ZHAO, K. CHAROONSRISAWAD, R. M. ICHIYAMA, S. CHAKRABARTY; Univ. of Leeds, Leeds, United Kingdom

Abstract: Surface electromyography (sEMG) is used to identify muscle fatigue, leading to increased muscle activity and a shift to its lower-frequency components. These feature changes provide insights into muscle recruitment patterns and putative pathways that control, helping investigate muscle fatiguability and underlying neural processes. Currently, most fatigue research uses isometric tasks, and pathways are examined using transcranial magnetic stimulation and electrical stimulation, providing insight on certain parts of fatigue processes. However, knowledge of the processes leading to fatigue demands research of dynamic tasks. In our study median frequency (MDF) and root mean square (RMS) characteristics of sEMG signal, during fatigue-inducing (FI) exercise, was analysed. Participants completed four sets of dynamic (15 curls in 30 seconds) and static (30-second hold at a 90º elbow angle) tasks with decreasing rest periods (90-30 seconds) between sets with a 4kg dumbbell. Eight upper limb muscles, in 15 healthy volunteers (19-39yrs, 5 females). All participants self-reported measure of exhaustion, using Borg RPE (Rating of Perceived Exertion), and 6 also conducted a Maximum Voluntary Contraction (MVC) before and after the FI exercise. All participants reported a significant increase in Borg RPE ($P < 0.0001$, one-sided t-test) at the end of set 4 (8±1.7). MVC output significantly decreased post-FI exercises ($P < 0.0001$, two-sided permutation t-test). Across both static and dynamic segments of the task, an overall decrease in MDF and an increase in RMS was observed across all muscles in all subjects. The difference, in mean of MDF and RMS, between the first and last set of the task was significant ($P < 0.0001$, two-sided permutation t-test). However, variability and the gradient of change in MDF and RMS between sets differed for individual muscles in both tasks, and these parameters changed differentially for each muscle as the FI exercise progressed. Our findings suggest this protocol induces muscle fatigue, as indicated by the change in RPE and MVC; along with change in MDF and RMS in both the dynamic and static segments of the task. Fatiguability of the muscle was also observed in both segments. We propose that fatiguability can be better defined using the combination changing gradient measures of RMS and MDF, providing details about muscle activity and fibres recruitment affected by fatigue induction. These measures might also provide mechanistic information about the compensatory mechanisms employed by the nervous system to cope with generation of the required force even when a muscle is fatigued.

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Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.19/J37

Topic: E.09. Motor Neurons and Muscle

Title: Distribution of voluntary drive within triceps surae muscles varies with ankle position

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Abstract: Although triceps surae (TS) muscles share a common insertion and act together to plantarflex the ankle, prior research has shown minimal shared common inputs within these muscles. Each TS muscle exhibits distinct changes in motor unit (MU) firing and electromyography (EMG) amplitude in response to altered ankle position. However, how the voluntary neural drive of TS muscles, which is directly linked to muscle force generation, changes with ankle position remains unknown. Intramuscular coherence (IntraCoh) in the delta band (0-5 Hz) was computed for TS muscles to assess voluntary drive during isometric plantarflexions (PFs) at various ankle positions. Thirteen healthy young adults participated in the study. High density EMG was used to extract MU activity from the medial (SOLM) and lateral soleus (SOLL), and medial (GM) and lateral gastrocnemii (GL). Participants performed maximal voluntary contractions (MVC) followed by submaximal trapezoidal isometric PFs at 20% MVC at three ankle positions: 20°PF, 0°PF (neutral), and 20° dorsiflexion (DF). Z-transformed pooled MU coherence over the plateau was compared using a two-way ANOVA. MVC torque at 0°PF (716±355 Nm) and 20°DF (790±448 Nm) were greater than that at 20°PF (383±225 Nm) (both $p < 0.01$). We observed a contrasting pattern in Delta IntraCoh of GM and GL as ankle position changes, and Delta IntraCoh increased linearly as muscle length increased for SOLM and SOLL. At 20°PF, Delta IntraCoh of GM was greater than that of GL, and both were greater than SOLL and SOLM (all $p = 0.00$). Thus, there was greater voluntary drive to gastrocnemii (GAS) compared to soleus (SOL) at a shortened position. At 0°PF, Delta IntraCoh was greatest in GM and lowest in GL, and SOLM exhibited greater Delta IntraCoh than SOLL (all $p = 0.00$). This suggests greater voluntary drive to the medial heads compared to lateral heads of TS at a neutral position. At 20°DF, Delta IntraCoh was greatest in SOLL but lowest in GM, and SOLM exhibited greater Delta IntraCoh than GL (all $p = 0.00$). This indicates greater voluntary drive to SOL compared to GAS when muscles are lengthened. In contrast to findings at a neutral position, greater voluntary drive was observed in the lateral heads compared to the medial heads

of the TS at a lengthened position. The altered distribution of voluntary drive within TS muscles in response to changes in ankle position can be attributed to modulations of the two motoneuron synergies that innervate TS muscles. The higher muscle spindle density and the uniarticular nature of SOL may also contribute to a greater sensitivity to changes in ankle position compared to the biarticular GAS, as the latter is affected by both knee and ankle positions.

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Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.20/K1

Topic: E.09. Motor Neurons and Muscle

Title: Neural modulation of biceps femoris motor units during isometric knee flexion and hip extension tasks

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Abstract: BACKGROUND AND AIM: Task-dependent activation in biarticular muscles have consistently been reported for the rectus femoris and gastrocnemius. However, evidence regarding the biceps femoris long head (BF_{lh}) is controversial, showing both similar and non-uniform activation patterns when comparing knee flexion and hip extension movements. These discrepancies may be attributed to methodological issues in surface electromyography (EMG), especially amplitude cancellation. To overcome these issues, in this study we decomposed high-density surface EMGs from the BF_{lh} muscle to investigate whether the discharge rate of motor units and common synaptic oscillations to spinal motor neurons are modulated differently during knee flexion and hip extension tasks. **METHODS:** Seventeen healthy men performed isometric knee flexions and hip extensions with trapezoidal ramp feedback set at 20% of maximal voluntary isometric contraction (MVC). Tasks were performed with the knee and hip in a neutral position. High-density surface EMGs (2 grids of 32 channels) were acquired from BF_{lh}. EMGs were decomposed into motor unit spike trains using a convolutive blind-source separation algorithm, and the mean discharge rate (MDR) was calculated during the plateau (30s), separately for each task. Additionally, the motor units' z-coherence was calculated to estimate common synaptic inputs within delta (1-5 Hz), alpha (5-15 Hz) and beta (15-35 Hz) bands. For this analysis, only participants with a minimum of four identified motor units per task were included (n= 9). Linear mixed models (LMM) were applied to compare the effect of the knee flexion and hip extension on MDR, and Wilcoxon signed-rank tests were used to compare the z-coherence area under the curve between tasks, separately for delta, alpha and beta bands. **RESULTS:** No differences were observed in the MDR between knee flexion and hip extension

tasks (LMM; F = 0.008; P = 0.927). Wilcoxon signed-rank tests also did not show significant differences when comparing the z-coherence area under the curve between tasks for the delta (P = 0.203), alpha (P = 0.425), and beta (P = 0.734) bands. **CONCLUSIONS:** Our results reveal that the motor units of the BF_{lh} are similarly modulated during isolated knee flexion and hip extension isometric tasks at low force levels. Understanding this can offer valuable insights for sports and rehabilitation, particularly in choosing resistance exercises. Additionally, for robotic prosthetics design, the uniformity in BF_{lh} activation across tasks suggests that assistive devices could be developed to accommodate a variety of movements without requiring specific adjustments for each task.

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Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.21/K2

Topic: E.09. Motor Neurons and Muscle

Title: Optimizing Muscle Activation via Transcutaneous Nerve Stimulation: Spatial and Temporal Analysis of M-Wave and H-Reflex in Hand Muscles

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Abstract: Transcutaneous nerve stimulation (TNS) was used to target the median and ulnar nerves to evoke muscle responses. Previous research has indicated that TNS can delay fatigue onset by activating both superficial and deep muscle fibres, which offers advantages over traditional functional electrical stimulation techniques. This study builds on earlier work to assess whether TNS can induce naturalistic muscle activation patterns through reflex pathways. We explored the effects of varying stimulation intensity and location on the efferent and afferent pathways. Using a 2 × 8 electrode grid on the upper arm, we could selectively stimulate neural pathways to trigger M-waves and H-reflexes in the hand muscles, assessing individual finger forces and muscle responses via high-density EMG and force transducers. Our analysis focused on the spatial/temporal distribution of these responses, especially the H-reflex, to identify the optimal stimulation parameters for clinical TNS applications. For each of the 15 study participants, we identified and used to 4-7 pairs of stimulation electrodes that successfully evoked a motor response. We successfully elicited an H-reflex in 12 participants, with maximum amplitudes ranging from 6-50% of the maximum M-wave. In most participants, the primary muscle activation regions recorded on the EMG grid for the M-wave and H-reflexes were highly

correlated, whereas distinct regions were observed in a few regions. For some participants, both the maximum amplitude and the required stimulation intensity varied, whereas for others, the recruitment curves were remarkably consistent. This suggests that altering the stimulation location and intensity may differentially recruit Ia afferent fibres, thereby influencing the H-reflex magnitude and distribution across muscle groups in some participants. The variability in the H-reflex response highlights the potential of personalized stimulation protocols aligned with physiological motor unit recruitment for sustained activation and functional recovery. These insights underscore the importance of precise nerve-targeted stimulation strategies in rehabilitation. Further research on the nuanced effects of TNS parameters on neuromuscular recruitment and recovery in neuromuscular impairments is required.

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Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.22/K3

Topic: E.09. Motor Neurons and Muscle

Title: Multimodal dataset of hand gestures in different arm orientations

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Abstract: Humans use their hands to perform a variety of intricate tasks for activities of daily living (ADLs), and they are the primary means of interaction with the environment. The loss of hand function greatly diminishes a person’s ability to independently perform ADLs. The restoration of hand function through prosthesis that can perform basic functions like grasping, pointing, and picking, would assist people with ADLs and assume some functional independence. Such dexterous prosthetic hands require intuitive, multichannel control interfaces, which can be achieved using high-density electromyography (HDEMG). The research in myoelectric controllers has been greatly assisted by the availability of multiple open source datasets of EMG activity for various hand gestures. This has enabled the training of AI based myoelectric controllers which perform well when evaluated for limited function. A common limitation of these datasets and consequently the AI models is the constrained environment of data collection. Typically, participants perform hand gestures while maintaining a fixed arm pose to simplify data collection and ensure consistency across participants. However, naturalistic movements of the hands during ADLs are not performed in such a constrained manner. Before developing a myoelectric classifier that can decode unconstrained hand gestures, we need a dataset that captures EMG signals corresponding to hand gestures performed in an unconstrained

manner. To address this limitation in current research, we have developed a rich dataset of naturalistic hand gestures performed in an unconstrained manner. This will be a valuable asset for further development of myoelectric classifiers by other researchers.Our dataset consists of HDEMG data (128 channels covering the dorsal and ventral sides of the forearm), and optical motion capture. 20 participants performed various movements of wrist (flexion, extension, ulnar and radial deviation) and fingers (hand opening, closing and pinching). These gestures were performed again in different forearm orientations (supinated, middle and pronated). At the beginning and end of each session, we also collected HDEMG data corresponding to the maximum voluntary forces (MVF) a participant could exert with isometric wrist flexion and extension. The MVF data assists with normalizing and comparing HDEMG data across different sessions and participants. Each participant completed three sessions, on different days, to record HDEMG data over time. This allowed me to create a robust evaluation dataset to assess the generalizability of AI models across multiple days for the same participant and across different participants.

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Poster

PSTR291: Human Motor Units and Physiologic Recordings

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Title: Assessing F wave Characteristics Using the Compound Muscle Action Potential (CMAP) Scan Technique

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Abstract: Split hand is a special clinical feature in amyotrophic lateral sclerosis (ALS), which is described as preferential wasting of the abductor pollicis brevis (APB) and first dorsal interosseous muscles with relative preservation of the abductor digiti minimi (ADM) muscle. The pathophysiology of the phenomenon remains unclear. Researchers have reported different F wave characteristics in terms of persistence, index of F repeaters and index of total F repeaters, between the APB and ADM muscles in ALS that suggests subclinical alterations in the anterior horn cells. Nevertheless, there are limited normative data on the differences of F waves between the two muscles. In this study, we examined F characteristics in APB and ADM muscles using a new technique based on the compound muscle action potential (CMAP) scan recording.
Twenty-four healthy subjects participated in the study. The CMAP scan was applied to record progressive muscle responses to electrical stimuli, ranging from the supramaximal strength to the intensity below the threshold of the lowest motor unit. F waves were obtained from the late responses of the CMAP recording and conditioned by removing the baseline drift from the afterpotential of the CMAP waveform. The amplitude, latency, and stimulating intensity were measured for each F wave. Repeater F waves were detected using the unsupervised machine learning technique in each muscle. The persistence of F waves, F wave activating threshold, index of F repeaters and index of total F repeaters were calculated.
An average of 200 F waves per muscle were extracted from the CMAP scan recording. We observed longer F-M latency and lower activating F-threshold in the ADM muscles (F-M latency: APB: 25.43±2.39 ms, ADM: 26.15±2.32 ms, $p<0.05$; F-threshold: APB: 7.68±8.96 % of CMAP, ADM: 2.35±2.42 % of CMAP, $p<0.05$). Comparison of the indices of F repeaters and total F repeaters between the two muscle groups indicated significant difference in index of F repeaters (APB: 0.18 ± 0.049, ADM: 0.13 ± 0.047, $p<0.005$) and index of total F repeaters (APB: 0.35 ± 0.078, ADM: 0.27 ± 0.086, $p<0.005$). Such changes may reflect the differences of the nerves innervating the muscles.
This study provided normative data on F wave characteristics in the APB and ADM muscles based on the CMAP scan recording. The new F wave analysis can be potentially combined with the CMAP scan based motor unit number estimation technique to assess and track motoneuron function and degeneration to improve understanding of the split hand syndrome and ALS prognosis.

Disclosures: X. Li: None. **M. Chen:** None. **P. Barkhaus:** None. **S. Nandedkar:** None. **P. Zhou:** None.

Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.24/K5

Topic: C.06. Neuromuscular Diseases

Title: Effects of neuromuscular electrical stimulation on intermuscular EMG coherence across rotator cuff and deltoid muscles in people with rotator cuff disease

Authors: *X. LI, H. ZHU, L. GRIFFIN;
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Abstract: Rotator cuff disease (RCD) is a common reason for anterolateral shoulder pain. Intermuscular electromyographic (EMG) coherence quantifies the shared oscillatory inputs to motoneuron pools of a pair of muscles in the frequency domain. Neuromuscular electrical stimulation (NMES) increases the motor-evoked potential and facilitates the activation of the motor cortex. Eight symptomatic adults (49.4 ± 7.9 yrs) performed three sessions of shoulder contractions. In each session, they performed 30s isometric contractions at 30° scaption at 25% (low load), 50% (mid load), and 75% (high load) of maximal voluntary contraction. The order of loads was randomized for each trial to minimize learning effects. Intramuscular EMG was recorded from the supraspinatus (SS), and surface EMG was recorded from the infraspinatus (IS) and middle deltoid (MD) muscles. Between the first and second sessions, participants received a 10-min sham treatment followed by a 10-min rest; between the second and third sessions, they received a 10-min NMES treatment followed by a 10-min rest. For both treatments, electrodes were placed on the SS and IS, with intensity set to 0 mA for the sham and adjusted to the maximum comfort level for NMES. The electrical impulse was delivered at 30 Hz (pulse width = 0.2 ms) in an intermittent cycle of 4s on and 6s off. Z-transformed pooled coherence of each muscle pair (SS-IS, SS-MD, and IS-MD) in the delta (2-5 Hz) and beta (15-35 Hz) bands at baseline, after sham treatment, and after NMES treatment were compared with a two-way repeated-measures ANOVA (Time x Force) with Bonferroni post-hoc analysis. No significant differences in intermuscular coherence were observed in both bands between baseline and after sham treatment across force levels. After NMES, there was a main effect of Time for both delta- (p=0.02) and beta-band (p<0.01) coherences across muscle pairs. There was also an interaction effect between Time and Force (p=0.04). Post-hoc analysis revealed that SS-IS delta- (p=0.04) and beta-band (p=0.03) coherences increased at mid-load and high-load force levels. The SS-MD beta-band coherence (p=0.03) increased at low-load force levels. For IS-MD, beta-band coherence was also found to increase at the mid-load force level. Our findings highlight the therapeutic potential of NMES in managing RCD through modulating neuromuscular control patterns. Particularly, the increase in beta-band coherence across three muscle pairs indicates the excitability effect of NMES on the corticospinal tract. Increased coherence between stabilizer muscle pairs (SS-IS and IS-MD) may help prevent humeral head migration, solving a potential mechanism under RCD.

Disclosures: X. Li: None. **H. Zhu:** None. **L. Griffin:** None.

Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.25/K6

Topic: I.04. Physiological Methods

Support: Coulter-Drexel Translational Program

Title: First in-human tests with intramuscular braided multielectrode probes for advanced clinical electrodiagnosis

Authors: *T. KIM[1], C. CHEWACHUTIRUNGRUANG[2], A. P. BORISYUK[2], B. BINDER-MARKEY[3], S. F. GISZTER[4];
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Abstract: We have developed an intramuscular Braided Multi-Electrode Probe (BMEP) for advanced clinical electrodiagnostic with support from Coulter-Drexel translational research awards. There are three main types of commercially available clinical EMG needle electrodes: monopolar, concentric, and single fiber. These are widely used in various clinical fields, such as Neurology, Physical Medicine & Rehabilitation (PM&R), etc. for intramuscular electrodiagnosis. All three types of electrodes have just a single channel for EMG recording. In the standard-of-care procedure, the EMG needle must be inserted into target muscles multiple times to obtain standard-of-care numbers of single motor unit (SMU) recordings for diagnostic decisions. This repetition increases discomfort and sensitives pain levels. To significantly reduce the number of penetrations and increase reliability and objectivity of test results, our strategy is to add multichannel capability to the EMG needle. We braid microwires onto the current clinical EMG needle form. The multichannel probe increases numbers of recorded SMUs across the distributed channels. The in-parallel acquisitions also provide an opportunity to use statistical measures among channels. We chose the Natus 28G monopolar needle as the base for braiding microwire designs. At SfN in 2022 and 2023, we demonstrated intramuscular BMEPs (12 Ch braid + 1 Ch needle) were able to record 9 ~ 11 different SMUs while the needle tip could pick up only 2 SMUs in for the same 30s timeframe. We confirmed that this capability is consistent in muscles of frogs and rats, though the shapes of SMU waveforms and the lengths of SMU time windows were different from each other. We are now embarking on first-in-human tests. At SfN 2024, We will demonstrate needle robustness tests in results of BMEPs subject to multiple mechanical insertion tests in rat skin, needed preceding the human tests. We also plan to show SMUs recorded with BMEPs and Natus monopolar needles in upper extremity and lower extremity of healthy human subjects, SMU comparisons between multichannel on braid and the clinical needle tip, and insertion discomfort level comparisons between BMEPs and Natus monopolar needles. PM&R doctors' feedback will also be described.

Disclosures: T. Kim: None. **C. Chewachutirungruang:** None. **A.P. Borisyuk:** None. **B. Binder-Markey:** None. **S.F. Giszter:** None.

Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.26/K7

Topic: I.04. Physiological Methods

Support: Public Interest Incorporated Foundation The Japan Science Society The Sasakawa Scientific Research Grant
Tateishi Science and Technology Foundation

Title: Characteristics of Motor Unit Activity from Different Types of Electrodes in Multi-Channel Surface Electromyography

Authors: *M. ITO[1], K. NOGI[1], T. KOKUBUN[2,3];
[1]Grad. Sch. of Hlth., Med., and Welfare, Saitama Prefectural Univ., Saitama, Japan; [2]Physical Therapy, Saitama Prefectural Univ., Koshigaya-Shi, Japan; [3]Graduate School of Health, Medicine, and Welfare, Saitama Prefectural University, Koshigaya, Japan

Abstract: In recent years, it has become possible to acquire musculo-neurophysiology data based on electrophysiological methods using surface electromyography (sEMG). Among these, analyzing motor unit (MU) activity by the decomposition technique using multi-channel sEMG is the most attractive topic in that field. The aim of this study was to clarify the differences in MU data acquired by different multi-channel sEMG electrodes.The subjects were five healthy adults on the dominant side and two hemiplegic stroke patients on the paralyzed side. A four-channel sEMG sensor (Galileo; 2,222Hz) and a 64-channel high-density sEMG sensor (HDsEMG; 2,000Hz) were used. The target muscle was the biceps brachii. The tasks consisted of a maximal voluntary isometric contraction with elbow flexion (MVC) followed by a target tracking task at 25%MVC and 50%MVC normalized by MVC. The muscle activity during the target tracking task was decomposed to detect the motor unit action potentials (MUAP). For the motor unit data, we analyzed the identified MU number and discharge rate.The number of identified MU was not significantly different between the two electrodes in the 25%MVC ($p = 0.93$). However, the HDsEMG showed a significantly lower number of identified MU at 50%MVC ($p = 0.04$). The discharge rate was not significantly different between the two electrodes in 25%MVC and 50%MVC ($p=0.07$, $p=0.27$). In stroke patients, the number of identified MU in HDsEMG was higher than Galileo for both 25%MVC and 50%MVC. Few MUs were identified with the Galileo in the 25%MVC.In the HDsEMG of healthy subjects, as in previous studies, the high intensity of muscle activity was decomposed into only a low number of identified MU. This was due to the different decomposition algorithms used for each electrode.HDsEMG could not be identified due to superimposed on the strong muscle contraction by healthy subjects because it identifies based on the firing timing of MUAP. In contrast, Galileo identified a MU if the individual MUAP from the recorded compound action potentials matched the template waveform. Therefore, a variety of motor units were identified by

the strong muscle activity. On the other hand, the MUs of stroke patients on the paralyzed side of Galileo could not discriminate enough number of MU, but HDsEMG could discriminate. In subjects with small MVC like stroke patients, HDsEMG is more suitable because the amplitude of the compound action potentials is small. Thus, the accuracy of MU analysis using sEMG depends on the maximum voluntary contraction strength of the subject and the level of muscle contraction task we set. We propose that equipment should be selected based on these considerations.

Disclosures: M. Ito: None. **K. Nogi:** None. **T. Kokubun:** None.

Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.27/K8

Topic: E.09. Motor Neurons and Muscle

Support: Simon Foundation
Azrieli Foundation

Title: Flexible control of motor units across a range of human upper-limb arm muscles

Authors: *T. OYA[1], A. PRUSZYNSKI[2];
[2]Physiol. and Pharmacol., [1]Western Univ., London, ON, Canada

Abstract: All voluntary movements are ultimately achieved by activating spinal motoneurons via descending commands. It is often assumed that the descending commands are relatively uniformly distributed across a given motoneuron pool, and that this ‘common drive’ simplifies voluntary control by dictating a rigid pattern of motoneuron recruitment and discharge across the pool. Several previous studies have demonstrated occasional violations of such a rigid control scheme, bringing into questions its universality (Nardone et al. 1989, Wakeling et al. 2006). A recent report suggests that such violations routinely occur in proximal arm muscles of a non-human primate when producing rapidly changing target forces and that primary motor cortex may be in direct control of this more flexible recruitment strategy (Marshall et al. 2022). Here we tested whether violations of rigid control are routine for human arm muscles controlling the finger, wrist and elbow joints. Similar to the non-human primate study, we instructed participants to generate isometric contractions tracking either ramp-and-hold (rise time: 2s; steady state time: 3s) or chirp (0-3 Hz in 6s) force profiles. Force levels were set at 10-25% MVC at the joint of interest. Single motor units were recorded via high-density multi-contact intramuscular probes (bipolar 16ch - Myomatrix: Chung et al. 2023) placed in the first dorsal interosseus, extensor digitorum communis, and triceps brachii muscles. We successfully recorded and isolated approximately 10 motor unit action potentials per electrode. To examine whether motor units

respond as a function of a common underlying input, we correlated the discharge rate of motor unit pairs for the two tasks. Consistent with deviations from rigid control, we found that the firing rate relationship for most motor unit pairs was not well explained by one-dimensional manifold (i.e. a line). This dispersion was observed in all muscles that we examined. These results further the idea that violations of rigid control of motor units are common. Such a scheme would permit efficient deployment of motor units to achieve required force output by taking advantage of mechanical properties of the innervated muscle fibres.

Disclosures: T. Oya: None. **A. Pruszynski:** None.

Poster

PSTR291: Human Motor Units and Physiologic Recordings

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR291.28/K9

Topic: I.04. Physiological Methods

Support: Boehringer Ingelheim Fonds PhD fellowship (SW)
Swiss National Science Foundation (SNSF) Eccellenza Grant (181239) (PR)
Swiss National Science Foundation (SNSF) Project Grant (175667) (PR)

Title: FlyTrack, simultaneously measuring 3D poses and muscle activity in freely behaving *Drosophila*

Authors: *V. STIMPFLING[1], S. WANG-CHEN[2], J. S. PHELPS[3], P. RAMDYA[3];
[1]SV, EPFL, Lausanne, Switzerland; [2]Brain Mind Inst., EPFL, Lausanne, Switzerland; [3]EPFL, Lausanne, Switzerland

Abstract: Animal behavior arises from coordinated neural signals. However, these behaviors are first filtered by nonlinearities arising from passive viscoelastic properties of the musculoskeletal system and physical interactions with the environment. Thus, a direct mapping from neural activity to observed behaviors requires an understanding of the intermediate transformation imparted by the body's muscles and skeleton. Although tethering has facilitated the discovery of neural and muscle activity underlying behavior, it is even more crucial (and difficult) to unravel this transformation in an unrestrained setting in which the full capacity of postural and kinematic muscles are engaged. To address this challenge, we have built an optical setup capable of recording accurate 3D poses and underlying muscle activity in untethered flies at high framerates. To achieve this, we are leveraging the genetic toolkit available for studying the fly, _Drosophila melanogaster_. Specifically, we are using muscle driver lines to express genetically encoded calcium sensors. In our setup, flies walk freely in a linear corridor with prism mirrors as walls. This enables the capture of three views (two side and one ventral) using a single infrared-

sensitive high-speed camera. This camera is mounted on a translating stage to follow the animal as it locomotes. These three camera views are then used to obtain triangulated 3D poses. Simultaneously we leverage an epifluorescence module to record muscles activity readout as changes in GCaMP-related fluorescence. We use a muscle map derived from an X-ray based reconstruction of the fly to align muscle signals with the animal's 3D pose, allowing us to measure the activity of individual muscles. Ultimately, we plan to combine these data with existing fly morphology datasets to model muscle-based actuation in our detailed neuromechanical model of the adult fly, NeuroMechFly. In summary, FlyTrack opens up the possibility of uncovering the contribution of muscle activity in fly behavior toward the ultimate goal of obtaining a complete understanding of how neural signals give rise to actions in a physically complex world.

Disclosures: V. Stimpfling: None. **S. Wang-Chen:** None. **J.S. Phelps:** None. **P. Ramdya:** None.

Poster

PSTR292: Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR292.01/K10

Topic: F.07. Biological Rhythms and Sleep

Support: NIH 1RF1AG077570

Title: A unique sleep phenotype and reduced orexin-expressing neuron number in the Tg-SwDI mouse model of Alzheimer's disease

Authors: Y. WU[1], N. R. BHAT[2], ***M. LIU**[3];
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Abstract: The bi-directional interactions between sleep disturbance and Alzheimer's disease (AD) and AD-related dementias (ADRD) are not fully understood. We performed a sleep study on Tg-SwDI mice, a model of cerebral amyloid angiopathy (CAA), and age-matched wild-type (WT) control mice and identified that Tg-SwDI mice spent significantly more time in non-rapid eye movement sleep (NREM sleep or NREMS) (44.6±2.4% in Tg-SwDI versus 35.9±2.5% in WT), and had a much shorter average length of wake bout during the dark (active) phase than WT controls (148.5±8.7 s in the Tg-SwDI versus 203.6±13.0 s in WT). Histological analysis revealed stark decreases (~34%) of orexin immunoreactive (orexin-IR) cell number and soma size in the brain of these Tg-SwDI mice (Cell number: 2187±97.6 in Tg-SwDI versus 3318±138.0 in WT. Soma size: 109.1±8.1 μm^2 in Tg-SwDI versus 160.4±6.6 μm^2 in WT), while

the number and soma size of melanin-concentrating hormone (MCH) immunoreactive (MCH-IR) cells remain unchanged (Cell number: 4257±273.7 in Tg-SwDI versus 4494±326.8 in WT. Soma size: 220.1±13.6 μm^2 in Tg-SwDI versus 202.0±7.8 μm^2 in WT). The apoptotic cell death marker cleaved caspase-3 immunoreactive (Caspase-3-IR) percentage in orexin-IR cells was significantly higher in Tg-SwDI mice (15.6±3.4% in Tg-SwDI versus 2.47±0.8% in WT), while the Caspase-3-IR percentage in MCH-IR cells was insignificant between Tg-SwDI and WT groups (2.67±0.24% in Tg-SwDI versus 2.08±.5 in WT). This selective loss of orexin-IR cells could be responsible for the abnormal sleep phenotype in Tg-SwDI mice. Further studies are needed to determine the cause of the selective death of orexin-IR cells and its potential effects on cognition impairments in this mouse model.

Disclosures: Y. Wu: None. **N.R. Bhat:** None. **M. Liu:** None.

Poster

PSTR292: Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR292.02/K11

Topic: F.07. Biological Rhythms and Sleep

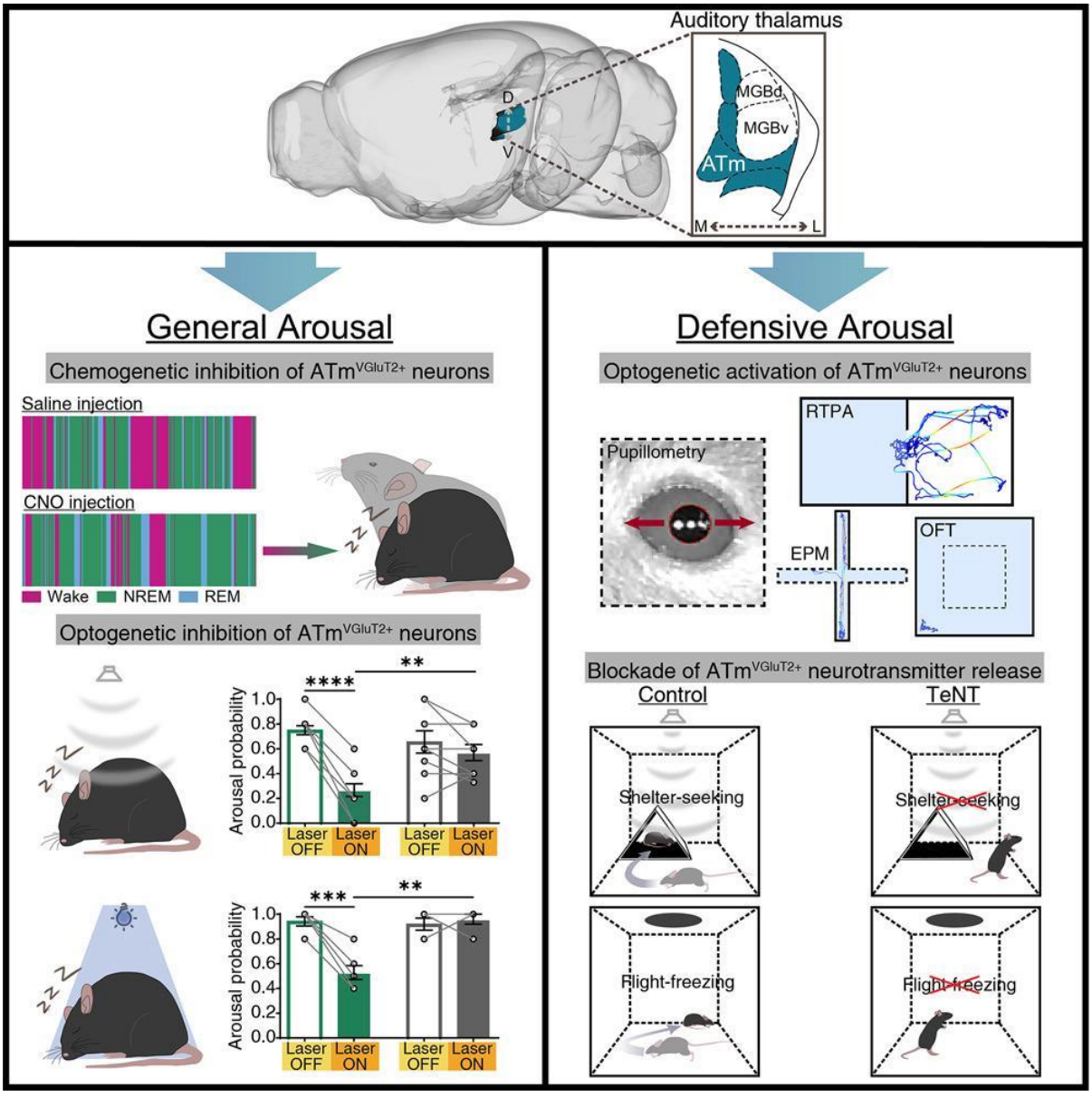
Support: National Natural Science Foundation of China 31871057
National Natural Science Foundation of China 32070993
National Natural Science Foundation of China 81527901

Title: A common thalamic hub for general and defensive arousal control

Authors: Y. WANG[1], L. YOU[1,2], K. TAN[1], ***M. LI**[1], K. YUAN[1];
[1]Sch. of Med., [2]Tsinghua Univ., Beijing, China

Abstract: A common thalamic hub for general arousal and defensive arousalIntroduction: Specific motivated behaviors are dependent on both general arousal and specific arousal. General arousal has a general purpose and is the basis of motivated behaviors. On top of general arousal, specific arousal can be induced by internal needs or external cues, which are associated with specific motivational states. Likewise, the expression of defensive responses to alerting sensory cues also requires both general arousal and a specific arousal state associated with defensive emotion. However, it remains unclear whether these two forms of arousal can be regulated by common brain regions.We discovered that the non-primary auditory thalamus in mice is a thalamic hub controlling both general and defensive arousal. The spontaneous activity of non-primary auditory thalamus was correlated with and causally contributed to wakefulness. In sleeping mice, its sustained population responses were predictive of sensory-induced arousal, the likelihood of which was markedly decreased by the inhibition of non-primary auditory thalamic neurons or multiple downstream pathways. In awake mice, the

neuronal activation further heightened arousal that accompanied by excessive anxiety and avoidance behavior. Notably, blocking its neurotransmission abolished alerting stimuli-induced defensive behaviors. These findings may shed light on the comorbidity of sleep disturbances and abnormal sensory sensitivity in specific brain disorders.**Highlights:** 1. Non-primary but not primary auditory thalamus is a wake-promoting nuclei.2. Non-primary but not primary auditory thalamus can induced sensory-evoked arousal.3. Non-primary thalamus can induce defensive emotion and required for defensive behavior expression .
URL: https://doi.org/10.1016/j.neuron.2023.07.007



Disclosures: Y. Wang: None. **L. You:** None. **K. Tan:** None. **M. Li:** None. **K. Yuan:** None.

Poster

PSTR292: Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR292.03/K12

Topic: F.07. Biological Rhythms and Sleep

Support: Stanford Propel Fellowship (BJB)
NIH R00 AA025677 (WJG)
Whitehall Foundation Research Grant 2022-05-99 (WJG)
Brain Research Foundation Seed Grant (WJG)

Title: Extended amygdala neuropeptide circuits linking sex-specific psychostimulant effects on body temperature and sleep/circadian rhythms

Authors: *B. J. BUSH, E. ROGERS, A. MORNINGSTAR, Y. MA, W. J. GIARDINO; Psychiatry and Behavioral Sci., Stanford Univ., Palo Alto, CA

Abstract: Sleep is key for socioemotional function, and substance use disorders are associated with dysregulated sleep and circadian rhythms. The neuronal mechanisms underlying these associations remain understudied, especially in women. Intriguingly, body temperature fluctuations are a key feature of sleep/circadian changes as well as acute drug exposure. Several brain structures are implicated in interactions between psychostimulant effects, stress, and sleep/wake regulation, including the bed nucleus of the stria terminalis (BNST), a core component of the extended amygdala network. The BNST regulates emotional responses to stress and consists of many distinct cellular populations, including neurons expressing tachykinin2 (*Tac2*), the gene encoding the neuropeptide Neurokinin B (NKB). Tac2/NKB signaling drives the hypothalamic-pituitary-gonadal reproductive axis and is implicated in social stress as well as hot flashes. Thus, we hypothesize that drug effects on sleep and circadian rhythms may be mediated by Tac2-BNST neurons regulating emotional arousal and sex differences in body temperature. We used wireless telemetry systems for chronic locomotor activity and body temperature monitoring in male and female mice, finding that females displayed enhanced acute cocaine-induced locomotor stimulation and hypothermia. Ongoing studies seek to assess sex differences in Tac2-BNST circuit organization and neuronal activity responses to cocaine, using anterograde viral tracing and cFos immunohistochemistry in Tac2-Cre/tomato mice. Furthermore, chemogenetic modulation of Tac2-BNST neurons with estrous cycle monitoring in female mice will delineate hormonal-dependent roles of Tac2-BNST neurons in cocaine effects on thermoregulation and sleep/wake balance. Overall, these studies will add to our understanding of sex differences in psychostimulant interactions and improve the development of therapeutic strategies for treating disorders of stress and sleep.

Disclosures: B.J. Bush: None. **E. Rogers:** None. **A. Morningstar:** None. **Y. Ma:** None. **W.J. Giardino:** None.

Poster

PSTR292: Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR292.04/K13

Topic: F.07. Biological Rhythms and Sleep

Support: 2017YFE0126500
81861138013

Title: Claustrum, as a key brain structure mediating general anesthesia

Authors: *S. LI[1], F. YANG[2], B. LU[3];
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Abstract: Despite the widespread utility of general anesthetic drugs in clinical medicine and scientific research, the mechanism underlying general anesthesia, characterized as loss of consciousness, remains a long-standing mystery. Here, we show that the *Bdnf*-e6-expressing neurons in the claustrum, described as a 'seat of consciousness', is critical general anesthesia. Mice with selective disruption of BDNF production from promoter VI (*Bdnf*-e6$^{-/-}$ mice) exhibited the significant anesthetic deficits. In wild-type mice, either chemogenetic inhibition of claustral neurons or deletion of *Bdnf* gene decreased depth and duration of drug induced anesthesia. In *Bdnf*-e6$^{-/-}$ mice, chemogenetic activation of claustral *Bdnf*-e6 expression neurons could reverse the deficits in propofol-induced anesthesia. Single-nucleus sequencing identified a specific population of *Bdnf*-e6-expressing glutamatergic neurons whose functions are required for general anesthesia. Our results demonstrate that claustrum is a key brain area critical for general anesthesia.

Disclosures: S. Li: None. **F. Yang:** None. **B. Lu:** None.

Poster

PSTR292: Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR292.05/K14

Topic: F.07. Biological Rhythms and Sleep

Support: Neuroscience Academy Denmark, Lundbeckfonden

Title: Unraveling the intimate relationship between brain temperature dynamics and infraslow oscillations during natural sleep and state transitions

Authors: *A. TSOPANIDOU[1], P. C. PETERSEN[2], C. KJAERBY[3];
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Abstract: Brain temperature is highly dynamic and intricately linked to brain state transitions as it is the effect of active warming through local neuronal activity and passive cooling due to blood flow dynamics. Infraslow oscillations of sigma power (8-15 Hz) occur during Non-Rapid Eye Movement (NREM) sleep (~1-2 cycles/min) and create sleep microstructures associated with neuronal synchronization and arousal levels. So far, the relationship between infraslow sigma rhythm and brain temperature remains unexplored. In this project we aim to elucidate how brain temperature correlates with the window of neuronal synchronization and arousal created by the infraslow sigma power rhythm during natural sleep and state transitions. To investigate this, we employed simultaneous recordings of brain temperature and Electroencephalography (EEG) acquired sigma power oscillations from the S1 primary somatosensory cortex in C57Bl6 mice (N= 4-6 per group, mixed gender). Our findings indicate a close correlation between infraslow sigma power oscillatory dynamics and brain temperature during sleep. Specifically, during NREM sleep, brain temperature oscillates in synchrony with the sigma power rhythm. Brain temperature reliably increases at Rapid Eye Movement (REM) sleep onset following the transient sigma power increase that precedes REM onset. Sleep-to-wake transitions are marked by brain temperature increases, closely tied to sigma power descents. In conclusion, our findings highlight the intimate relationship between brain temperature dynamics and infraslow oscillations, which present a compelling basis for further exploration and potential clinical implications. Understanding the interplay between brain temperature dynamics and infraslow oscillations could pave the way for novel therapeutic interventions in various neurological conditions, which are marked by dysregulation of infraslow rhythms, such as Alzheimer's disease.

Disclosures: A. Tsopanidou: None. **P.C. Petersen:** None. **C. Kjaerby:** None.

Poster

PSTR292: Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR292.06/K15

Topic: F.07. Biological Rhythms and Sleep

Support: NS122589
NS073613

Title: Transcriptional, anatomical, and functional divergence in lateral hypothalamic inhibitory neurons underlying innate and motivated behaviors

Authors: *S. K. PINTWALA[1,2], E. ARRIGONI[3], P. FULLER[4];
[1]Neurolog. Surgery, Univ. of California, Davis, Davis, CA; [2]U.C. Davis School of Medicine, Davis, CA; [3]Neurol., BIDMC and Harvard Med. Sch., Boston, MA; [4]U.C. Davis Sch. of Med., Davis, CA

Abstract: Single cell transcriptomics has advanced our understanding of the transcriptional heterogeneity of individual cell types in discrete nuclei, but cannot provide spatial context, including efferent organization, an essential component for many biological questions. Here, we merged single nuclei RNA sequencing (snRNA-seq) of vesicular GABA transporter neurons (VGAT; *slc32a1*) in the lateral hypothalamus (LH^{VGAT}) with a retrograde tracer to test if transcriptional heterogeneity correlates with neuroanatomy. LH^{VGAT} neurons projecting to the ventral tegmental area (VTA; $LH^{VGAT\text{-}VTA}$) were identified by delivering the retrograde tracer (rgAAV2-CAG-FLEX-rc [Jaws-KGC-GFP-ER2]) to the VTA of VGAT-IRES-cre mice (n=4). Fluorescent microscopy revealed that GFP-expressing retrogradely-labelled VGAT neurons were distributed throughout the LH between AP -1.0 and -2.0mm (from bregma). Hypothalamic tissue was extracted and isolated nuclei were fluorescently sorted for GFP prior to sequencing. snRNA-seq was performed with 10X Chromium using a custom reference library appended with the *gfp* transgene (produced in-house). Unsupervised clustering of 214 *slc32a1*+ nuclei yielded two distinct clusters (VGAT-1, VGAT-2), one expressing *gfp* (VGAT-2, n=130 nuclei) hence representing the $LH^{VGAT\text{-}VTA}$ subpopulation. Cluster VGAT-2 was enriched in, and exclusively expressed several genes including: *atp1a2* (p=4.93E-54, Negative Binomial Exact Test with the Benjamini-Hochberg procedure), *slc6a11* (p=1.07E-51), *slc7a10* (p=1.09E-42), *ntsr2* (p=4.44E-37) and *gpr371l* (p=9.21E-31). This research aims to reconcile transcriptional divergence observed in LH^{VGAT} neurons with neuroanatomy. Next, we will leverage this knowledge using intersectional techniques to understand how genetic diversity and neuroanatomy influence a range of animal behaviors known to be regulated by the LH, in particular LH^{VGAT} neurons.

Disclosures: S.K. Pintwala: None. **E. Arrigoni:** None. **P. Fuller:** None.

Poster

PSTR292: Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR292.07/K16

Topic: F.07. Biological Rhythms and Sleep

Support: PAPIIT-DAGAPA BG200524
Joint Canada-Israel Health Research 109928-002 Program

CONAHCyT CVU 757156
PAEP-UNAM 2024

Title: Light on the hypothalamus

Authors: *I. J. ROMERO VERA, E. SANTACRUZ, R. M. BUIJS;
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Abstract: Environmental signals govern all life on Earth, but the light has the most influence on our physiology and behavior. There are different processes that light can influence, like body temperature or mood, but the activity rhythm is the most obvious. But how does the light switch on or off our activity and influence our physiology? Hereto, retinal projections reach several structures in the brain, especially one structure that has a central place in transmitting light information, the suprachiasmatic nucleus (SCN). In addition, several other areas, from the hypothalamic preoptic area to the superior collicus, can receive light input from the retina and influence biological responses. Considering retinal input, the SCN has special features: 1) It receives a dense retina input that allows SCN synchronization with the dark-light cycle to impose its rhythm to other structures. 2) The SCN does not need this retinal input to transmit its “light” signal to the brain. However, after the SCN lesion also, light cannot change the activity of physiology anymore. This raises the question of whether the retina input to the hypothalamus is mainly to synchronize the SCN or whether it also directly influences hypothalamic functions. Our hypothesis is that retinal projections reach, through the SCN, structures that regulate activity (like the ventrolateral preoptic nucleus), while the SCN also has the capacity to inhibit activity without light. When retinal fibers pass through the SCN, an SCN lesion would remove the retinal projections to other hypothalamic targets. To answer this, we used a male and female adult Wistar rats receiving an intravitreal injection of cholera toxin (CTb). In the second group, in addition to the retinal tracer, the animals received a unilateral or bilateral electrolytic lesion in the SCN. After seven days, animals were sacrificed to observe the retina projections with CTb. We observed that there are structures innervated by the retina that have not been reported before. We also observed differences in the retinal projections of animals with SCN lesions compared to the control group. These results indicate that anatomically, retinal fibers can reach other structures and exert its function passing through the SCN . Therefore, the function of these retinal fibers passing through the SCN needs to be established. This work was carried out under the master's program in Biological Sciences at UNAM.

Disclosures: I.J. Romero Vera: None. **E. Santacruz:** None. **R.M. Buijs:** None.

Poster

PSTR292: Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR292.08/K17

Topic: F.07. Biological Rhythms and Sleep

Title: Continuous Monitoring of Sleep States Following Disruptions in Vitamin K Intake

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Abstract: Significance: In rodents, diets with long-term low vitamin K intake are associated with behavioral deficits in learning and memory, and a marked hypoactivity resulting in a lack of exploratory behavior. Additionally, evidence suggests a link between diets lower in vitamin K and neurodegenerative disorders. In the brain, vitamin K plays an important structural role in cell membranes, through its actions in sphingomyelin and protein synthesis. However, little is understood about vitamin K intake and sleep architecture, which greatly impacts the resulting cognitive deficits and hypoactivity associated with disruptions in vitamin K intake. **Aim:** Determine sleep architecture during periods of deficient and fortified vitamin K intake. **Approach:** Sleep architecture after disruptions in vitamin K intake was scored by continuously monitoring electroencephalogram (EEG) and electromyography (EMG) signals. Male and Female C57Bl6 mice were implanted with wireless physiological telemetry devices and underwent two-week periods of baseline, vitamin K deficient, recovery, and vitamin K fortified changes in diet. **Results:** Preliminary analysis suggests that during periods of vitamin K deficiency, male and female mice spent less time awake and more time in deep (slow wave) sleep during the dark phase when they should normally be awake and active. During the light phase, there is a possible increase in time spent in paradoxical sleep (e.g., rapid-eye-movement sleep). **Conclusions:** Diets low in vitamin K intake seem to influence sleep architecture in male and female mice. Sleep architecture was rescued after mice spent two weeks back on the control diet. A vitamin K-fortified diet does not seem to influence sleep architecture. These findings will be understood further through the quantification of brain sphingolipids and cognitive behavioral assays in automated home cage systems during periods of low vitamin K intake.

Disclosures: L.R. Wilson: None. **J.D. Cushman:** None. **L. Li:** None.

Poster

PSTR292: Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR292.09/K18

Topic: F.07. Biological Rhythms and Sleep

Support: UAB Palliative Research Enhancement Project

Title: A pilot feasibility and efficacy study of transdermal auricular vagus nerve stimulation for treating insomnia and stress in breast cancer patients receiving palliative care

Authors: *M. DO[1], W. TYLER[2];
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Abstract: While the purpose of sleep is heavily debated, it is widely accepted that quality sleep is essential to the well-being of every individual. It has been shown to decrease stress, improve depressive moods, enhance memory consolidation, reduce inflammation, and improve one's overall health. Insomnia is a common problem experienced by patients with breast cancer, affecting about 40% of cancer survivors. This is a critical concern with cancer clinicians as it can affect the overall health of those with breast cancer or those recovering from breast cancer. Benzodiazepines are commonly prescribed to treat insomnia in breast cancer patients, but these drugs come with negative side effects and a high risk of abuse. Transauricular Vagus Nerve Stimulation (taVNS) is a non-invasive and non-pharmacologic intervention that could potentially be used as an alternative to treat insomnia. taVNS is safe and well-studied neuromodulation device that delivers low-intensity pulsed electrical currents to the vagus nerve through the external ear. This neuromodulation device has demonstrated efficacy in treating insomnia, stress, anxiety, pain, depression, inflammation reduction, and other diseases. Therefore, this intervention could serve as a safe, critical intervention to aid in breast cancer recovery and issues associated with breast cancer diagnosis. In this study, we aim to investigate the influence of taVNS to address insomnia in breast cancer patients receiving palliative and supportive care services. Specifically, we aim to evaluate the feasibility of using taVNS to treat insomnia in patients with breast cancer. We also aim to evaluate the efficacy of repeated, nightly taVNS on sleep quality, anxiety, and cancer-related fatigue. Additionally, we aim to evaluate the changes on inflammation markers, IL-6, IL-10, C-reactive protein (CRP), and fibrinogen, and changes on cortisol levels. We expect that 30 patients with breast cancer and insomnia will be enrolled and undergo taVNS to address insomnia, quantified by various sleep related outcome measures, with an estimated recruitment rate of 70%, eligibility rate of 70%, completion rate of 80%, and follow up rate of 80%. We hypothesize that patients will report significantly improved sleep (minimally clinically significant change of 6 points on the Insomnia Severity Index (ISI)), with possible improvements in anxiety, depression, and cancer related fatigue after two weeks of taVNS. We also expect to see decreases in inflammation markers and cortisol levels.

Disclosures: M. Do: None. **W. Tyler:** None.

Poster

PSTR292: Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR292.10/K19

Topic: F.07. Biological Rhythms and Sleep

Support: CDMRPL – 19-0-PR192622
W81XWH-22-2-0038

Title: Insights into the circadian rhythm utilizing wearable sleep tracking devices

Authors: *A. POLLATOU[1,2,3,4], A. PENAFIEL[5,2,1], S. YALEWAYKER[6,4,2], E. R. CHERAGHPOUR[7,3,2], S. SKEETE[8,3,4], E. METZGER[5], J. K. WERNER, Jr.[9,2,4,10];
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Abstract: Background: Sleep dysfunction and circadian misalignment are prevalent health concerns associated with adverse health outcomes such as obesity, cardiovascular disease, and cognitive and/or psychological deficits. Despite their widespread impact, the assessment of circadian rhythm remains a challenge in both clinical and non-clinical settings. Recent advancements in wearable technology present a promising avenue for addressing this gap by offering a promising approach to estimating circadian phase and amplitude. Although core body temperature is one of the most reliable circadian phase biomarkers, tracking core body temperature over several days is intrusive and challenging and therefore impractical for widespread use. Wearables that track movement and skin temperature have emerged as viable alternatives as they allow for less intrusive assessment of diurnal rhythms over extended periods of time. This study aims to explore the utility of wearable devices in assessing circadian rhythms.Methods: Participants wore temperature and actigraphy trackers for a minimum of 2 weeks. We collected minute-to-minute data on skin temperature and sleep estimates, from which we derived the sleep midpoint and circadian phase by fitting a cosine curve to the data with a cosinor analysis.Results: In the initial analysis involving N=17 participants (aged 27-45, with 35% female representation) in our ongoing study, the average sleep midpoint was determined to be 3:02:54 AM (SD=58 min) falling within the healthy range (defined as the midpoint of sleep between 2:00 AM and 4:00 AM). Cosinor analysis further indicated a mean mesor of 32.33 ºC (SD= 0.89) and amplitude of 2.86 (SD=1.14). The mean acrophase occurred at 2:15:02 AM (SD=34.5 min) with a peak temperature 34.5 ºC (SD=0.83) . The midpoint of sleep shows a positive correlation with the acrophase, whereas the amplitude exhibits a strong relationship with the mesor (inversely) and the temperature at zenith (positively). These relationships suggest the potential of utilizing indices derived from wearable devices to inform circadian rhythms.Conclusions: Wearable devices can conveniently measure circadian rhythm disruption in the real world and can offer valuable insights that could aid in identifying and addressing the extent of circadian disruption to facilitate clinical management. We propose that the metrics derived from wearable technology hold promise for informing circadian rhythms, in a non-obtrusive and continuous way, pending further validation, as suggested by our initial analysis.

Our presentation will include ongoing analysis with optimized techniques for data preprocessing for the Oura ring device.

Disclosures: A. Pollatou: None. **A. Penafiel:** None. **S. Yalewayker:** None. **E.R. Cheraghpour:** None. **S. Skeete:** None. **E. Metzger:** None. **J.K. Werner:** None.

Poster

PSTR292: Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR292.11/K20

Topic: F.07. Biological Rhythms and Sleep

Support: NIH R01HL151853

Title: Microbiota and sleep regulation: the role of liver macrophages in sleep and fever induced by microbial molecules

Authors: *L. KAPAS[1], K. C. BUCKLEY[2], A. R. MASSIE[3], E. SZENTIRMAI[3];
[1]Washington State University, Spokane, spokane, WA; [3]Elson S. Floyd Col. of Med., [2]Washington State Univ., Spokane, WA

Abstract: Introduction: Emerging evidence suggests that the intestinal microbiota is a source of sleep-promoting signals. Bacterial metabolites and components of the bacterial cell wall are likely mediators between the intestinal commensal flora and sleep-generating mechanisms in the brain. Microbial molecules translocate from the gut lumen to the host circulation, impacting host physiology. We previously demonstrated that mimicking the translocation of the bacterial cell wall component lipopolysaccharide (LPS) by injecting it directly into the portal vein induces sleep and fever in rats. Liver macrophages (Kupffer cells) are the primary targets for translocated LPS in the host. Therefore, we examined the role of liver macrophages in LPS-induced sleep and fever by using Kupffer cell-depleted rats.
Methods: Male Sprague-Dawley rats were implanted with chronic intravenous catheter in their portal vein and instrumented to monitor sleep-wake activity, body temperature and locomotion. Kupffer cell depletion was achieved by the intraportal administration of 1 ml clodronate-containing liposomes (CCL). Control rats received intraportal saline injection. Four days after the induction of macrophage depletion, we tested the effects of 1 µg/kg intraportally administered LPS. All treatments were performed 20-30 min before dark onset. Sleep, body temperature and motor activity were recorded for 23.5 h after each treatment. Data were analyzed by using ANOVA followed by Tukey's Honestly Significant Difference test.
Results: CCL treatment eliminated Kupffer-cells from the liver confirmed by the absence of Clec4F mRNA expression, a specific Kupffer cell marker. Macrophage populations in other organs were not affected, as indicated by unchanged CD68 and F4/80 expression in the spleen

and white adipose tissue. Intraportal injection of LPS induced 50% increase in non-rapid-eye movement sleep in the first six hours after the treatment in the control group (baseline: 95.5 ± 7.6 vs. LPS: 143.7 ± 5.7 min), while the increase was only 16% in the Kupffer-cell depleted group (baseline: 104.4 ± 5.1 vs. LPS: 121.5 ± 8.4 min). LPS suppressed rapid-eye movement sleep in both groups of rats. LPS-induced fever was attenuated by ~0.2 °C in the macrophage-depleted animals.
Conclusions: The results suggest that liver macrophages play a major role in sleep responses induced by LPS translocated into the portal circulation, and they also contribute to LPS-induced febrile responses.

Disclosures: L. Kapas: None. **K.C. Buckley:** None. **A.R. Massie:** None. **E. Szentirmai:** None.

Poster

PSTR292: Sleep Regulation: Anatomy, Physiology, Neurochemistry

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR292.12/K21

Topic: F.07. Biological Rhythms and Sleep

Support: NIH R01HL151853

Title: Prostaglandins induce sleep through peripheral sleep-promoting mechanisms

Authors: *E. SZENTIRMAI[1,2], K. C. BUCKLEY[3], A. R. MASSIE[3], L. KAPAS[3];
[1]Washington State Univ., Spokane, WA; [2]Department of Translational Medicine and Physiology, Washington State University, Spokane, WA; [3]Dept. of Translational Med. and Physiol., Washington State Univ., Spokane, WA

Abstract: Introduction: Signals from peripheral tissues play a critical role in coordinating sleep-wake activity with the metabolic, nutritional and immune status of the organism. Recent findings highlight the intestinal microbiota as a key source of sleep-promoting signals. Previously, our work demonstrated that intraportal lipopolysaccharide (LPS) administration induces sleep and fever in rats by stimulating Kupffer-cell prostaglandin production, an effect blocked by COX inhibition. As hepatic vagus afferents express PGE_2 receptors, we investigated whether intraportal PGE_2 and arachidonic acid (AA) administration could mimic LPS effects.Methods: Male rats were surgically implanted with intraportal catheter and instrumented with EEG and EMG electrodes and an intraabdominal transmitter to monitor sleep-wake activity, body temperature and locomotion. On the control day, the animals received intraportal injections of 0.3 ml saline. On the treatment day, rats were treated with intraportal injections of 300 μg PGE_2. After a recovery period of 5 days, the same animals received vehicle on the control day, and 600 μg arachidonic acid on the following day. All treatments were performed 5-20 min before dark onset. Sleep, body temperature and motor activity were recorded for 23.5 h after each treatment.

Data were analyzed by using ANOVA followed by Tukey's Honestly Significant Difference testResults: PGE_2 and AA significantly increased non-rapid eye movement sleep (NREMS) and reduced body temperature during the 12-hour post-injection period. PGE_2 and AA induced approximately a 22% and 36% rise in NREMS, respectively (PGE_2 baseline: 236.2 ± 31.0 min, treatment: 287.1 ± 29.5 min, $p < 0.01$; AA baseline: 264.6 ± 30.5 min, treatment: 358.6 ± 34.0 min, $p < 0.05$). Additionally, there was a trend toward increased REMS in response to AA (baseline: 34.7 ± 7.9 min, treatment: 47.1 ± 4.1 min, $p = 0.057$). PGE_2 suppressed EEG SWA, and during the light phase, an increase in body temperature was observed. While there were tendencies toward decreased motor activity, neither PGE_2 nor AA administration led to statistically significant changes.Conclusions: The results suggest that the sleep-inducing effects of PGE_2 are mediated by a sensory mechanism located in the liver and/or in the portal vein wall. Hepatoportal prostaglandin-sensitive pathways likely contribute to sleep modulation by the intestinal microbiota.

Disclosures: E. Szentirmai: None. **K.C. Buckley:** None. **A.R. Massie:** None. **L. Kapas:** None.

Poster

PSTR293: Lateral Hypothalamus and Forebrain Circuits

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR293.01/K22

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH-NIDDK Grant DK118000

Title: Melanin concentrating hormone neuronal signaling and ethanol intake in female rats

Authors: *G. C. E. MADU, E. E. NOBLE;
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Abstract: Melanin concentrating hormone (MCH) is a 19- amino acid neuropeptide produced predominantly in the lateral hypothalamus (LH) and zona incerta (ZI) that has been implicated in elevated intake of both food and drugs of abuse. Alcohol is both a source of kilocalories as well as a drug of abuse, which relates it to both the hedonic and homeostatic aspects of feeding. Prior research shows that pharmacological injection of MCH increases both chow and ethanol consumption in males, however whether these findings are generalizable to females remains unknown. Furthermore, whether the MCH neuronal signaling, similar to MCH pharmacological injection, modulates alcohol intake remains to be determined. Therefore, we investigated the impact of chemogenetic activation of MCH neurons using an MCH promoter driven excitatory Designer Receptor Exclusively Activated by Designer Drugs (DREADDS) approach on ethanol intake in females. Female Wistar rats (n=12) were virogenetically injected with MCH-DREADDs in the LH and ZI and tested for chow, ethanol, or chow and ethanol together intake at

the onset of the dark cycle in a within- subject's design. In a separate cohort (n=16), pharmacological injection of 0, 5, or 10 micrograms intracerebroventricular (ICV) MCH was administered and chow and ethanol intake measured. Results show that activation of MCH DREADDs receptors in females increases ethanol intake but not chow when animals were offered both together. When either were offered alone, however, chemogenetic activation of MCH neurons increased both chow and ethanol intake. In contrast, when given the option to consume chow or ethanol, ICV MCH administration preferentially increased chow intake but not ethanol. Taken together, our findings show differential impacts of MCH neuronal activation and pharmacological injection in female rodents, where MCH neurons preferentially increase ethanol ingestion and MCH ICV pharmacology preferentially increases chow intake.

Disclosures: G.C.E. Madu: None. **E.E. Noble:** None.

Poster

PSTR293: Lateral Hypothalamus and Forebrain Circuits

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR293.02/K23

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH T32 #5T32NS076401-19
NIH T32 #5T32DA007281-25
NIH F31 #1F31DK135233-01
NIH R01 NIH #1RO1DK129366-01

Title: Melanin-concentrating hormone neuron efferents in the NAc mediate the reward value of food and do not induce REM sleep

Authors: *K. FURMAN[1,2,3], H. C. LYONS[4], L. BARON[3,1], J. R. EVANS[1,3], T. CHA[1], J. MANNA[1], L. ZHU[4], J. MATTIS[4,2], C. R. BURGESS[1,2,3];
[1]Michigan Neurosci. Inst., [2]Neurosci. Grad. Program, [3]Mol. & Integrative Physiol., [4]Dept. of Neurol., Univ. of Michigan, Ann Arbor, MI

Abstract: Animals must make informed decisions about what and how much to eat in order to maintain energy balance. Yet homeostatic need is not the sole factor in the decision to eat. Non-homeostatic motivators to eat are common, such as craving of sugary or fatty foods even when sated. Dysregulation of such non-homeostatic motivators can contribute to the development of eating disorders. Melanin-concentrating hormone (MCH) neurons of the lateral hypothalamus (LH) and zona incerta are a relevant neural target for both homeostatic and non-homeostatic motivators to eat. MCH neurons project to many brain areas including the arcuate nucleus, nucleus accumbens (NAc), and cerebral cortex, and have a role in numerous behaviors including feeding, sleep, learning, and reward. Injection of MCH peptide into the NAc increases feeding,

and chemogenetic activation of MCH neurons that project to the NAc slightly increase food intake in male but not female mice.We hypothesize that MCH projections to the NAc promote hedonic motivations to consume food but do not have a role in sleep-wake regulation. To address this hypothesis, we instrumented MCH-ChR2 mice with EEG/EMG headcaps and optic fibers placed either over the MCH neurons in the LH or their terminals in NAc, and investigated how optogenetic stimulation affected feeding and sleep behavior in different behavioral contexts. When optogenetic stimulation was delivered continuously (473nm, 20Hz, 1s ON 4s OFF for 3hr), we observed that mice with stimulation of MCH neurons in the LH (n = 11) spent more time in REM sleep as well as transitioned into REM sleep bouts more frequently ($p < 0.05$), while mice with terminal stimulation in the NAc (n= 13) did not show a sleep effect. This continuous stimulation did not significantly increase feeding, regardless of optic fiber location. However, when given the opportunity to choose between a port which delivers both food and acute optogenetic stimulation or a port which delivers stimulation alone, mice with terminal stimulation in the NAc had a significant preference for food paired with stimulation ($p<0.0001$), while mice with cell body stimulation of all MCH neurons in the LH did not. The preference for NAc terminal stimulation developed within the first day but strengthened with increasing days of stimulation. Additionally, we observe a sex difference in both groups, with male mice seeming to develop a stronger preference for stimulation-paired food regardless of whether they received stimulation of MCH terminals in NAc or MCH cell bodies in LH. These findings begin to elucidate a mechanism by which MCH neurons differentially regulate feeding and arousal as a function of downstream projection area.

Disclosures: K. Furman: None. **H.C. Lyons:** None. **L. Baron:** None. **J.R. Evans:** None. **T. Cha:** None. **J. Manna:** None. **L. Zhu:** None. **J. Mattis:** None. **C.R. Burgess:** None.

Poster

PSTR293: Lateral Hypothalamus and Forebrain Circuits

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR293.03/K24

Topic: F.08. Food and Water Intake and Energy Balance

Support: Cancer Research Society
Medical Research Fund
CIHR- Canada Graduate Scholarship
Memorial University of Newfoundland

Title: Sex-specific role of PGE2 EP2 receptors on MCH neurons in high-fat diet-induced cognitive impairment

Authors: *S. E. C. CAMPBELL[1], M. HIRASAWA[2];
[1]Div. of Biomed. Sci., Mem. Univ. of Newfoundland, St. John's, NL, Canada; [2]Div. of Biomed. Sci., Mem. Univ., St John's, NL, Canada

Abstract: Chronic consumption of Western diet (WD), high in fat and sugar, can result in diet-induced obesity as well as anxiety and memory impairment. However, the mechanisms behind this memory impairment remain unclear. Melanin-concentrating hormone (MCH) neurons in the hypothalamus are known to promote obesity by increasing food intake and decreasing energy expenditure. Previously, our lab has demonstrated that WD induces an inflammatory mediator prostaglandin E2 (PGE2) in the brain, which activates MCH neurons via the EP2 receptor (EP2R), resulting in a vicious cycle of WD intake and weight gain in a sex-dependent manner. As MCH can also modulate memory and mood, we hypothesized that EP2R signaling in MCH neurons mediates WD-induced anxiety and memory impairment. To test this, male and female mice with EP2R deletion in MCH neurons (KO) and EP2R f/f littermates were fed chow or WD for 3 months. The open field test (OFT) showed that WD decreased locomotor activity, while increasing anxiety in both male and female mice. The novel object recognition test is currently ongoing to assess hippocampal-dependent memory. To evaluate synaptic function, fEPSPs were recorded at CA3-CA1 synapses in brain slices. Basal fEPSP was inhibited by WD but not affected by EP2R KO or sex. Paired-pulse facilitation (PPF) was lower in female KO mice compared to other groups, suggesting a higher release probability, which was abolished by WD. To assess activity-dependent synaptic plasticity, 100Hz, 100 pulse afferent stimulation was applied to induce post-tetanic potentiation (PTP) and long-term potentiation (LTP). In females, PTP was lower in KO than f/f regardless of diet, while in males, both KO and WD increased PTP. On the other hand, LTP was not significantly affected by genotype, sex, or WD. Our results suggest that MCH EP2Rs could have a modulatory effect on hippocampal synaptic transmission in a sex-specific manner. This research provides novel insight into the potential role of PGE2 signaling in MCH neurons in diet-induced memory impairment.

Disclosures: S.E.C. Campbell: None. **M. Hirasawa:** None.

Poster

PSTR293: Lateral Hypothalamus and Forebrain Circuits

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR293.04/K25

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NSERC

Title: High-fat diet-induced plasticity of biphasic EPSC in MCH neurons

Authors: M. S. CHOWDHURY, S. C. BOWES, *M. HIRASAWA;
Biomed. Sci., Mem. Univ., St John's, NL, Canada

Abstract: Melanin concentrating hormone (MCH) neurons in the lateral hypothalamus coordinate energy balance by increasing food intake and decreasing energy expenditure. These cells are activated by high-fat diet (HFD) and promote further weight gain, leading to diet-induced obesity. Glutamate plays a key role in shaping MCH activity, which may undergo plasticity upon consumption of HFD. Here, we examined the role of ionotropic glutamate receptors and transporters in mediating and modulating excitatory signaling to MCH neurons and the effect of HFD in this mechanism by using *in-vitro* patch-clamp electrophysiology in acute rat brain slices. Upon presynaptic train stimulation (50Hz, 20 pulses), we identified biphasic EPSCs consisting of fast and slow components, which were mediated by distinct receptor types: Fast EPSC is primarily mediated by AMPA receptors (AMPAR) and kainate receptors (KAR), whereas slow EPSC by NMDAR, AMPAR and KAR. To test the effect of HFD on biphasic EPSCs, male SD rats (7-10 weeks old) were fed with high fat, high sugar western diet (WD) for 1 week. WD had no effect on short-term plasticity of fast EPSC, suggesting that release probability is unaltered. On the other hand, slow EPSCs progressively increased during train stimulation, which could be due to reduced glutamate uptake in WD condition, resulting in increased spillover of synaptic glutamate. Hence, we tested the effect of DHK, a blocker of astrocytic glutamate transporter GLT-1, which had no effect on EPSCs in both chow and WD groups. As KAR was found to underlie both fast and slow EPSCs, the KAR antagonist UBP310 was tested in different dietary conditions. UBP310 inhibited both fast and slow EPSCs in chow-fed group, whereas it decreased slow EPSC but had no effect on fast EPSC in WD-fed group. These results suggest a lack of change in glutamate uptake via GLT-1 while the contribution of synaptic KAR is selectively reduced by WD without a significant change in peri/extrasynaptic KAR. This would favor high frequency synaptic inputs and alter synaptic integration. Thus, chronic dietary fat intake may modulate the glutamatergic excitatory signaling in MCH neurons, and such alteration may be relevant in diet-induced obesity.

Disclosures: M.S. Chowdhury: None. **S.C. Bowes:** None. **M. Hirasawa:** None.

Poster

PSTR293: Lateral Hypothalamus and Forebrain Circuits

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR293.05/K26

Topic: F.08. Food and Water Intake and Energy Balance

Support: R01 DK103676

Title: Fasting increases protein kinase A (PKA)-dependent CREB phosphorylation in lateral hypothalamus (LH) orexin neurons

Authors: *P. SARKAR, P. X. DIAZ MUNOZ, D. M. SIEGEL, V. H. ROUTH;
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Abstract: The neuronal circuitry involved in food-motivated behavior following caloric restriction is poorly understood. Previously we found that caloric restriction increases activation of lateral hypothalamic (LH) orexin glucose-inhibited (GI) neurons in low glucose. This was associated with an orexin-dependent increase in glutamatergic signaling onto the dopamine neurons in the ventral tegmental area (VTA) and the motivation to seek food. Using brain slice electrophysiology, we showed that pertussis toxin and Rp-cAMP (a protein kinase A [PKA] inhibitor) blocked activation of LH-GI neurons in low glucose, suggesting that the Gαi/o-adenylyl cyclase-cAMP-PKA pathway mediates glucose sensing in these neurons. Therefore, we hypothesized that preventing PKA activation during fasting would blunt fasting-induced activation of LH-GI neurons in low glucose and subsequently glutamate plasticity and motivation to seek food. To begin to test this hypothesis, we determined whether 1) fasting increased phosphorylation (p) of LH CREB, the downstream target of PKA; and 2) overexpression of the endogenous PKA inhibitor, PKI, in orexin neurons would prevent fasting-induced pCREB. Mice expressing cre-recombinase in orexin neurons were stereotaxically injected into the LH with cre-dependent adeno-associated PKI overexpression virus (AAV-PKI) or control AAV. Orexin immunohistochemistry was used to validate AAV expression only in orexin neurons. After 2 weeks post-injection, the AAV-PKI and -control mice were fasted overnight and the LH was dissected for Western blot analysis. There was a 2.8-fold ($p<0.005$) increase in LH pCREB in the fasted compared to fed mice injected with control AAV. (n=4). This effect was completely absent in fasted vs fed mice injected with AAV-PKI (n=3). These data suggest that fasting increases pCREB in orexin neurons; an effect that is blocked by the endogenous PKA inhibitor, PKI. This supports our hypothesis that fasting targets the glucose sensitive signaling pathway in LH orexin-GI neurons. Moreover, since the PKA pathway is common to many neurotransmitters and hormonal signals of energy balance, this pathway may be a site of convergence for metabolic signaling. Supported in part by R01 DK103676.

Disclosures: P. Sarkar: None. **V.H. Routh:** None.

Poster

PSTR293: Lateral Hypothalamus and Forebrain Circuits

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR293.06/K27

Topic: F.08. Food and Water Intake and Energy Balance

Support: FONDECYT Regular 1200578
FONDECYT REGULAR 1240616

Title: Context modifies the effect of orexin/dynorphin neurons and orexin peptides on palatable food intake and sucrose demand

Authors: J. JARA[1], L. LUARTE[1], C. SANDOVAL[2], F. MUÑOZ[1], J. TESKE[3], ***C. PEREZ-LEIGHTON**[1];
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Abstract: The orexin (OX) and dynorphin peptides are co-released from OX/DYN neurons located in the lateral hypothalamus (LH) to several brain sites, where they regulate food intake and motivated behaviors. In the paraventricular nucleus of the hypothalamus (PVN), the Dynorphin-A_{1-13} (DYN) and orexin-A (OXA) peptides increase the intake of non-palatable food, but only DYN increases palatable food intake and OXA blocks this effect. However, we still do not fully understand how OX/DYN neurons, OXA and DYN peptides in PVN regulate palatable food intake and if their actions extend to motivated behaviors for palatable food. This study aimed to determine whether OX/DYN neurons and OXA in PVN regulate palatable food intake and demand for sucrose. Experiments were conducted in adult male C57BL6J mice with free access to standard rodent food and water. Intake of palatable foods was modelled using a cafeteria (CAF) diet which gave mice unlimited access to human palatable foods. Demand for sucrose was measured using a behavioral economic test that measured demand intensity (hedonic value of sucrose) and flexibility (decrease in sucrose intake over increasing price). First, we show that access to sucrose reduced by 27.3% the number of active OX/DYN neurons in the LH that project to the PVN ($p<0.05$, n=4). Second, PVN administration of the dual OX receptor antagonist TCS1102 (TCS) increased CAF intake by 39.9% ($p<0.05$, n=8), without further increasing the orexigenic effect of DYN administration in PVN. Third, we show that chemogenetic inhibition of OX/DYN neurons reduced demand intensity by 134.3% ($p<0.05$, n=8) without altering demand flexibility. Finally, the administration of OXA or TCS into the PVN did not impact sucrose demand. Together, these data suggest that while OX/DYN neurons in PVN contribute to the regulation of palatable food intake, OXA and DYN in PVN do not interact to regulate intake of free-access palatable food and that OXA in PVN does not impact sucrose demand. These data suggest that the regulation of eating behavior by OXA and DYN peptides in PVN might be dependent on the context of food access (free vs. operant). Ongoing experiments are determining whether the expression of splicing isoforms of OXA and DYN receptors in the hypothalamus correlates with the preference for a CAF diet or sucrose demand flexibility and intensity.

Disclosures: J. Jara: None. **L. luarte:** None. **C. Sandoval:** None. **F. Muñoz:** None. **J. Teske:** None. **C. Perez-Leighton:** None.

Poster

PSTR293: Lateral Hypothalamus and Forebrain Circuits

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR293.07/K28

Topic: F.08. Food and Water Intake and Energy Balance

Support: R01 DC007703
F31 DC019863

Title: The contribution of the orexin connection to licking behavior and cortical palatability responses

Authors: *K. C. MAIGLER[1], A. SURENDRAN[1], K. BROWNING[1], A. YAMANAKA[3], D. B. KATZ[2];
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Abstract: Primary taste cortex (gustatory cortex, GC) taste-response activity evolves in a well-characterized set of 3 states, the third of which reflects the hedonic value of a taste (palatability) and drives the taste decision. Our prior work has demonstrated that GC works with the lateral hypothalamus (LH) to produce the dynamic taste response. To further dissect the involvement of LH, we dissect the role of orexin+ cells, which are specific to LH and uniquely situated to influence cortical palatability activity. In the current work we first test the hypothesis that the LH orexin→GC connection is influencing palatability-dependent consumption behavior. We use transgenic Orexin-cre rats to optogenetically silence the orexin signal to GC in a brief access task. We found that the rats with their LH orexin afferents silenced consumed more aversive quinine and less palatable sucrose compared to those without silencing. We then go on to directly test whether orexin signal affects palatability-related neuronal activity in GC by recording single-unit activity in GC during passive taste delivery. To strictly test how orexin affects palatability processing in GC, we compare the inhibition of orexin(+) cells to that of orexin(-) cells and a nonspecific neuronal inhibition. Orexin-cre rats received bilateral LH virus injections of either AAV-CAG-FLEX-ArchT, AAV-FLEXoff-CAG-ArchT, or AAV-CAG-ArchT. Following the surgery recovery (virus infusions, GC opto-trodes implantation and IOCs), rats received 3 sessions of 30 trials each of water, 0.3M sucrose, 0.1M NaCl, 0.1M citric acid, and 5mM quinine; orexin-projections were inhibited on half of the trials. After controlling for the density of the connection for each of these groups of neurons in GC and the related proportion of neurons affected by the laser, we found inhibiting any connection to GC reduces the magnitude of palatability correlations and alters the onset of palatability-related firing states. Therefore, while inhibiting orexin does alter palatability behavior in active licking, our stringent comparison of different LH→GC connections suggests disruption of any substantial LH connection to GC will affect palatability processing and related behavior.

Disclosures: K.C. Maigler: None. **A. Surendran:** None. **K. Browning:** None. **D.B. Katz:** None.

Poster

PSTR293: Lateral Hypothalamus and Forebrain Circuits

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR293.08/K29

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH HL127673
NIH HL153274

Title: Metabolic regulation of appetitive behavior: Role of lateral hypothalamic area circuits engaged by leptin

Authors: *U. SINGH[1], B. TOTH[2], G. DENG[1], H. CUI[1];
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Abstract: Heterogeneous groups of neurons within the lateral hypothalamic area (LHA) regulate appetitive behavior, yet the precise mechanism by which LHA neurons perceive peripheral metabolic cues and translate them into food-motivated behavior remains incompletely understood. In this study, we sought to elucidate the role of LHA neurons expressing leptin receptors (LepRs)—a subset of LHA GABAergic neurons—in appetitive behaviors using male mice under ad libitum fed or calorically restricted conditions. We first deleted LepRs from the LHA by targeted microinjection of either AAV-Cre-GFP or AAV-GFP (control) into the LHA of LepR flox/flox male mice (n=6-7/gp). Notably, we observed that the specific deletion of LepRs from the LHA heightened motivation toward a palatable diet under a progressive ratio (PR) paradigm within an operant chamber. Furthermore, chemogenetic activation of LHA LepR-expressing neurons reduced homeostatic intake of either regular chow or a palatable diet while increasing exploratory behaviors (5-7/gp). Using viral-mediated anterograde tract-tracing (n=3), we found that LHA LepR-expressing neurons innervate brain regions implicated in motivated behavior, such as the ventral tegmental area (VTA), further underscoring their significance in regulating motivated feeding behaviors. Additionally, *in vivo* fiber photometry unveiled a marked increase in calcium signaling in LHA LepR-expressing neurons upon exposure to palatable high-fat food pellets and subsequent consummatory behavior in ad libitum fed mice (n=4). Conversely, a similar response was observed with chow diet only under calorie-restricted condition. These findings pinpoint the LHA as a critical site where the adipocyte-derived metabolic hormone leptin exerts its influence on coordinated control of appetitive and consummatory behaviors to meet bodily energy demands.

Disclosures: U. Singh: None. **B. Toth:** None. **G. Deng:** None. **H. Cui:** None.

Poster

PSTR293: Lateral Hypothalamus and Forebrain Circuits

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR293.09/K30

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH grant HL153274

Title: Sex-specific control of appetitive motivation by the medial prefrontal cortex to lateral hypothalamus circuit

Authors: *M. PHAM[1], B. BARNHART[2], L. SCHMIDT[2], U. SINGH[1], G. DENG[3], H. CUI[4]; [1]Neurosci. & Pharmacol., [3]Neurosci. and Pharmacol., [2]Univ. of Iowa, Iowa City, IA; [4]Neurosci. and Pharmacol., Univ. of Iowa Roy J and Lucille A Carver Col. of Med., Iowa City, IA

Abstract: Dysregulation of appetitive control often results in disordered eating that can lead to serious clinical conditions such as obesity and anorexia nervosa, which disproportionately affect women over men. While homeostatic eating is primarily governed by the hypothalamic and brainstem circuits capable of sensing the body's energy status, appetitive motivation toward food is also influenced by the higher brain regions involved in cognitive control, reward evaluation, and decision-making processes that evidently differ between men and women. However, the neural circuits responsible sex-specific control of appetite remains largely unknown. In this study, we assessed the impact of neuronal projections from the medial prefrontal cortex (mPFC) to the lateral hypothalamic area (LHA)—two brain regions known to be involved in emotional and cognitive controls and reward function—on homeostatic feeding and appetitive motivation in both sexes of mice. We found that selective chemogenetic activation of LHA-projecting mPFC neurons resulted in decreased and increased hedonic motivation toward a palatable high-fat diet (HFD) in males and females, respectively, without affecting homeostatic feeding during the dark cycle, indicating a sex-specifically opposing role of LHA-projecting mPFC neurons in the regulation of hedonic drive for palatable food. In vivo fiber photometry revealed an increase in calcium signaling in LHA-projecting mPFC neurons upon feeding and stressful stimuli. These findings underscore the importance of the mPFC-LHA circuit in hedonic feeding and appetitive control, which may underlie the context-dependent sex-specific regulation of appetitive behaviors.

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Poster

PSTR293: Lateral Hypothalamus and Forebrain Circuits

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR293.10/K31

Topic: F.08. Food and Water Intake and Energy Balance

Support: Supported by gifts for research from Cain-Stanley & Co.

Title: Contralateral $GABA_A$ agonist injection into the Lateral Hypothalamus suppresses Lateral Septum-induced feeding

Authors: *I. GABRIELLA[1], V. NAMBIAR[2], C. KUANG[3], A. VENKATRAGHAVAN[4], B. STANLEY[5];
[1]Psychology, UC, Riverside, Riverside, CA; [2]Bioengeneering, UCSD, San Diego, CA; [3]Cell. and Mol. Biol., Univ. of California, Riverside, Riverside, CA; [4]Neurosci., UC Riverside, Riverside, CA; [5]Depts of Psychology & Mol. Cell Systems Biol., Univ. California, Riverside, Riverside, CA

Abstract: It is important to understand the neural circuits that control eating behaviors, especially since more than 40% of adults in the U.S. are either overweight or obese. Previous research has shown that the Lateral Septum (LS) and the Lateral Hypothalamus (LH) are connected both anatomically and functionally. This study aimed to determine whether the connection between the LS and the LH associated with eating is ipsilateral, contralateral, or both via activating and inactivating $GABA_A$ receptors. We bilaterally implanted cannulas in adult male rats' LS and LH. The rats received either a $GABA_A$ agonist (muscimol; 0.3µg/0.3µl) or an antagonist (picrotoxin 0.1µg/0.3µl) in both brain regions to elicit contrasting responses, such as inducing feeding in one brain region while inhibiting it in the other. The injections were administered either ipsilaterally, contralaterally, or bilaterally in a counterbalanced design to investigate which pathway could suppress the induced feeding. The data was assessed by a 2-way repeated measures ANOVA. Results showed that feeding initiated by a unilateral muscimol injection in the LS (n=13) could be significantly suppressed by a contralateral (2^{nd} hr: $p=0.03$, 3^{rd} hr: $p=0.02$) and a bilateral (1^{st} hr: $p<0.01$, 2^{nd} hr: $p=0.03$, 3^{rd} hr: $p<0.01$) administration of muscimol into the LH (Main effect: $F_{(3,11)}=3.67$, $p=0.02$, $\eta_p^2=0.23$). Post hoc analysis revealed that the mean difference between food intake induced by unilateral muscimol injection in the LS and contralateral muscimol injection in the LH was 7.4±3 g ($p=0.03$) during the second hour, and 8±2.9 g ($p=0.02$) during the third hour. However, the ipsilateral administration did not significantly suppress food intake. A unilaterally injected picrotoxin dose into the LH (n=11) was not significantly suppressed by either an ipsilateral, contralateral, or bilateral injection of picrotoxin in the LS. These results indicate that LH signals can override LS signals, placing the LH downstream from the LS and that this connection is contralateral, not ipsilateral. This study contributes to future research about overweight and obesity trends by providing new knowledge on the specific connections between LS and LH.

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Poster

PSTR293: Lateral Hypothalamus and Forebrain Circuits

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR293.11/K32

Topic: F.08. Food and Water Intake and Energy Balance

Support: HKRGC_GRF 14112523
HMRF Grant 09203236

Title: Mu opioid receptor in BNST regulates food intake with region selectivity

Authors: *Y. ZHU[1], S. WANG[1,2], X. ZHAO[1], W. YUNG[3], Y. KE[1];
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Abstract: As a constituent of the extended amygdala, the bed nucleus of the stria terminalis (BNST) comprises multiple subregions and serves as a central hub that broadly integrates and sends signals to other brain regions, thereby playing a paramount role in a wide array of behaviors and functions, such as anxiety, mating, and feeding behavior. On the other hand, BNST also possesses a rich profile of neuropeptides and neural modulator receptors, including those for endogenous opioids. Nonetheless, the functional significance of opioid receptor activation in the BNST remains largely unclear. In this study, we found substantial expression of μ-opioid receptor (MOR) in BNST neurons. By intracranial administration of its agonist DAMGO, we observed that the activation of MOR in BNST induced a voracious food intake in rats. Furthermore, we explored the cellular effects of DAMGO on BNST neurons by conducting whole-cell patch-clamp recording in brain slice. The drug was delivered via micro-perfusion through a separate glass pipette to achieve focal activation on the patched neurons. We found that DAMGO mainly exerted a profound inhibitory effect on BNST neurons, even at nanomolar level. However, this postsynaptic inhibition was not uniform across all BNST neurons but differentially influenced its subregions, with the greatest impact observed in the anteromedial and anterolateral parts of BNST, while only minimally affecting the oval part of BNST. In summary, our results suggest that the activation of MOR in the BNST regulates food intake by selectively modulating the subregions within the BNST.

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Poster

PSTR293: Lateral Hypothalamus and Forebrain Circuits

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR293.12/K33

Topic: F.08. Food and Water Intake and Energy Balance

Support: NIH Grant DC019348
NIH Grant NS122892
NSF Grant IOS1652432

Title: Leptin Receptors in Sensory and Limbic Forebrain Regions of the Mouse

Authors: C. R. CANEPA, J. A. KARA, ***C. C. LEE**;
Comparative Biomed. Sci., LSU Sch. of Vet. Med., Baton Rouge, LA

Abstract: Leptin receptors (LepRs) in the central nervous system enable the control of physiological functions related to hunger and satiety, particularly through their functional regulation of the hypothalamus. Interestingly though, LepRs are also expressed ubiquitously throughout the body and brain, including the cerebral cortex and hippocampus. These forebrain regions can be profoundly affected by satiety, particularly those involved in higher-order sensory, motor, cognitive, and memory functions. However, a cell-type specific characterization of LepR expression in these forebrain regions is necessary to begin assessing their potential functional impact. Consequently, we examined LepR expression on neurons and glia in the forebrain using a LepR-Cre transgenic mouse model. LepR-positive cells were identified using a 'floxed' viral cell-filling approach and immunohistochemical co-labeling for cell-type specific markers, i.e. NeuN, VGlut2, GAD67, Parvalbumin, Somatostatin, 5-HT3R, WFA, S100β, and GFAP. We found that LepR-positive cells were localized to the lower layers (primarily layer 6) in the sensory cortical areas and hippocampus. The lower layer cells exhibited non-pyramidal morphologies, with further analysis suggesting that the majority were neurons, while the remainder were identified as astrocytes (GFAP) or other glial cells. Many of LepR-positive cells co-expressed parvalbumin, while none expressed somatostatin or 5-HT3R. In contrast, all hippocampal LepR expressing cells were neurons, while none co-expressed GFAP. Therefore, our data suggest that leptin can potentially influence neural processing in higher forebrain regions associated with sensation and limbic-related functions.

Disclosures: C.R. Canepa: None. **J.A. Kara:** None. **C.C. Lee:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.01/K34

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant R21 MH121772

Title: Novel Context Induced Activation of the Claustrum in Rats

Authors: *K. W. HE, R. G. PARSONS;
Stony Brook Univ., Stony Brook, NY

Abstract: The claustrum (CLA) is a subcortical structure between the insular cortex and striatum. It has been found to participate in a variety of fundamental brain functions including processing sensory information, attention, sleep, and memory. Prior work from our lab showed that the claustrum was activated during the expression of auditory fear in rats. However, this study was not able to discern whether the activation was related to the expression of auditory fear or exposure to the novel context in which testing occurred. Here, we examined freezing behavior and cFos expression of claustrum in male and female rats using Pavlovian fear conditioning. In Experiment 1, male rats were conditioned in Context A and received 2 tone-shock pairings. On testing day, both groups were exposed to Context B (novel context), while one group of animals received 4 presentations of the tone, and the other were only exposed to the context. We found that the tone-exposed group froze more than the novel group during testing, yet there was no significant difference between groups in cFos expression; and both groups showed elevated cFos expression in CLA relative to naïve rats. In Experiment 2, male and female rats were conditioned in Context A using the same parameters as Experiment 1. One group of rats (familiar) was exposed to Context B at 24 hours and again at 48 hours, while the other group (novelty) received a single context exposure at 48 hours. We found that rats in the novelty group showed significantly higher cFos expression in CLA than rats in the familiar group and that this effect did not differ between sexes. Our data indicate that the claustrum is activated by exposure to a novel environment, but not by the expression of fear. Ongoing experiments will determine if prior conditioning drives the activation of CLA and the role of claustrum in the attentional and mnemonic processes that support fear learning.

Disclosures: K.W. He: None. **R.G. Parsons:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.02/L1

Topic: G.01. Fear and Aversive Learning and Memory

Support: R21 MH121772

Title: The Influence of Context Pre-exposure and Shock Number on Sex Difference in Contextual Fear Conditioning

Authors: *Z. ANDERSON[1], R. G. PARSONS[2];
[1]Stonybrook Univ., Stonybrook, NY; [2]Psychology, Stony Brook Univ., Stony Brook, NY

Abstract: Contextual fear conditioning occurs when a relationship forms between a novel environment and an aversive stimulus, so that when the individual is re-exposed to the environment, there is a conditioned fear response. This form of learning has been used extensively in the laboratory, as it holds special relevance to fear and anxiety-based disorders, which often involve the expression of fear in contexts where the aversive stimulus was not encountered. Females show a higher incidence of anxiety disorders, making the study of how sex influences contextual fear in rodent models of high importance. Prior research comparing males and females is mixed, with some studies showing that males exhibit higher levels of contextual fear than females, others reporting the opposite pattern, and others reporting no difference. These discordant findings are often suggested to be the result of parametric differences between studies, but the determinative factors are not well understood. Here, we tested whether varying the number of trials and pre-exposure to context would mitigate sex differences in contextual fear. In the experiment, male and female rats were divided into two groups: one group was pre-exposed to the context, while the other was not. Each group was further subdivided, with animals either receiving a single shock or 6 shocks during training. A day later, rats were tested in the context in which shock occurred, followed the next day by testing in a novel context to measure the generalization of context fear. Our findings indicate that the observation of higher levels of freezing in an aversive context in males was maintained across groups trained with different numbers of shocks and that males also showed higher levels of freezing behavior in a novel context. Pre-exposure to the training context before conditioning showed no significant effect on contextual fear behavior expressed between the sexes.

Disclosures: Z. Anderson: None. **R.G. Parsons:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.03/L2

Topic: G.01. Fear and Aversive Learning and Memory

Support: R21 MH121772

Title: Sex difference in the activation of prelimbic projections to the ventrolateral periaqueductal gray following contextual fear expression

Authors: *K. VAZQUEZ[1], R. G. PARSONS[2];
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Abstract: There are projections originating from multiple regions of the medial prefrontal frontal cortex (mPFC) that terminate across the different columns of the periaqueductal gray (PAG). Even though both mPFC and PAG have been implicated in regulating fear expression, knowledge about the function of the connections between the mPFC and PAG is scant. In

addition, there have been several studies examining the effect of sex on contextual fear conditioning, but how and whether the neural mechanisms of contextual fear differ is unclear. To begin to understand these questions, we infused a viral retrograde tracer into the ventrolateral PAG in male and female rats and trained half of them in a contextual fear conditioning task, while the other half was exposed to the same context without receiving shock. The following day, all rats underwent a 10-minute context test in the conditioning chamber. 1 hour after testing, rats were perfused and the brains were harvested. Immunohistochemistry was performed and data were analyzed by counting the number of cells that were labeled by the viral tracer and cells that were positive for EGR1 in the anterior cingulate cortex (ACC), prelimbic cortex (PL), and infralimbic cortex (IL). Male rats that underwent training and testing showed a significant increase in the proportion of viral infected cells that express EGR1 in the PL compared to rats that had only received context exposure. There was no significant difference between trained and control in female rats on the same measure. There were no differences between groups in the proportion of tracer labeled cells that were EGR1 positive in the ACC or IL. Our findings suggest that PL projections to the vPAG are involved in the expression of fear and that differences in the activation of this pathway may underlie sex differences in the expression of contextual fear.

Disclosures: K. Vazquez: None. **R.G. Parsons:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.04/L3

Topic: G.01. Fear and Aversive Learning and Memory

Support: College of Wooster Copeland Fund
College of Wooster SOREP Funding

Title: The 5-HT1A receptor agonist 8-OH-DPAT prevents the formation and recall of a contextual fear memory at near and remote time points.

Authors: L. FILIPPI[1], **R. MEHTA**[1], N. KATZENMEYER[1], B. RAMESH[1], M. SHIMIZU[1], *A. ZUNIGA[2];
[1]The Col. of Wooster, Wooster, OH; [2]Col. of Wooster, Wooster, OH

Abstract: Our ability to form, store, and recall memories is critical for our survival. Memories of traumatic experiences or fearful events can promote defensive behaviors that allow organisms to better cope with similar situations in the future or avoid them all together. The retrieval of these memories at inappropriate times can be maladaptive, however. Indeed, anxiety disorders and PTSD are characterized in part by the inability of patients to suppress the recall of traumatic

memories, even under safe conditions. Although serotonin is most known for its role in affective disorders, expanding evidence suggests that serotonin and its receptors contribute directly and indirectly to learning and memory. The 5-HT1A receptor is of particular interest, as prior work has shown that modulation of this receptor affects contextual fear learning. Thus, the goal of this study was to examine the effects of a 5-HT1A receptor agonist on the formation and recall of a contextual fear memory. To test this, male and female C57BL/6J mice were used in two separate experiments. In experiment 1, mice were treated with the 5-HT1A receptor agonist 8-OH-DPAT (1mg/kg, I.P.) immediately prior to contextual fear conditioning, and then tested 24hrs or 15 days after training. In contrast, in experiment 2, mice were given 8-OH-DPAT (1mg/kg, I.P.) prior to the contextual fear conditioning test. As with experiment 1, mice were tested 24hrs or 15 days after fear conditioning. In experiment 1, we found that activation of 5-HT1A receptors inhibited the formation of a contextual fear memory, as demonstrated by a significant decrease in freezing 24hrs and 15 days after training. In experiment 2, we found that activation of these same receptors prior to the recall test inhibited memory recall, again as measured by decreased freezing. Importantly, memory recall was inhibited significantly more during the 15-day test, indicating that this receptor subtype might be more important for the recall of a remote fear memory compared to a more recent one. Our results provide further evidence for the involvement of the serotonergic system in the formation and recall of fearful memories and may provide future therapeutic targets for disorders such as PTSD.

Disclosures: L. Filippi: None. **R. Mehta:** None. **N. Katzenmeyer:** None. **B. Ramesh:** None. **M. Shimizu:** None. **A. Zuniga:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.05/L4

Topic: G.01. Fear and Aversive Learning and Memory

Title: Contextual Fear Conditioning and Brain Stimulatory Intervention in a Rat Model of Blast TBI

Authors: *L. COUGHLIN[1,2], M. MCGUIER[2], L. DWIEL[2], M. COMPANY[2], C. NOLLER[3], K. DISANO[3], P. E. HOLTZHEIMER[3], W. DOUCETTE[2];
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Abstract: Traumatic brain injury (TBI) is the most common neurological disorder. Exposure to the shockwave from an explosion can cause a blast TBI (bTBI). bTBI was the defining injury of the Iraq and Afghanistan conflicts; 1 in 5 veterans suffered a bTBI during deployment. Concerningly, the burden of bTBI extends beyond neurological symptoms; rates of PTSD are 3x

higher among veterans with bTBI. We theorize that bTBI-induced neural damage leads to the dysregulation of fear memory and fear behavior circuits. We employed a preclinical model of bTBI and a context-based fear conditioning paradigm to characterize post-blast fear behavioral phenotypes and corresponding neural activity biomarkers. We hypothesize that bTBI disrupts frontal-striatal-limbic systems and neural biomarkers of blast injury will correspond to individual differences in context-based fear memory expression and extinction, which may be ameliorated by deep brain stimulation (DBS) intervention. Thus, we evaluated the potential of infralimbic cortex (IL) DBS to reduce context-based fear behavior. N=53 male Sprague-Dawley rats received a blast injury; N=30 got a sham blast. N=24 blasted rats were surgically implanted with an electrode to record local field potential (LFP) data before and after conditioning and intervention. All rats underwent context-based fear conditioning, receiving 3 foot shocks over 8 minutes. For one cohort (N=18 blast, N=10 control) a 20 second auditory tone was paired with each shock. After conditioning, N=6 blast rats received a five-day IL DBS intervention. Finally, all rats underwent 20 minutes of context re-exposure to assess freezing behavior. During context re-exposure, blast-injured rats in one cohort froze for a significantly greater percentage of time overall compared to controls ($p=0.0427$). Another cohort had no between-group differences in freezing. The N=6 IL-stimulated bTBI rats spent a lower percent of time frozen than controls with a trend towards significance ($p=0.0557$). Our findings align with previous literature that reports mixed findings for a bTBI phenotype for context fear memory and expression and hint at the potential of IL DBS to reduce fear expression. Analysis of the tone/shock-paired cohort is ongoing; behavioral and neural data will be presented. We anticipate increased freezing upon context re-exposure and will compare this to freezing during subsequent tone re-exposure. Lastly, LFP data from DBS and pharmacological interventions may reveal circuit-level changes that reflect fear behavior changes, allowing for the development of novel therapies that address the neural mechanisms underlying the fear-related consequences of bTBI.

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Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.06/L5

Topic: G.01. Fear and Aversive Learning and Memory

Title: Effects of Oxytocin on Contextual Fear Expression via the Central and Basolateral Amygdala in Male Rats

Authors: *M. KANEKO, A. A. LACKAN, T. Q. YATES, R. J. RAGER-AGUIAR, K.-C. LEONG;
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Abstract: Though fear can be adaptive, maladaptive fear processing can result in detrimental and debilitating outcomes. Unfortunately, there are currently limited pharmacological interventions for fear-related disorders, making it imperative to explore options. Recent studies have investigated the anxiolytic properties of the neuropeptide oxytocin (OXT) to confront stress and fear-related behaviors. The present study examines the ability for OXT to attenuate contextual fear expression in male rats and identify the role of amygdala subnuclei (central amygdala, CeA, and basolateral amygdala, BLA) in mediating this effect. Male Sprague-Dawley rats (N = 57) underwent a contextual fear conditioning paradigm in which they received short, intermittent foot shocks (1.0 mA, duration of 1s) during the first two days of conditioning. On test day, freezing behavior was recorded as a measure of fear expression upon exposure to the fear-conditioned context. In Experiment 1, animals (n = 22) received peripheral injections of either OXT (1 mg/kg or 3 mg/kg) or saline (0.9% NaCl) 30 minutes prior to testing. In Experiment 2a, animals (n = 19) received one of two treatments, an intra-CeA infusion of OXT (0.5 μg at a volume of 0.5 μL/side) or saline, followed by Experiment 2b in which animals (n = 16) received either intra-BLA infusion of OXT (0.5 μg at a volume of 0.5 μL/side) or saline, both administered five minutes before testing. A two-way ANOVA revealed that peripheral injection of OXT in Experiment 1 did not have an effect on contextual fear expression while intra-CeA infusion of OXT in Experiment 2a confirmed the attenuation of the fear response. The effect of intra-BLA infusion of OXT in Experiment 2b was also explored, contrasting with the results of intra-CeA infusion. Overall, our results first demonstrate that OXT may attenuate contextual fear expression depending on the route of administration. Furthermore, intra-amygdala infusion of OXT suggests that this effect may be modulated within the CeA. These findings suggest that OXT may reduce retrieval of contextual fear memories within the CeA, though route of administration must continue to be considered. This proposes OXT as a potential therapeutic remedy for reducing fear expression in contexts where fear memories have been established.

Disclosures: M. Kaneko: None. **A.A. Lackan:** None. **T.Q. Yates:** None. **R.J. Rager-Aguiar:** None. **K. Leong:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.07/L6

Topic: G.01. Fear and Aversive Learning and Memory

Support: KAKENHI Grant 21K15751
KAKENHI Grant 23K27533

Title: The effect of electrical stimulation of the subthalamic nucleus on the expressionof contextual fearin rats

Authors: *A. NAKAJIMA[1,2], T. NEMOTO[3], H. IWAMURO[4], N. HATTORI[3], Y. SHIMO[3]; [1]Juntendo Univ. Nerima Hosp., Tokyo, Japan; [2]Neurology, Juntendo University, Tokyo, Japan; [3]Neurol., Juntendo Univ., Tokyo, Japan; [4]Dept. of Neurosurg., Juntendo Univ., Tokyo, Japan

Abstract: Although deep brain stimulation (DBS) of the subthalamic nucleus (STN) is an established therapy for treatment-refractory obsessive-compulsive disorder (OCD), its therapeutic mechanisms remain unclear. Unilateral STN stimulation may be as effective as bilateral stimulation. To evaluate this hypothesis, we induced lesions to the left side of the context-conditioned rats at the behavioral level. We evaluated rats for obsessive-compulsive ideation under high-frequency left-STN stimulation (frequency: 130 Hz, pulse width 60 microseconds, stimulus current 1mA for 60 seconds). Freezing during acclimation (first 5 min of the test) and startle measurements were compared among the sham, lesioning (only inserted electrode), and stimulation groups. Both behavioral measurements were expressed as the difference in post-test and pre-test scores. We used the Kruskal-Wallis test to evaluate differences between the groups. Fourteen rats underwent stereotaxic brain surgery and were divided into the STN-DBS (n = 5), sham (n = 5), and lesioning (n = 4) groups. No significant differences were found in the fear response measurements (freezing time: $H(3) = 0.32$, $p = 0.87$; startle response: $H(3) = 0.66$, $p = 0.84$). Fear-conditioned rats did not show significant improvements in compulsive behavior following STN-DBS compared with the sham or lesioning groups. While previous studies have suggested improvements with stimulation in other brain regions, this study did not find the same effect with left STN stimulation. It is unclear how DBS affects the OCD loop and which target stimulation is the most effective. The efficacy of STN-DBS for OCD may vary based on hemisphere stimulation and may be less effective than stimulation in other regions, such as the bed nucleus of the stria terminalis.

Disclosures: A. Nakajima: None. **T. Nemoto:** None. **H. Iwamuro:** None. **N. Hattori:** None. **Y. Shimo:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.08/L7

Topic: G.01. Fear and Aversive Learning and Memory

Title: Contextual fear memory formation induces synaptic potentiation at hippocampal afferents in the central amygdala

Authors: M. KARLOCAI[1], B. BARABAS[2], K. MÜLLER[3], ***N. HAJOS**[1];
[1]Indiana Univ. Bloomington, Bloomington, IN; [2]Indiana Univ., Bloomington, IN; [3]Inst. of Exptl. Med., Budapest, Hungary

Abstract: Contextual fear learning is a memory process, during which an originally neutral context becomes associated with a threat. Cortical areas, including the hippocampus play a key role in controlling contextual fear memory formation, yet the function of one of the main output station of fear circuits, the central amygdala (CeA) is less clear in this cognitive operation. Recently we have shown that CeA-projecting vasoactive intestinal polypeptide (VIP)-expressing neurons in the ventral periaqueductal gray (vPAG) regulate contextual fear memory acquisition, but not the recall. As the primary source of contextual information is the hippocampus, we aimed to uncover the synaptic mechanisms underlying vPAG VIP neuron-driven changes at ventral hippocampal (vHC) fibers in the CeA. We hypothesized that VIP axon terminals from the vPAG would potentiate vHC inputs on CeA neurons thus inducing LTP. In line with this prediction, we found that in 60% of slices, LTP was successfully induced by pairing the excitation of vHC afferents and vPAG VIP axons. Furthermore, we compared the AMPA/NMDA receptor-mediated current ratio at vHC afferent in CeA neurons between fear conditioned and control animals and found a significant increase after fear learning. The fear learning induced increase in the AMPA/NMDA ratio could be prevented, however, by suppressing the activity of vPAG VIP neurons using chemogenetics. The freezing during contextual fear memory recall positively correlated with the AMPA/NMDA ratio at vHC afferents in the CeA. Our data thus show that i) CeA networks receive enhanced synaptic inputs from the vHC upon contextual fear memory formation and ii) the vPAG VIP inputs on CeA neurons is necessary for contextual fear learning by potentiating the vHC synapses.

Disclosures: M. Karlocai: None. **B. Barabas:** None. **K. Müller:** None. **N. Hajos:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.09/L8

Topic: G.01. Fear and Aversive Learning and Memory

Title: Thalamo-hippocampal circuit mechanisms for contextual fear generalization

Authors: *Q. WU;
CAS Ctr. for Excellence in Brain Sci. and Intelligence Technol., Shanghai, China

Abstract: Thalamo-hippocampal circuit mechanisms for contextualfear generalizationAuthorsQingge Wu[1,2,#], Yumian Li[1,2,#], Shishuo Chen[12], Jing Liu[1], Chun Xu[*]; [1]Institute ofNeuroscience, Key Laboratoty of Brain Cognition and Brain-inspried Intelligence Technology,CAS Center for Excellence in Brain Science and Intelligence Technology, Chinese Academyof Sciences, Shanghai, China; [2]College of Life Sciences, University of Chinese Academy ofSciences, Beijing, China.**DisclosuresQingge Wu**: None. **Yumian Li**: None. **Shishuo Chen**: None. **Jing Liu**: None. **Chun Xu**: None**Abstract**Proper expression of defensive behaviors is critical for animal survival in a volatile world (i.e.,context). Context fear generalization is a fundamental cognitive function and involves brainareas such as medial prefrontal cortex, thalamus and hippocampus. The nucleus reuniens (NRe),as a thalamic relay of projections from mPFC to hippocampus, plays a key role in the feargeneralization. However, it remains largely unclear how NRe participates in fear generalizationvia regulating the activity of the hippocampus. Using viral tracing, chemogenetic manipulation,and in vivo calcium imaging, we characterized two largely separate subpopulations of NReneurons targeting the dorsal and ventral part of the ventral hippocampus ($vCA1^{dorsal}$ and$vCA1^{ventral}$), respectively. Chemogenetic manipulation showed that the $vCA1^{dorsal}$ -projectingneurons regulated the accuracy and generalization of contextual fear memory, whereas the$vCA1^{ventral}$ -projecting neurons modulated the overall strength of contextual fear memory.Furthermore, miniscope calcium imaging data showed that the context discrimination of$vCA1^{dorsal}$ neurons at both single-cell and population levels decreased after fear conditioning,in a NRe dependent manner. Taken together, these results reveled a thalamo-hippocampalcircuit that is specifically promoting fear generalization.

Disclosures: Q. Wu: None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.10/L9

Topic: G.01. Fear and Aversive Learning and Memory

Support: NSF/IOS 1924732

Title: Role of primary auditory cortex in fear learning during recent and remote memory retrieval

Authors: *S. WASBERG[1], N. COOK[2,3], M. E. NORMANDIN[4], I. MUZZIO[5]; [1]The Univ. Of Iowa Neurosci. Grad. Program, Iowa City, IA; [2]Neurosciences Inst., UTSA, San Antonio, TX; [3]University of Iowa, Iowa City, IA; [4]Psychological And Brain Sci., Univ. of Iowa, Iowa City, IA; [5]Univ. of Iowa, Iowa City, IA

Abstract: Some sounds play an adaptive function since their detection allows animals to avoid threatening situations by converting this sensory input into adaptive actions. In the auditory cortex (AC), neurons display learned responses that distinguish the importance of sounds at a population level: Responses to familiar sounds decrease (sensory-specific adaptation or SSA), while responses to novel sounds increase. Therefore, when a sound is associated with a negative outcome, AC neurons continue to respond strongly to the sound, akin to a novel stimulus, suggesting a learning mechanism that blocks SSA. This indicates that brain circuits might enhance the signal-to-noise ratio in favor of more relevant sounds. According to the systems consolidation theory, memories initially require communication between subcortical and neocortical regions. As memories consolidate in the neocortex, they can be retrieved with less involvement of subcortical inputs. Although this theory has garnered support for episodic memories, it remains particularly controversial for emotional memories reliant on the amygdala, a subcortical area crucial for fear learning and memory. Additionally, the role of the primary AC in retrieval of fearful sound associations is contentious, with its contribution to remote memories being unclear. To address these gaps in knowledge, we manipulated the complexity of two behavioral tasks to explore the AC's role in fear learning using simple and complex sounds. Our findings indicate that principal cells in the AC are vital for flexible retrieval of remote fear representations, regardless of task complexity. Notably, AC involvement is apparent only when animals are exposed to sequential learning of associations with different valences. Furthermore, we examined the evolution of sound-responsive AC cells during recent and remote retrieval using calcium imaging in freely moving mice. Our results reveal the importance of distinct cell types in retrieving a fearful complex sound. We are currently exploring how long-range projections between the AC and lateral amygdala affect different cell types in the AC over time. Understanding the neural representations and dynamics in the AC during retrieval of learned fear will be pivotal in clarifying the circuits involved in fear memory and their impact in anxiety disorders.

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Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.11/L10

Topic: G.01. Fear and Aversive Learning and Memory

Support: SNSF Grant 310030_204587 / 1

Title: A cortical auditory afferent to the posterior insula contributes to auditory-cued fear learning

Authors: D. OSYPENKO[1], S. PALCHAUDHURI[2], O. KOCHUBEY[3], ***R. SCHNEGGENBURGER**[3];
[1]Univ. of Lausanne, Lausanne, Switzerland; [2]New York Univ., New York, NY; [3]Ecole Polytechnique Federale de Lausanne (EPFL), Lausanne, Switzerland

Abstract: During fear learning, neurons in specific brain areas form associations between an aversive stimulus (US), and a sensory cue (CS). Plasticity of the CS representation has been observed in the amygdala; however, other brain regions likely contribute with CS-plasticity to fear learning. The posterior insular cortex (pInsCx) processes multiple types of sensory information including interoceptive signals, and has been implicated in valence coding; furthermore, a pInsCx region that processes auditory information has been identified (Insular auditory field; IAF). Here, we study a possible function of this cortical area in fear learning. Single-unit recordings during auditory-cued fear learning (habituation - training - recall sessions on subsequent days) showed that partially overlapping sub-populations of pInsCx neurons code for the US, and develop responses to the CS ("CS-learner units"). Many CS-learner units were inhibited by freezing, and transiently increased their AP-firing when movement is re-initiated. Thus, during CS-driven fear recall, when mice respond with freezing, CS-learner units responded transiently to tone beeps, embedded in a tonically decreased firing rate caused by freezing. CS learner units were preferentially localized in an ~ 500 µm stripe at the pInsCx - S2 border. Circuit tracing revealed that the auditory thalamus (MGm) and primary auditory cortex (A1) send axons precisely to this area where they make robust glutamatergic synapses, identifying this area as the IAF. To investigate whether local synaptic plasticity at these auditory afferents underlies CS-plasticity, we first measured the AMPA/NMDA ratio (A/N), which revealed an increase of A/N following fear learning selectively at synapses from the A1. Second, we recorded single units in the pInsCx and inhibited A1 axons optogenetically. This revealed that CS-responses in the pInsCx were affected significantly more during fear recall than before fear learning, which shows that excitatory synapses from A1 are the substrate for CS-plasticity in the pInsCx. Finally, bilateral inhibition of A1 afferents to the pInsCx during CS-presentation upon fear memory recall caused a moderate, but significant decrease in CS-induced freezing. On the other hand, suppressing the CS-driven activity of excitatory pInsCx neurons non-selectively did not affect fear memory recall. Our study shows that a learned representation of aversively - motivated tones (CS) in the pInsCx contributes to fear memory recall. Moreover, we identify two auditory inputs to the IAF, and show that CS-engaged pInsCx neurons additionally code for the break of aversively-motivated freezing behavior.

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Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.12/L11

Topic: G.01. Fear and Aversive Learning and Memory

Support: PSC-CUNY Enhanced R01MH118441

Title: Safety conditioning improves auditory discrimination relative to fear conditioning in female mice

Authors: *R. KOLARIC[1], E. LIKHTIK[2];
[1]Biol., CUNY Grad. Program In Neurosci., New York, NY; [2]Biol., Hunter Col., CUNY, New York, NY

Abstract: Stress-related disorders such as post-traumatic stress disorder (PTSD) are more prevalent in women than men and are characterized by fear generalization. Similarly, in auditory fear conditioning (FC), pairing an auditory conditioned stimulus (CS) with an aversive unconditioned stimulus (US) leads to generalization of fear to non-conditioned tones, increases representation of the aversive tone at the auditory cortex (Weinberger, 2007), and increases the perceptual threshold for tone discrimination (Dunsmoor & Paz, 2015). Safety conditioning (SC), which establishes an association between a cue and safety, is one promising approach to mitigate excessive fear expression and improve discrimination of threat from non-threat (Nahmoud et al., 2021). However, it is not known whether SC improves perceptual tone discrimination relative to FC. To address this, we first tested the efficacy of fear vs safety learning in female mice (BL6JC57 PV-Cre Ai9) using paired tone CS-US for FC (n=6), an explicitly unpaired CS and US for SC (n=7), and US only for contextual FC (CFC, n=5). Mice were habituated to the context, followed by 2 days of training (5 trials/day), when a CS (4kHz, 30s) was either explicitly paired (FC) or explicitly unpaired (SC) with the US (0.6mA, 1sec). The CFC group received only the shocks US. The next day, all groups underwent a retrieval phase, and defensive freezing to context and tone were assessed. The SC group suppressed freezing during the safety CS relative to context alone ($p<0.01$), indicating that female mice learned about the safety cue. Notably, mice in the FC group froze similarly to context and cue ($p>0.05$). Next, we questioned whether SC vs FC leads to similar levels of behavioral generalization to non-conditioned auditory cues. Two new groups of mice first underwent 3 days of FC or SC training as described above, at the end of which the SC group (n=5) showed significantly less freezing during the safety CS than context alone ($p=0.01$). The next day we tested auditory discrimination, when mice were presented with the trained CS (4kHz) and two novel tones (1.6kHz, 17kHz). The SC group showed less overall freezing to tones than the FC group. Further, the SC group showed less freezing to the trained CS than to novel tones whereas the FC group showed similar levels of freezing across all tones. Thus, we propose that SC enhances auditory discrimination of novel stimuli in female mice, suggesting that SC may be a useful therapeutic approach to battle maladaptive fear generalization in women. Note that experiments with male mice are ongoing.

Disclosures: R. Kolaric: None. **E. Likhtik:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.13/L12

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant MH077681

Title: Engagement of hippocampal acetylcholine signaling during appetitive and aversive social learning

Authors: *I. M. ETHERINGTON, Y. S. MINEUR, M. R. PICCIOTTO;
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Abstract: Major depressive disorder (MDD) is frequently accompanied by changes in memory function, including bias in favor of negative events and associations, as well as structural changes in the hippocampus. Human brain imaging has linked depression to elevated levels of acetylcholine (ACh), and pharmacological elevation of ACh can induce depression or depression-relevant behaviors in humans and animal models, respectively. We hypothesize that changes in ACh signaling in the hippocampus may contribute to a negative bias in memory function. Using fiber photometry with a genetically-encoded fluorescent sensor (GRAB-ACh) in C57BL6 mice, we measured ACh levels in dorsal and ventral hippocampus during exposure to acute aversive stimuli and found transient elevation proportional to aversive stimulus intensity in both regions. Using a retro-AAV-packaged Cre-dependent calcium sensor in the hippocampus of ChAT-IRES-Cre mice, we found that activity of cholinergic terminals in dorsal hippocampus follows a similar pattern. To determine whether ACh signaling differs during learning or expression of negative versus positive associations, we adapted a bidirectional social olfactory conditioning paradigm to compare positive and negative learning within individual animals. In this task, male mice are repeatedly exposed to negative social experience (social defeat) or positive social experience (interaction with female conspecifics) in the presence of one of two equally-preferred odor cues. Olfactory preference is measured before and after conditioning using a T-maze with the conditioned odorants presented at the ends of the goal arms. We found that pairing with positive and negative social stimuli produces changes in odor preference in favor of the positively-paired odorant. Ongoing work will evaluate hippocampal ACh levels across phases of the bidirectional olfactory learning task and future studies will determine whether changes in ACh signaling are sufficient to produce valence-specific changes in the strength of conditioned olfactory preference.

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Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.14/L13

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NINDS IRP

Title: Threat memory encoding by distinct subsets of basal forebrain cholinergic neurons

Authors: *F. LUO[1], G. V. WATKINS[2], L. JIANG[3], N. S. DESAI[2], D. A. TALMAGE[3], L. W. ROLE[2];
[2]Circuits, Synapses, and Mol. Signaling Section, [3]Genet. of Neuronal Signaling Section, [1]Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: Recent studies reveal that distinct subsets of basal forebrain cholinergic neurons (BFCN) are inscribed into a threat memory engram. BFCNs are distributed along the rostro-caudal axis of the adult forebrain including the medial septum (MS), diagonal band (DB, vertical and horizontal limbs), the substantia innominate (SI), ventral pallidum (VP), and nucleus basalis (NBM). Neurons of the NBM/SI and MS/DB provide the major cholinergic input to the basolateral amygdala and the hippocampus respectively. As both the hippocampus and the BLA are essential network components of threat processing, we asked if MS/DB neurons would show similar changes in intrinsic excitability to those of the NBM/SI following threat learning. We expressed an activity dependent and cre dependent (ADCD) viral construct in the MS/DB and NBM/SI of Chat-Cre x Fos-tTA/GFP mice. This allowed us to tag cholinergic neurons that were transcriptionally activated by training (ADCD -red tagged) as well as those that were transcriptionally activated by training AND reactivated by recall (both Red-ADCD & Fos GFP tagged). We compared physiological properties of MS/DB neurons with those of the NBM/SI in 2 sets of mice. The control set did not undergo behavioral training. The second set underwent tone-shock paired training and then, 72 hrs later were tested for their recall to tone. Within 3 hrs of recall we analyzed physiological properties of MS/DB and the NBM/SI cholinergic neurons, blinded to behavioral condition. We analyzed mice that “learned” to associate the tone with threat and freeze in response to the tone (freezing increase of >10% of pre tone levels) and mice that did not separately. The electrophysiological profile of reactivated cholinergic neurons in strong performing differed from those in behaviorally naive mice, although different subsets of properties were altered in MS/DB vs. NBM/SI. Perhaps most interesting, when we compared MS/DB and NBM neurons activated by training, but not reactivated by recall, we found that NBM neurons in this group had properties more similar to the control NBM neurons whereas the comparable MS/DB neurons more closely resembled their reactivated counterparts then the control group. Both the activated and reactivated neurons of MS/DB and NBM/SI in poor performing mice resembled the neurons in mice not subject to behavioral training. Overall these studies suggest that while both MS/DB and NBM/SI cholinergic neurons are engaged by threat learning, the encoding of this process- both in training and post recall -- may involve different

subsets of physiological features that subserve a net increase in excitability, compared with behaviorally naive neurons.

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Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.15/L14

Topic: G.01. Fear and Aversive Learning and Memory

Support: HHMI

Title: Parabrachial Calca neurons mediate second-order conditioning

Authors: *S. PARK[1], A. ZHU[2], F. CAO[3], R. D. PALMITER[4];
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Abstract: Learning to associate cues, both directly and indirectly, with biologically significant events is essential for survival. Second-order conditioning (SOC) involves forming an association between a previously reinforced conditioned stimulus (CS1) and a new conditioned stimulus (CS2) without the presence of an unconditioned stimulus (US). The neural substrates mediating SOC, however, remain unclear. In the parabrachial nucleus, *Calca* gene-expressing neurons, which react to the noxious US, also respond to a CS after its pairing with a US. This observation led us to hypothesize their involvement in SOC. To explore this possibility, we established an aversive SOC behavioral paradigm in mice and monitored *Calca* neuron activity via single-cell calcium imaging during SOC and subsequent recall phases. These neurons were activated not only by CS1 following its association with the US but also by CS2 after SOC. Chemogenetically inhibiting these neurons during second-order associations attenuated SOC. These findings suggest that activating the US pathway in response to a learned CS plays an important role in forming the association between the old and a new CS, promoting the formation of second-order memories.

Disclosures: S. Park: None. **A. Zhu:** None. **F. Cao:** None. **R.D. Palmiter:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.16/L15

Topic: G.01. Fear and Aversive Learning and Memory

Support: Australian Research Council Future Fellowship (FT190100697)

Title: The acquisition and consolidation of second-order conditioned fear in rats requires neuronal activity and plasticity in the prelimbic cortex

Authors: *L. SAAVEDRA CARDONA[1], J. A. LEAKE[2], R. F. WESTBROOK[1], N. M. HOLMES[1];
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Abstract: Rats quickly learn to fear a stimulus (e.g., a light) that signals a brief but painful foot-shock. The acquisition of this first-order conditioned fear requires activation of NMDA receptors in the basolateral amygdala complex (BLA) and its consolidation requires the synthesis of new proteins. Rats also learn to fear the associates of first-order conditioned stimuli, such as a sound paired with the already-conditioned light. The acquisition of this second-order conditioned fear also requires NMDA receptor activation in the BLA; but its consolidation does not require new protein synthesis. Given the importance of protein synthesis for stabilizing information in memory, here we investigated whether consolidation of second-order conditioned fear requires protein synthesis in a component of the circuitry involved in retrieving and expressing first-order conditioned fear, the prelimbic cortex (PL). Specifically, we examined whether the acquisition of second-order fear requires neuronal activity in the PL; and, if so, whether the consolidation of this fear requires *de novo* protein synthesis in the PL. In each experiment, female Long-Evans rats were exposed to light-shock pairings in stage 1 to establish first-order fear of the light (measured in freezing); and to tone-light pairings in stage 2 to establish second-order fear of the tone. To assess the role of the PL in the acquisition of second-order fear, we used micro-infusions of the GABA agonist, muscimol, to temporarily inhibit neuronal activity in the PL; or the NMDA receptor antagonist, D-APV, to block NMDA receptors in the PL. We found that both approaches disrupted the acquisition of second-order fear: relative to vehicle-infused controls, rats that received either infusion immediately prior to stage 2 froze significantly less to the tone in the final drug-free test. Next, we examined whether the consolidation of second-order fear requires protein synthesis in the PL using micro-infusions of the protein synthesis inhibitor, cycloheximide. Importantly, rats that received this infusion immediately after the stage 2 training session froze significantly less to the tone at test (compared to vehicle-infused controls). Together with previous findings, these results show that the acquisition of second-order fear requires activation of NMDA receptors in both the BLA and PL; but that the consolidation of second-order fear requires *de novo* protein synthesis in the PL only. Our future work will examine how the BLA and PL communicate to establish second-order conditioned fear, and whether the protein synthesis requirement for consolidation of second-order fear in the PL depends on this communication.

Disclosures: L. Saavedra Cardona: None. **J.A. Leake:** None. **R.F. Westbrook:** None. **N.M. Holmes:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

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Program #/Poster #: PSTR294.17/L16

Topic: G.01. Fear and Aversive Learning and Memory

Support: Australian Research Council Future Fellowship (FT190100697)
National Centre for Scientific Research (CNRS), France
ANR-20-SFRI-0001, France

Title: The dorsal hippocampus is necessary to integrate and retrieve memories in sensory preconditioning

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Abstract: The ability to integrate information acquired at different times enables animals and humans to respond appropriately in novel situations. This type of integration can be studied using sensory preconditioning protocols in rats. In a standard protocol, rats are exposed to pairings of two neutral stimuli (S1 and S2) in stage 1 and, 24 hours later, to pairings of S1 and an aversive unconditioned stimulus (US, e.g., foot shock) in stage 2. Finally, during stage 3, rats exhibit fear responses when tested with S2 even though it was never directly paired with the US, showing that they had integrated the S2-S1 and S1-US information acquired in stages 1 and 2. Several studies have implicated the hippocampus in aspects of this protocol (Busquets-Garcia, 2018; Iordanova et al., 2011; Wimmer & Shohamy, 2012) . However, as these studies have used a range of designs and parameters, the precise role of the hippocampus in sensory preconditioning remains to be determined. To address this gap in knowledge, the current study systematically examined the involvement of the hippocampus in encoding, integrating and retrieving the different types of associations that form across the three stages of an audio-visual sensory preconditioning protocol. Using micro-infusions of the $GABA_A$ receptor agonist, muscimol, we inhibited the dorsal hippocampus (dH) of male Long-Evans rats during stage 1 or stage 2, or at the time of testing with the S1 or S2. We found that inhibiting the dH during stage 1 had no effect on test levels of fear (measured in freezing) to S2 or S1. By contrast, inhibiting the dH during stages 2 or 3 disrupted preconditioned fear to S2 without affecting directly conditioned fear to S1. Subsequent experiments showed that the role of the dH in establishing sensory preconditioned fear: 1) involves activation of NMDA receptors in stage 2 (confirmed using micro-infusion of the NMDA receptor antagonist, DAP5); 2) is distinct from that of the perirhinal cortex (PRh) which is also involved in stage 1; and 3) likely relates to information

exchange between the PRh and basolateral amygdala complex (BLA - examined by combining inhibition of the dH with an assessment of neuronal activity in the PRh and BLA). Together, our results confirm that the hippocampus plays a critical role in sensory preconditioning and identify this role with regulation of activity in regions that encode the S2-S1 (PRh) and S1-US (BLA) memories. Thus, they shed light on how sensory and emotional memories are integrated such that events never paired with danger become frightening.

Disclosures: M. Talaron: None. **S. Killcross:** None. **E. Coutureau:** None. **N.M. Holmes:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.18/L17

Topic: G.01. Fear and Aversive Learning and Memory

Support: Marie Sklodowska-Curie grant agreement 956414

Title: Dorsal raphe nuclei/ventrolateral periaqueductal grey and cerebellar fastigial nucleus interactions modulate danger response during fear learning

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Abstract: Fear learning and extinction are crucial for survival and lead to disease, if impaired. Recently, it has been demonstrated that the cerebellum is bidirectionally controlling fear learning via the fastigial nucleus (FN) to ventrolateral periaqueductal grey (vlPAG) pathway in rodents (Frontera et al., 2020, Lawrenson et al., 2022). The mechanisms of the cerebellar influence on fear learning still need to be elucidated.Using a combined anterograde/retrograde viral tracing strategy, we characterized a dorsal raphe nuclei (DRN)/vlPAG pathway that projects on the FN. In extracellular recordings during a modified pavlovian fear conditioning paradigm, we found that this pathway is facilitating danger responses in the FN during fear learning. We also identified cross-correlated pairs between FN and DRN/vlPAG neurons, which form a functional loop between DRN/vlPAG and FN. We are now further characterizing this midbrain-cerebellar loop in fear learning and extinction.

Disclosures: J. Urrutia: None. **C. Léna:** None. **D. Popa:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

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Topic: G.01. Fear and Aversive Learning and Memory

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National Science Foundation (to EE)

Title: Tensor decomposition of neural population activity supporting contingency judgement learning

Authors: *J. STEINHAUSER[1], J. PASTORE[3], K. SHULER[4], E. PAPALEXAKIS[2], E. KORZUS[3];
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[3]Psychology, Univ. of California Riverside, Riverside, CA; [4]UC Riverside, Riverside, CA

Abstract: Learning the distinction between environmental cues representing threat or safety is critical for survival. Understanding how appropriate judgements of the conflicting environmental cues are learned to maintain correct cue-behavioral outcome relationships is defined as contingency judgement learning (CJL). We are investigating the neural mechanisms within the prefrontal cortex that are implicated in CJL using a fear discrimination learning paradigm. We hypothesized there to be unique populations of neurons within the prelimibic region (PL) of the medial prefrontal cortex (mPFC) that are necessary for CJL. Understanding the underlying neuronal dynamics during CJL of threat and safety is of importance due to its implications in maladaptive fear responses, such as overgeneralized fear.We employ contextual fear conditioning followed by fear discrimination learning. The current CJL behavioral paradigm consists of 1 day of fear conditioning and a subsequent 8 days of contextual fear discrimination learning where mice are exposed to two similar, yet distinct contexts that have unique cues signaling danger and safety. To investigate prefrontal cortex network dynamics associated with CJL, we used calcium imaging in freely behaving mice and recorded calcium signals across CJL. Then we used tensor decomposition as an unsupervised technique that decomposes multidimensional arrays into latent factors. Tensor decomposition is a valuable method to represent large datasets with multiple factors into concise components that model specific aspects of the data. Here, we represented our data as a 3-mode tensor with neurons' spiking activity, time within a trial, and the total number of trials as the 3 factors. Tensor decomposition uncovered several unique neuronal populations predicting different phases of learning. Specifically, there is a population of neurons in PL that are highly engaged during different phases of CJL, another population of neurons coactive before fear conditioning, and a third population maintaining responses to a neutral context. Overall, these results are informative of

the prefrontal neuronal dynamics associated with CJL and provide valuable insight into the different populations of neurons that are selectively activated during different phases of CJL.

Disclosures: J. Steinhauser: None. **J. Pastore:** None. **K. Shuler:** None. **E. Papalexakis:** None. **E. Korzus:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

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Topic: G.01. Fear and Aversive Learning and Memory

Support: DoD/ARL W911NF-23-1-0145
Brain and Behavior Research Foundation
Brain and Behavior Research Foundation
NIH/NIMH 5R01MH106617
National Science Foundation

Title: Learning-specific neuronal community detection and its dynamics associated with contingency judgement learning of safety and threat

Authors: *K. SHULER, J. PASTORE, J. STEINHAUSER, E. PAPALEXAKIS, E. KORZUS; UC Riverside, Riverside, CA

Abstract: The ability to learn predictive contingencies is critical in many real-world scenarios and is relevant to individual survival. However, more ambiguous situations can make it difficult to determine accurate cue-outcome relationships. In certain stressor-related mental disorders such as posttraumatic stress disorder, some of the hallmark symptoms may be caused by disruptions in learning proper contingencies in the presence of conflicting environmental cues. To be able to provide more focused therapeutics, it is key to identify the neural processes and specific brain regions involved in this learning process, contingency judgment learning (CJL). In previous rodent research, the medial prefrontal cortex (mPFC) has been identified as an important brain region for modulatory control over fear expression through its direct connections to the amygdala, which is especially relevant in CJL involving threat/safety predictions. The prelimbic (PL) region of the mPFC has furthermore been implicated in guiding behavior in ambiguous situations as well as discriminating between safe and threatening stimuli. Thus, we expected PL to be particularly important for CJL of threatening and safe cues. Using calcium imaging in freely behaving mice, we observed the neural activity within PL during a CJL paradigm to test this hypothesis. Mice were exposed to three stimuli that predicted different outcomes: a neutral stimulus (no outcome), an aversive CS+ context (threat outcome, paired with foot shock), and a CS- context that is similar to CS+ but distinct (safety outcome). The CS-

context is initially ambiguous due to contextual similarities to CS+ and mice generalize fear to CS- even though a foot shock is never received in this context. Throughout multiple days of exposure to all contexts, mice gradually learn that the CS- context does not predict danger but is predictive of safety. We analyzed the calcium data using a novel application of graph-based community analysis. A graph representation of our data was created based on the functional relationships between individual neurons' activity. Community analysis was then used to identify communities, or subpopulations of neurons, in the graph that were frequently coactive across the CJL paradigm. We detected communities in PL that were active during different phases of CJL predicting stimulus-outcome relationships. Notably, one PL community activity pattern was engaged across early and late phases of CJL that corresponded to learning to disambiguate CS- from CS+ and that CS- predicts safety while CS+ predicts danger. This finding supports that the neural network in PL facilitates successful CJL involving threatening and safe cues.

Disclosures: K. Shuler: None. **J. Pastore:** None. **J. Steinhauser:** None. **E. Papalexakis:** None. **E. Korzus:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.21/L19

Topic: G.01. Fear and Aversive Learning and Memory

Support: NSERC
CFI

Title: Role of medial prefrontal cortex in mediating relief learning

Authors: *L. R. THIVIERGE, H. LEHMANN, N. M. FOURNIER;
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Abstract: Maladaptive fear can emerge from incorrectly attributing danger to an otherwise nonthreatening stimulus. To date most of this work has focused on mechanisms involving fear extinction, in which the repeated presentation of a previously threat-associated cue no longer serves as a reliable predictor of danger. However, animals can learn to associate a cue with the absence of danger in other ways. Past work has shown that environmental stimuli encountered immediately following the cessation of an aversive event can provide a signal of relief. Both human functional neuroimaging and animal studies have shown that offsetting an acute noxious and threat-evoking stimulus can activate brain regions that regulate appetitive and aversive defensive responses, such as the medial prefrontal cortex (mPFC). However, the specific role of the mPFC in relief learning remains unclear. In the present study, rats underwent presentation of either 6 explicit forward pairings (threat conditioning) of a tone and foot shock (5000 Hz, 12 s)

or 6 explicit backward pairings (relief conditioning) of a shock and tone. Twenty-four hours later, a recall test was conducted by presenting the tones (CS) in the original training environment. We found that the relief-conditioned group showed significantly lower freezing during the presentation of the test CS tones compared to the threat-conditioned group. Immunohistochemical labelling observed higher numbers of Fos+ cells in the mPFC of relief-conditioned rats than in threat-conditioned rats or those that underwent fear extinction. Moreover, we also observed greater expression of GluA1 phosphorylation at Ser831 in mPFC homogenates. In future experiments, we will examine activity in projections to the mPFC in relief-conditioned rats following infusion of a viral GFP-conjugated retrograde tracer. This work will help improve our understanding of the neural circuitry involving relief learning and can provide insight into how the mPFC can regulate and control behavior in response to threatening stimuli.

Disclosures: L.R. Thivierge: None. **H. Lehmann:** None. **N.M. Fournier:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.22/L20

Topic: G.01. Fear and Aversive Learning and Memory

Title: Spiking dynamics in the basolateral amygdala during discriminative fear learning and recall

Authors: *D. MAGYAR[1,2,3,4], Z. REÉB[5,6], N. HAJOS[7,3,2];
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Abstract: The basolateral amygdala, a key structure in the brain's limbic system, plays a crucial role in fear learning and memory formation. Located in the temporal lobe, it receives sensory information from various cortical and subcortical regions. Through its intricate network of connections, particularly with the thalamus and prefrontal cortex, the basolateral amygdala integrates sensory information to promote fear responses. Studies have shown that interfering the function of this region impairs the acquisition and expression of fear-related behaviors, highlighting its significance in adaptive responses to threatening stimuli. Understanding the mechanisms underlying fear learning in the basolateral amygdala holds implications for treating

anxiety disorders and post-traumatic stress disorder. In our experiment, we employed 64-channel silicon probe electrophysiological recordings in head-fixed mice to monitor single-neuron activity from distinct nuclei within the amygdala during repeated presentations of two neutral stimuli (tone), one of which was co-terminated with an aversive input (tail shock).We observed significant alterations in neuronal responses during and following tone-shock pairings and when the two distinct tones were presented alone during the recall phase. Specifically, we noted increased firing rates and enhanced responsiveness to the tone associated with shock in neurons within the basolateral amygdala. These results provide insights into the neural dynamics during tone-shock association and recall and emphasize the dynamic nature of amygdala activity in promoting emotional behaviors. Such knowledge is instrumental in elucidating the pathophysiology of anxiety disorders and devising targeted therapeutic interventions aimed at modulating amygdala function.

Disclosures: D. Magyar: None. **Z. Reéb:** None. **N. Hajos:** None.

Poster

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Program #/Poster #: PSTR294.23/L21

Topic: G.01. Fear and Aversive Learning and Memory

Support: JSPTPN Grant T32 NS115704

Title: Unveiling the role of Inusla-BNST circuit in salience processing and fear discrimination

Authors: *A. SHIPMAN, J. LITTLE, S. CENTANNI;
Translational Neurosci., Wake Forest Univ. Sch. of Med., Winston-Salem, NC

Abstract: Post-Traumatic Stress Disorder (PTSD) prompts the misattribution of neutral stimuli as threatening, blurring the discrimination between reality and traumatic memories. A novel pathway implicated in this salience misattribution is the Insula-BNST circuit. The Insula, part of the salience network, governs interoceptive sensations toward stimuli, while the bed nucleus of the stria terminalis (BNST) processes emotionality involving threat monitoring. This study aims to elucidate the role of the Insula-BNST circuit in discerning the saliency and discrimination between fearful and safe stimuli, providing distinct stress-circuit biomarkers for novel therapeutic interventions for stress-related disorders. Utilizing discriminatory fear conditioning in rodents, a routine paradigm for fear learning, we trained mice to associate auditory cues with respective stimuli. A conditional auditory tone (80 dB, 10s) is paired with a shock (0.8 mA, 1s) and referenced as a fearful cue (CS+). A secondary tone (60 dB, 10s) is not paired with a shock ("safety cue", CS-). 24 hours after the training session, mice were presented with the same auditory tones for CS+/CS- in the absence of foot shock to test for conditioning. Using *in vivo*

fiber-photometry, we measured real time neuronal activity in the Insula-BNST circuit during the training and testing sessions of fear conditioning. To do this, we unilaterally injected a retrograde GCaMP virus into the BNST, and a fiber optic implant was ipsilaterally inserted into the Insula. During the training session, GCaMP activity of Insula-BNST neurons was highest during the fearful experience, the shock. This heightened activity was maintained during CS+ phase of the testing period despite lacking a shock. Interestingly, this heightened activity for CS+ during the testing session sustained even after the tone ended which could indicate a measure of anticipatory anxiety in waiting for the shock to occur. The CS- phase had similar inactivity during both sessions. All together, these results suggest that the Insula-BNST circuit may play a role in processing the saliency and discrimination between fearful and safe stimuli. This could indicate that BNST projecting Insula neurons associate cues to fearful experiences and then recall that experience to the cue even in non-stressful environments. Future experiments will further establish this pathway in the larger stress circuitry which will improve diagnoses and early intervention therapeutics for stress-related disorders.

Disclosures: A. Shipman: None. **J. Little:** None. **S. Centanni:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

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Program #/Poster #: PSTR294.24/L22

Topic: G.01. Fear and Aversive Learning and Memory

Support: NS R35111562 (GT)

Title: Investigating the neural correlates of maladaptive taste learning in a mouse model of autism

Authors: *C.-H. WU[1], G. TURRIGIANO[2];
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Abstract: Animal models of autism spectrum disorder (ASD) exhibit a wide range of sensory processing and learning deficits. However, the extent to which the cortical plasticity that modifies learned behavior based on updated sensory experiences is impacted in these animals remains incompletely understood. Here, we address this question using conditioned taste aversion (CTA), a paradigm of associative learning wherein animals learn to avoid a taste following its association with gastric malaise. A notable feature of CTA is that the learned aversion will subside if the conditioned taste is later uncoupled from the negative outcome. This malleable process makes CTA and its “extinction“ a well-suited model for probing the (in)flexibility of associative memory. We employed in vivo two-photon calcium imaging in a head-fixed CTA paradigm to examine learning-induced changes in taste-evoked neuronal

activities in the mouse anterior insular cortex, a brain region critical for CTA. We found that in wild-type mice, the number of insular neurons evoked by different tastants and the magnitude of their responses to these tastants remain stable and comparable throughout the learning process. However, the taste-related tuning properties of these neurons underwent a dynamic transformation during memory extinction. At the early stage of memory retrieval, most neurons displayed unimodal and selective responses to either the conditioned or the control tastant, but this discriminability rapidly degraded during the extinction of the taste aversion. Our findings thus support the mounting evidence that neuronal representations of taste in the anterior insular cortex are heavily modulated by experience-dependent changes in taste valence. We then went on to investigate whether such neural adaptability is impaired in a mouse model genetically depleted of Shank3, an important ASD-associated postsynaptic scaffold protein. Our preliminary results indicated that Shank3 deletion leads to stronger but less reliable taste-evoked neuronal activities in the insular cortex during memory retrieval. These functional changes may compromise the selectivity of taste-evoked responses in these neurons, behaviorally resulting in defective learned taste aversion that is more susceptible to extinction. Our ongoing research aims to further elucidate the cell-type specific alterations in synaptic and intrinsic properties that could explain the abnormal taste responsivity in these mutant animals. Ultimately, our work will provide novel insight into the cellular and systems mechanisms underlying maladaptive sensory learning in ASD.

Disclosures: C. Wu: None. **G. Turrigiano:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

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Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH grant DC006666
NIH grant DC007703

Title: Inhibition of basolateral amygdala alters experience-induced latent enhancement of taste aversion learning

Authors: *J.-Y. LIN[1], D. B. KATZ[1], V. L. FLORES[2];
[1]Psychology, Brandeis Univ., Waltham, MA; [2]Psychology, Furman Univ., Greenville, SC

Abstract: Animals readily learn to avoid a taste if its consumption leads to aversive post-ingestive consequences (e.g., malaise), a type of associative learning termed conditioned taste aversion (CTA). The acquisition of CTAs can occur at different rates. For instance, if animals are exposed to benign taste stimuli before aversion learning, their CTAs are acquired at a faster rate

than those without prior taste exposures (latent enhancement [LE] of CTA). Subsequently, we found, through in-vivo electrophysiology, that benign experience likely enhances CTA learning by improving taste coding in the gustatory cortex (GC) – taste responses become more reliable but also more discriminable between tastes. Given the close interaction between GC and the basolateral amygdala (BLA) in both taste coding and CTA learning, we tested the hypothesis that the occurrence of LE requires a functional BLA to assist GC taste processing. Specifically, we examined the impact of BLA inactivation during prior taste exposure on subsequent CTA learning to a novel taste. Female Long Evans rats (N = 18) were first infected with pAAV-CaMKIIa-hM4D(Gi)-mCherry or control virus bilaterally into BLA, and three weeks later received an implantation of intra-oral cannula for taste delivery. After recovery, the rats were water-restricted and exposed to a taste battery (water, 0.1M NaCl, and 0.02M citric acid) for 3 days before receiving CTA training to 0.3 M sucrose. During taste exposure, BLA was inhibited by activating the inhibitory designer receptors exclusively activated by designer drugs through CNO (2%) injections. Surprisingly, rather than attenuating LE, BLA inactivation resulted in even stronger CTA acquisition (i.e., higher LE) compared to rats with intact BLA function during taste exposure. This pattern of results suggests a complex BLA involvement in GC taste processing, likely to be context-dependent. Future research will explore how BLA inhibition during taste exposure impacts GC responses toward novel taste stimuli, shedding light on the nature of the involvement of BLA in the integration of taste experience with subsequent associative learning.

Disclosures: J. Lin: None. **D.B. Katz:** None. **V.L. Flores:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

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Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant F9913770402

Title: Basolateral Amygdala Ensembles Supporting the Persistence of Stress-Enhanced Fear Memory

Authors: *J. COLOM-LAPETINA[1], M. HAFENBREIDEL[2], R. SANDO[3], G. RUMBAUGH[1], A. MAXIMOV[4], C. A. MILLER[5];
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Abstract: Stress leads to the enhancement of memory in both humans and animals. This stress-enhanced memory has relevance to psychiatric disorders such as posttraumatic stress disorder (PTSD), which is marked by perseverant memories of trauma. Our group previously developed a stress-enhanced fear memory (SEFM) protocol that produces PTSD-like characteristics in inbred male and female mice, including recent and remote memory that is both enhanced and resistant to extinction. Consistent with the human PTSD literature, SEFM mice also show heightened activity in the basolateral amygdala (BLA), a crucial node for regulation of affective memory. In this study, we sought to determine how acute stress impacts cellular ensembles in the BLA to influence the persistence of fear memories. To address this in male and female mice, we combined our SEFM paradigm with activity-dependent labeling of stress ensembles via intra-BLA injections of an AAV expressing the synthetic activity-dependent promoter eSARE, in which cells that are active during a 30-minute window at the time of trimethoprim lactate (TMP) administration are permanently labeled through expression of GcAMP6s fluoresence. Remote fear memory ensembles were labeled in these same mice by immunofluorescent identification of cells expressing the immediate early gene Arc during recall of the remote (30-day) SEFM. Control mice were exposed to the home cage in lieu of restraint stress. Both groups underwent the same auditory fear conditioning procedure. As expected, greater eSARE labeling was observed in the BLA when tagging occurred at the time of restraint stress, as compared to non-stressed controls exploring the homecage. SEFM mice also exhibited elevated Arc immunolabeling associated with remote SEFM recall, as compared to fear conditioning only controls. Further, SEFM mice displayed remote fear memory patterns of activity across the anterior-posterior axis of the BLA that differed from controls. Current analysis is focused on the overlap of these ensembles and correlations with behavior. Collectively, our findings are expected to provide insight into the neurobiology underlying differential vulnerability to stress.

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Poster

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Location: MCP Hall A

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Program #/Poster #: PSTR294.27/L25

Topic: G.01. Fear and Aversive Learning and Memory

Title: Deciphering the Role of the Anterior Pretectal Nucleus in Fear Generalization

Authors: *X. QIN[1], X. YANG[2], Y. WANG[3];
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Abstract: Posttraumatic stress disorder (PTSD) is a severe psychiatric disorder with limited effective treatment options. While the amygdala and its subdivisions have been the primary focus of studies in fear memory mechanisms due to their role in fear acquisition and extinction, the need for alternative targets is critical as interventions in these areas can impair essential functions such as threat detection and reward processing. Recent research has highlighted the potential role of thalamic and subthalamic nuclei in fear generalization, but their exact mechanisms remain unclear. The anterior pretectal nucleus (APtN), a midbrain structure with significant connections to these regions, shows promise in understanding these mechanisms due to its role in visual processing and its interaction with the zona incerta (ZI), which when attenuated, enhances fear generalization without affecting adaptive fear responses. Preliminary studies suggest a link between heightened APtN activity and generalized fear responses, leading to the hypothesis that targeting APtN activity might mitigate excessive fear generalization. This hypothesis is supported by the safety established in direct APtN stimulation in rodent models for other neural functions.Our research aims to establish a stable behavioral model for visual information-based fear generalization and investigate the neuronal activities within the APtN in relation to fear recall and generalization. We have developed a behavioral model by manipulating variables such as the interval between training and testing and environmental cues, which successfully induced comparable fear responses in altered contexts. Further, our experiments employing chemogenetic tools to modulate APtN activity have shown that its inhibition leads to reduced fear generalization, suggesting a direct role in fear modulation. Currently, we are exploring the role of AMPA receptor expression in the APtN, particularly the GluA2 subunit, in fear generalization, with preliminary data indicating that modulation of this receptor could influence fear responses. This ongoing work could open new therapeutic avenues for managing PTSD and related anxiety disorders by targeting less explored brain regions like the APtN.

Disclosures: X. Qin: None. **X. Yang:** None. **Y. Wang:** None.

Poster

PSTR294: Aversive Memory: Acquisition, Modification, and Expression II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR294.28/L26

Topic: G.01. Fear and Aversive Learning and Memory

Support: NRF2012R1A3A1050385
IBS-R001-D3

Title: Exploring Synaptic Engram Dynamics Between Ventral Hippocampus and Amygdala During Memory Formation

Authors: *I. HONG[1,2], Y. KIM[1,2], B.-K. KAANG[1,2];
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Abstract: Memory, a fundamental trait facilitating learning and knowledge accumulation, is orchestrated by ensembles of engram cells in the brain. While previous research confirmed the existence of these cells, the correlation between cellular and synaptic activities remains elusive. In our study, we aimed to explore the visualisations and molecular mechanisms of synaptic transmission and plasticity. Utilising the dual eGRASP technique in C57BL/6 mice, we investigated connections between pre-synapses in the ventral CA1 (vCA1) and dendrites in the basal amygdala (BA) during memory formation. Contextual cues activated vCA1, resulting in an increase in synaptic density in a neutral context (N=5 mice), whereas electric foot shocks specifically engaged BA, leading to observed synaptic strengthening predominantly in the contextual fear conditioning group (N=4 mice). Furthermore, the foot shock group exhibited not only increased synaptic density but also alterations in spine morphology, including an increase in spine length and volume. This underscores the significance of engram-to-engram synapses in associative memory. Subsequently, we achieved significant disruption of memory consolidation with four injections of anisomycin, resulting in freezing levels and engram cell activity nearing baseline. Our study aims to deepen our understanding of the physiological implications of synaptic transmission and plasticity through an exploration of synaptic engram. Additionally, we investigated the role of protein synthesis in memory formation by administering protein synthesis inhibitors (PSIs) to induce retrograde amnesia. The control group, injected with saline (N=9 mice, freezing level 13.61 ± 1.92%), and the single injection of anisomycin group (N=9 mice, freezing level 12.32 ± 2.42%), both exhibited fear responses upon optogenetic reactivation of engram cells. However, mice receiving multiple injections of anisomycin (N=6 mice, freezing level 1.44 ± 0.33%) or a single cocktail injection (anisomycin combined with cycloheximide; N=7 mice, freezing level 1.179 ± 0.25%) displayed impaired optogenetic memory recall, highlighting the essential role of synapses in memory storage. Collectively, engram cells, recognised as pivotal contributors to memory formation and retrieval, predominantly function at synaptic levels.

Disclosures: I. Hong: None. **Y. Kim:** None. **B. Kaang:** None.

Poster

PSTR295: Avoidance and Other Defensive Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR295.01/L27

Topic: G.01. Fear and Aversive Learning and Memory

Support: R01NS121253

Title: The role of the Parafascicular thalamic nucleus in avoidance behavior

Authors: *M. ROSHCHINA, H. H. YIN;
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Abstract: The parafascicular nucleus (Pf) is a component of the intralaminar thalamic system, implicated in variety of brain functions, including arousal, attention, motor initiation, and nociceptive processing. Previous research has demonstrated that optogenetic activation of Vglut2-expressing Pf neurons elicits robust head turning behavior followed by subsequent whole-body rotation. In the present study we employed closed-loop optogenetic stimulation, initiating upon the entry of mice into a designated area within an open field. We observed clear avoidance behavior: mice consistently avoided the area where they received stimulation. We analyzed physiological parameters commonly associated with states of arousal and fear, including pupil diameter, heart rate, and facial expressions. Kinematic analysis of facial expressions revealed a distinct facial grimace characterized by orbital tightening, bulging of the nose, and vibrissae pads. Additionally, we detected pronounced pupil constriction following optogenetic activation of Pf neurons. Collectively these results that prolonged Pf stimulation is associated with a painful sensation. Given that one of the primary inputs to the Pf originates from the superior colliculi (SC), a structure responsible for detecting potentially threatening stimuli and orchestrating defensive responses, we hypothesize that the pain-processing properties of the Pf may play a role within the SC avoidance circuitry. Indeed, upon stimulating projections from the SC to the Pf, we observed similar place avoidance behavior as with direct Pf stimulation. Collectively, our data suggest that the parafascicular nucleus (PF) is a critical node in the neural circuit for nociception and avoidance behavior.

Disclosures: M. Roshchina: None. **H.H. Yin:** None.

Poster

PSTR295: Avoidance and Other Defensive Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR295.02/L28

Topic: G.01. Fear and Aversive Learning and Memory

Title: Endocannabinoid and cortico-striatal control of avoidance in two avoidance tasks

Authors: *P. A. CASTELLANOS[1], S. E. PARDO[1], N. J. DELEON[1], A. GONZALEZ[1], J. F. CHEER[2], J. M. WENZEL[1];
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Abstract: Positive and negative reinforcement are fundamental drivers of behavior. While the involvement of cortical and striatal regions in positive reinforcement is well-documented, these systems' role in negative reinforcement remains less understood. In the rodent laboratory footshock avoidance tasks are commonly used to study negative reinforcement. Previous research using a lever-press avoidance task demonstrated that midbrain endocannabinoid signaling and striatal dopamine are integral for learning to avoid, but once the behavior is well-

learned these systems are no longer required to maintain avoidance. In the current study, we utilize pharmacological and DREADD manipulations to investigate the role of endocannabinoids and cortical (prelimbic cortex (PrL) and infralimbic cortex (IL)) and striatal (dorsomedial striatum (DMS) and dorsolateral striatum (DLS)) regions in well-learned avoidance in two tasks - a simple lever-press avoidance task in which rats press a lever to avoid a footshock and a platform-mediated avoidance task in which rats must choose between lever pressing for a food reward and shuttling to a platform to avoid shock. We found that, similar to ventral striatal dopamine antagonism, DREADD inhibition of the DMS or intra-DMS dopamine antagonism attenuated lever-press avoidance early in training, but not once the behavior was well-learned. Manipulations of PrL, IL, or DLS had no impact on lever-press avoidance. Similarly, systemic endocannabinoid antagonism attenuated avoidance learning, but not maintenance of well-learned avoidance. Interestingly, endocannabinoid antagonism in male but not female rats or PrL DREADD inhibition in male and female rats both decreased latency to the platform in the platform-mediated avoidance task, suggesting that endocannabinoid signaling and PrL activity are required for optimal avoidance behavior when rats must choose between food and avoidance. Together these data identify unique roles for endocannabinoid signaling and cortical and striatal regions in two distinct avoidance tasks.

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Poster

PSTR295: Avoidance and Other Defensive Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR295.03/L29

Topic: G.03. Motivation

Support: NIMH Grant R01MH114931
Templeton Foundation grant TWCF0366

Title: Devaluation of response-produced safety signals reveals circuits for goal-directed versus habitual avoidance in dorsal striatum

Authors: R. M. SEARS[1,2], E. C. ANDRADE[1], S. B. SAMELS[3], L. C. LAUGHLIN[1], D. MOLONEY[1], D. A. WILSON[4,5], M. R. ALWOOD[6], J. M. MOSCARELLO[6], *C. K. CAIN[7]; [1]Nathan Kline Inst., Orangeburg, NY; [2]New York University School of Medicine, New York, NY; [3]Drexel Univ. Col. of Med. Neurosci. Program, Philadelphia, PA; [4]New York Univ. Sch. of Med., New York, NY; [5]Nathan Kline Institute, Orangeburg, NY; [6]Texas A&M Univ., College Station, TX; [7]Emotional Brain Inst., Nathan Kline Inst. & NYU Sch. of Med., Orangeburg, NY

Abstract: Active avoidance responses (ARs) are instrumental behaviors that prevent harm. Adaptive ARs may contribute to active coping, whereas maladaptive avoidance habits are implicated in anxiety and obsessive-compulsive disorders. The AR learning mechanism has remained elusive, as successful avoidance trials produce no obvious reinforcer. We used a novel outcome-devaluation procedure in rats to show that ARs are positively reinforced by response-produced feedback (FB) cues that develop into safety signals during training. Males were sensitive to FB-devaluation after moderate training, but not overtraining, consistent with a transition from goal-directed to habitual avoidance. Using chemogenetics and FB-devaluation, we also show that goal-directed vs. habitual ARs depend on dorsomedial vs. dorsolateral striatum, suggesting a significant overlap between the mechanisms of avoidance and rewarded instrumental behavior. Females were insensitive to FB-devaluation due to a remarkable context-dependence of counterconditioning. However, degrading the AR-FB contingency suggests that both sexes rely on safety signals to perform goal-directed ARs.

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Poster

PSTR295: Avoidance and Other Defensive Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR295.04/L30

Topic: G.03. Motivation

Support: The Templeton World Charity Foundation
NARSAD Young Investigator

Title: Orexins as anxiety modulators in instrumental safety-seeking

Authors: *C. SILLER-PEREZ[1], E. C. ANDRADE[2], J. F. SMILEY[3], C. K. CAIN[4], R. SEARS[5];
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Abstract: Dissecting the neuromodulatory systems orchestrating adaptive coping behaviors is critical to understanding human anxiety. The orexinergic system, originating in the perifornical (PFH) and lateral hypothalamus (LH), modulates cognitive and emotional functions critical to survival through projections throughout the brain. One crucial target is the dopaminergic ventral tegmental area (VTA), which is essential for reinforcing behaviors that result in desired

outcomes. However, it is unclear if this circuit is important for reinforcing aversive instrumental behaviors such as active avoidance (AA) of threats. Evidence from other studies in the lab using the signaled AA (SigAA) shuttling task, suggests that AA is positively reinforced by achieving safety. Thus, we hypothesized that the LH-orexin to VTA projection would be required for safety-seeking, with safety being the 'rewarding' outcome. To test this hypothesis, adult Sprague Dawley rats received infusions of an orexin-specific viral vector containing an inhibitory opsin (AAV1-Ple112-Arch3.0-eYFP) into the PFH/LH, and optic fibers were implanted in orexin axon fields in the VTA. Following a 6-8-week incubation, rats were trained in SigAA. Animals were trained to avoid a foot-shock (1.0/0.7 mA males/females; 0.5 s) preceded by (and co-terminating with) a white noise warning signal (WS; 60 s). Rats were first trained with a single inescapable foot-shock delivered at the end of the 60 s WS presentation (Pavlovian trial). For all remaining trials, only failures to shuttle during the WS resulted in shock, which could be escaped by shuttling. If rats shuttled during the WS (an avoidance response), before the shock, a feedback (FB) cue was delivered (5 s, 80 dB). Thus, the FB cue becomes associated with safety—a safety signal. Rats received 15 trials per day until reaching a pre-defined criterion (80% successful avoidance); after, they were subjected to daily shock-free avoidance tests. Orexin to VTA axon terminals were inhibited (green laser 532 nm, 10 mW) during FB cue presentations only. On the first day, latency to avoid and avoidance responses were unaffected. However, inhibition on subsequent days increased latencies and impaired avoidance time- and session-dependent. These results suggest that 1) the FB cue is a reinforcer, perhaps through its association with safety, and 2) orexin communication with VTA is essential for safety-reinforced avoidance. Future studies will uncover the orexin system's role in adaptive coping behaviors and provide support for novel treatments of maladaptive coping, including active coping therapy combined with drugs to target the orexin system.

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Poster

PSTR295: Avoidance and Other Defensive Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR295.05/L31

Topic: G.04. Emotion

Support: NIMH Grant 1R01MH119384-01

Title: Behavioral Variability in the Platform-Mediated Avoidance Task

Authors: *D. PINEDA[1], S. HU[2], C. LI[3], M. ANGSTMAN[1], D. TITUS[4], A. S. WIDGE[5]; [1]Psychiatry and Behavioral Sci., Univ. of Minnesota, Minneapolis, MN; [2]Univ. of Minnesota, Woodbury, MN; [3]Psychiatry and Behavioral Sci., Univ. of Minnesota, Twin Cities, Minneapolis,

MN; [4]Dept. of Psychiatry and Behavioral Sci., Univ. of Minnesota, Minneapolis, MN; [5]Psychiatry, Univ. of Minnesota, Minneapolis, MN

Abstract: Excessive avoidance in the absence of threatening stimuli is a hallmark feature of psychiatric disorders such as generalized anxiety disorder and post-traumatic stress disorder. Understanding the factors influencing this behavior is crucial for the development of future therapies. In preclinical trials, the platform-mediated avoidance, or PMA, task serves as a relevant model for studying avoidance. While prior research have identified various factors influencing animal behavior, the causes of variability in avoidance during behavioral tasks requires further investigation. One such factor is the time taken to achieve stable task performance. Initially, behavior tends to be variable as animals learn and adapt strategies. Once mastery is attained, behavioral variability should decrease, indicating stable performance. Accurately determining the time required to reach stable behavior informs experimental designs, ensuring that interventions and analyses are conducted at optimal time points. Furthermore, hormonal levels driven by the estrous phase are known to impact learning in female rats. Previous literature suggests that estrogen and progesterone levels influence task strategies. Thus, we hypothesized that the rat estrous cycle phase would affect PMA task performance and behavioral variability, affecting the time to achieve stability. In this pilot study, male and female Long Evans rats underwent continuous training on the PMA task, with daily collection of estrous cycle phase samples using vaginal lavage. Preliminary findings indicate differences in task performance variability between males and females. Additionally, it appears that achieving stable behavior requires over 25 days of consistent task performance. These findings suggest that both duration and sex influence variability in avoidance behavior, crucial considerations for developing interventions for psychiatric disorders involving avoidance.

Disclosures: D. Pineda: A. Employment/Salary (full or part-time):; University of Minnesota. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Funding from the National Institute of Mental Health (1R01MH119384-01), Funding from the National Institute on Drug Abuse (RFA-NS-22-035). **S. Hu:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Funding from the National Institute of Mental Health (1R01MH119384-01). **C. Li:** A. Employment/Salary (full or part-time):; University of Minnesota. **M. Angstman:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Funding from the National Institute of Mental Health (1R01MH119384-01). **D. Titus:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Funding from the National Institute of Mental Health (1R01MH119384-01). **A.S. Widge:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds

come to an institution.; Funding from the National Institute of Mental Health (1R01MH119384-01). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Unlicensed intellectual property related to the topic of this poster. F. Consulting Fees (e.g., advisory boards); Consulting income from Abbott.

Poster

PSTR295: Avoidance and Other Defensive Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR295.06/L32

Topic: G.04. Emotion

Support: NIH Grant 1R01MH119384-01

Title: Sex-based differences in the formation and expression of active avoidance-approach strategies in the platform-mediated avoidance task

Authors: *C. J. LI[1], S. M. HU[1], D. G. PINEDA[1], M. R. ANGSTMAN[1], J. L. CHANG[1], E. HERNANDEZ[2], D. J. TITUS[1], A. S. WIDGE[1];
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Abstract: Women exhibit heightened susceptibility to anxiety and stress-related disorders such as PTSD or GAD. A key behavioral manifestation of these conditions is maladaptive avoidance, wherein individuals excessively engage in or withdraw from specific situations, activities, or stimuli, detrimentally impacting their well-being. Animal models are commonly employed to investigate the development of maladaptive avoidance and the formation of fear responses and memories associated with these disorders. Understanding the neurobiological underpinnings of these disorders is critical for identifying risk factors and developing appropriate sex-specific interventions. Despite the clear clinical relevance of examining sex differences in fear responses, the vast majority of pre-clinical research on fear learning and memory formation in the past has exclusively used male animals. The remaining studies that included both sexes primarily focused on differences in passive avoidance (ex. fear conditioning). However, in real-life scenarios, individuals must employ both passive and active avoidance strategies to effectively respond to environmental threats and stimuli. Thus, a large gap in the literature lies in the study of sex differences in active avoidance behaviors. Building on consistently demonstrated findings from previous studies, which show that females exhibit higher rates of freezing behavior than males, we hypothesized that females and males will develop and demonstrate active avoidance strategies that are different from one another. In this study, we utilized both sexes of Long Evans rats performing the platform-mediated avoidance (PMA) task, a clinically relevant behavioral paradigm designed to study active avoidance. Preliminary results demonstrate clear differences

in both learning and the expression of avoidance behaviors within the task. This research emphasizes the need for a more comprehensive understanding of sex-specific responses in anxiety-related disorders, providing valuable insights for developing targeted interventions and enhancing the translatability of findings to human contexts.

Disclosures: C.J. Li: None. **S.M. Hu:** None. **D.G. Pineda:** None. **M.R. Angstman:** None. **J.L. Chang:** None. **E. Hernandez:** None. **D.J. Titus:** None. **A.S. Widge:** None.

Poster

PSTR295: Avoidance and Other Defensive Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR295.07/L33

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIMH R01MH051399
Hope for Depression Research Foundation

Title: Sexually divergent traits in approach/avoidance biases transmute the psychedelic experience

Authors: *G. ROJAS[1], T. MARKOVIC[2], A. M. MINIER-TORIBIO[2], A. GODINO[2], F. J. MARTINEZ-RIVERA[3], E. J. NESTLER[2];
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Abstract: Females face a higher lifetime risk of developing anxiety- or fear-related disorders compared to males, yet the underlying mechanisms of this sex difference remain unclear. Approach-avoidance (A/A) strategies are essential for adaptive behavior, relying on emotional regulation and cognitive processing of associative threat learning to generate appropriate threat responses. Converging evidence implicates serotoninergic (5HTergic) neurotransmission in the medial prefrontal cortex (mPFC) in processing such reward vs threat scenarios, and 5HT2A receptors, integral to associative learning mechanisms, demonstrate sex differences in binding affinity. Fittingly, psychedelics are being studied to manage neuropsychiatric disorders characterized by inflexible associative learning via their 5HT2AR agonism, representing a promising and timely tool to understand mechanisms of learning and memory that demonstrate sexual divergence. In this study, we combine the platform-mediated avoidance (PMA) paradigm with adjunct exposure to LSD in female and male mice to explore the sex-specific effects of psychedelics under extinction conditions as a behavioral intervention. Further, we investigate the contribution of mPFC 5HT in signaling sex-specific approach or avoidance in the PMA test, as well as how such physiological activity is affected by psychedelics. Our initial findings highlight

sex as a primary factor underlying differential A/A strategies and their interaction with psychedelic treatment. To address whether the mPFC 5HT system signals stimulus-specific information or is generally recruited with salient experiences, we monitored 5HT release in the mPFC during reward and threat learning and retrieval following psychedelic-paired extinction learning using *in vivo* fiber photometry. We identified sex differences in baseline mPFC 5HT release driving reward-approach strategies during A/A conflict, a phenomenon enhanced after LSD treatment. Further, reduced avoidance behavior triggered by repeated LSD administration reflects changes in mPFC reward-related 5HTergic neurotransmission in females, but not males - possibly by sustaining mPFC 5HT release in response to salient shock-paired cues or rewards. These findings provide fundamental insight into sex-specific alterations in mPFC 5HT function that may influence the therapeutic outcomes of psychedelics in clinical applications.

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Poster

PSTR295: Avoidance and Other Defensive Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR295.08/L34

Topic: G.04. Emotion

Support: KAKENHI Grant 21K18238

Title: A Serotonergic Circuit Regulates Avoidance Contagion in Drosophila

Authors: *M. MARTINEZ CORDERA[1,2], H. KUROMI[1], K. UENO[1], M. SAITOE[1];
[1]Learning and Memory, Tokyo Metropolitan Inst. of Med. Sci., Setagaya, Japan; [2]Biol. Sci., Tokyo Metropolitan Univ., Hachioji, Japan

Abstract: Understanding the cellular and molecular mechanisms behind social behaviours such as empathy can lead to a better understanding of emerging diseases such as autism spectrum disorder, anxiety, or depression. We found that *Drosophila melanogaster* exhibits social behaviour that shares features with emotional contagion in mammals, suggesting that the brain function of emotion is conserved in animals in general.
We conducted experiments to measure heat avoidance in flies by presenting them with a heat barrier to cross. The flies avoided the barrier in a temperature-dependent manner. Moreover, when the flies are in a group, this avoidance is significantly increased compared to the avoidance of the flies when they are alone. This increase in heat avoidance requires visual input, disappears under stress conditions and requires social interaction in early adulthood, as it was absent in flies reared in isolation. These results strongly suggest that the collective effects on heat avoidance in *Drosophila melanogaster* are a form of emotional contagion.

Using transmission blockers, we found that inhibiting serotoninergic transmission suppressed this avoidance contagion, demonstrating a key role of serotonin in this behaviour. Furthermore, using activation markers, we found a cluster of serotoninergic neurons in the brain that showed traces of activation not only by experiencing heat shock itself, but also by observing the heat responses of others. Finally, using genetic tools, we found that either inhibiting these serotonergic neurons or knocking out serotonergic receptors in their projection areas disrupted the avoidance contagion.
In conclusion, our study in *Drosophila melanogaster* reveals the conserved neurobiological basis of social behaviour and emotional contagion. Social interactions significantly enhance heat avoidance, suggesting an emotional transfer similar to emotional contagion. Serotoninergic neurotransmission plays a central role, as its inhibition disrupts the avoidance contagion. We have identified a specific serotoninergic circuit that regulates this avoidance contagion. These findings advance our understanding of the evolutionary neural mechanisms underlying social behaviour, with implications for psychiatric disorders such as autism, anxiety and depression.

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Poster

PSTR295: Avoidance and Other Defensive Behaviors

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Topic: G.01. Fear and Aversive Learning and Memory

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Åhlénstiftelsen
OE & Edla Johansson
Stiftelsen för Zoologisk Forskning

Title: Experimental investigation into the ventromedial subthalamic nucleus (STN) in the context of behavioral avoidance

Authors: *E. RUBINO[1], G. SERRA[1], A. RICCI[1], S. DUMAS[2], A. J. WALLEN-MACKENZIE[1];
[1]Uppsala Univ., Uppsala, Sweden; [2]Oramacell, Paris, France

Abstract: The subthalamic nucleus (STN), as part of the basal ganglia, plays a critical role in movement. However, the STN is also associated with affective and associative functions.

Recently, optogenetic excitation of the STN in mice has been found to mediate aversion and aversive learning (our previous study: Serra et al., Cell Rep, 2023). This correlation between STN activation and aversion might be clinically relevant in context of deep brain stimulation (DBS) as the STN (STN-DBS) is an important DBS target in e.g. Parkinson´s disease and obsessive-compulsive disorder (OCD). Some patients receiving STN-DBS have reported negative side effects such as low mood state and depression. We reason that anatomical and functional dissection of the STN would help uncover neurobiological underpinnings of both positive and negative effects upon STN-DBS, potentially improving treatment outcomes. In primates, the STN structure has been divided into three anatomical-functional regions (motor, limbic, cognitive) giving rise to the so-called tripartite model. Specifically, limbic and affective functions have been associated with the ventromedial aspect of the STN, also called the "limbic tip". In the mouse, snRNASeq analysis has revealed a heterogeneous transcriptomic landscape within the STN with mRNA distribution giving rise to several discrete molecular profiles within the STN (e.g. our study: Wallén-Mackenzie et al., Comms Bio, 2020). In particular, the expression of Collagen Type XXIV Alpha 1 Chain (Col24a1) mRNA is distributed mainly in the ventromedial aspect of the mouse STN. To address the hypothesis that Col24a1-positive cells represent a limbic tip in the mouse STN, and that these Col24a1-positive neurons drive aversion, we created a new transgenic mouse line in which Cre recombinase is under control of the Col24a1 promoter. We are currently analyzing Col24a1-Cre mice in histological and behavioral paradigms, assessing if Col24a1-positive STN neurons drive behavioral aversion and/or other functions ascribed to the STN. Molecular, anatomical and behavioral data will be presented.

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Poster

PSTR295: Avoidance and Other Defensive Behaviors

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Program #/Poster #: PSTR295.10/L36

Topic: G.01. Fear and Aversive Learning and Memory

Support: NSERC Discovery Grant 506730

Title: Cell-type-specific control of innate defensive responses in the anterior hypothalamic nucleus

Authors: *Y. HONG[1], J. BANG[1], J. DIN[2], H. CHANG[2], J. KIM[3];
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Abstract: Animals rely on innate defensive responses to survive in natural habitats. Upon exposure to threat, animals engage in risk assessment behaviours during which they evaluate

their environment and subsequently undergo a decision-making process between freezing and fight or flight responses. While recent studies have highlighted the neural mechanisms underlying defensive attack and escape behaviours individually, it remains unclear how the overall sequence of innate defensive behaviours is achieved in the brain. Here, we demonstrate that the anterior hypothalamic nucleus (AHN) mediates innate defensive behaviours through cell-type-specific control of risk assessment and escape responses. In this study, we performed immunohistochemistry in addition to calcium-imaging and optogenetic manipulation paired with a wide range of behavioural assays to anatomically and functionally characterize the roles of GABAergic, CaMKIIa+, and glutamatergic AHN neurons in mediating innate defensive behaviours. First, our immunohistochemistry studies identified CaMKIIa+ AHN neurons as a heterogenous population of GABAergic and glutamatergic neurons. Through fiber photometry during a predator-evoked avoidance task, we showed that GABAergic and glutamatergic AHN neurons have distinct activity patterns during innate defensive behaviours. Furthermore, optogenetic stimulation of GABAergic AHN neurons evoked anxiety-related exploratory behaviours, whereas selective stimulation of either CaMKIIa+ or glutamatergic AHN neurons evoked escape responses. Lastly, we found that stimulation of each cell type carried negative valence and induced mild conditioned place aversion. Together, these results demonstrate a cell-type-specific control of innate defensive responses within the AHN, where GABAergic neurons mediate risk-assessment responses, and glutamatergic neurons mediate escape responses. Furthermore, our data provides preliminary insights into the role of CaMKIIa+ AHN neurons in mediating innate defensive responses.

Disclosures: Y. Hong: None. **J. Bang:** None. **J. Din:** None. **H. Chang:** None. **J. Kim:** None.

Poster

PSTR295: Avoidance and Other Defensive Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR295.11/L37

Topic: G.01. Fear and Aversive Learning and Memory

Support: Royal Society Dorothy Hodgkin Fellowship DHF\R\241009
Sainsbury Wellcome Centre Core Grant from the Gatsby Charitable Foundation and Wellcome (GAT3755 and 219627/Z/19/Z)
European Research Council grant (Consolidator #864912)

Title: A biophysical mechanism for changing the threat sensitivity of escape behaviour

Authors: *Y. LEFLER[1], G. FERREIRA[2], Y. WANG[1], T. BRANCO[1];
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Abstract: Animals faced with predatorial threats innately react by escaping to safety. Although escape is instinctive, it is also flexible enough to adapt to dynamic changes in the environment. For example, animals adapt to the risk of predation by increasing escape probability when there is a higher incidence of predator attacks. This flexibility is critical for maximizing the adaptiveness of behavioural choices, but the underlying mechanisms are unknown. It has recently been shown that activation of neurons in the dorsal periaqueductal gray (dPAG) is the main step for commanding escape initiation. Here we hypothesized that changing the excitability of dPAG neurons is a mechanism for implementing experience-dependent adaptations of escape behavior. To investigate this hypothesis, we developed a new paradigm for performing whole-cell recordings from dPAG neurons of mice escaping from threatening stimuli. Head-restrained mice navigate between a shelter and a threat zone and are exposed to threatening stimuli that cause escape-to-shelter responses. To modulate escape behavior we presented repeated stimuli, which caused an increase in the probability and vigor of escape, indicating a decrease in the escape threshold. Using this sensitization paradigm and whole-cell recordings we studied the cellular mechanisms of escape threshold modulation. We found that repeated presentation of escape-eliciting stimuli causes a subthreshold sustained depolarization that lasts for several minutes. This depolarization is large enough to reduce the amount of synaptic input needed to reach the action potential threshold. Consequently, subsequent threatening stimuli are more likely to elicit action potentials and therefore escape initiation. We further propose that the sustained depolarization arises from a local disinhibitory mechanism. Our findings present a cellular mechanism for rapid experience-dependent modulation of instinctive escape behavior. We suggest that this biophysical process at the level of single midbrain neurons may be a general mechanism for modulating behavior initiation in dynamic environments.

Disclosures: Y. Lefler: None. **G. Ferreira:** None. **Y. Wang:** None. **T. Branco:** None.

Poster

PSTR295: Avoidance and Other Defensive Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR295.12/M1

Topic: G.01. Fear and Aversive Learning and Memory

Support: Barrett Foundation
Faculty Development Grants, Mills College at Northeastern Univerrsity

Title: Relationships between body size, alternating current amplitude, and running responses during and after electricity application in C. elegans

Authors: *J. YOUNG;
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Abstract: When on agar to which certain amounts of alternating current is applied, C. elegans increases its movement speed and generally moves off of bacterial lawns (Tee, 2023). This is likely to represent an escape behavior, and the stimululs may elicit a brain state representing, or related to, the emotion of fear. When 30V alternating current is applied to the plate, the worms run while experiencing the voltage, whereas when 75V of alternating current is applied to the plate, the worms are immobilized during voltage application, but run after the voltage is turned off. 30V is the voltage that provides the most robust and consistent running response in the "N2" wild type strain (Tee, 2023). By recording electricity responses of an array of mutant strains, and with worms of different ages, we have found that there is a positive correlation between the size of worms and their running response to 30V of alternating current. Longer, skinnier worms have a stronger response than shorter, fatter worms. This relationship is seen both when measuring the increase in speed elicited by the voltage, and the degree to which voltage application triggers the worms to exit food lawns, which are normally their preferred location. We are also investigating the responses of worms of varying sizes to alternating current applied at voltages higher and lower than 30V to see, for example, whether higher voltages can elicit stronger responses in smaller worms than 30V can. Furthermore, some of the voltages tested are around 75V so that we can see if worm size has an effect on the somewhat different behavior triggered at that voltage range. This work aims to deepen our understanding of the mechanisms underlying this electricity response in C. elegans, providing greater insight into how electricity is interacting with worm bodies to elicit behavioral responses.

Disclosures: J. Young: None.

Poster

PSTR295: Avoidance and Other Defensive Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR295.13/M2

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: MOST 107-2320-B-002-040-MY3
MOST 108-2638-B-010-0020-MY2
MOST 110-2321-B-010-006
MOST 110-2320-B-002-013-MY3

Title: Input-specific disengagement of zona incerta in modulating defensive behavior

Authors: *F.-Y. HSIAO[1], P.-C. HO[1], S.-H. CHIU[2], S.-R. LEE[1], Y.-J. PENG[1], J.-T. WANG[1], H.-J. YAU[1];
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Abstract: Recent wave of studies has demonstrated that the zona incerta (ZI) is activated byrespective afferent inputs to regulates defensive behaviors as well as pain. Byemploying a biased retrograde AAV toward non-DA neurons in the ventral midbrain,we have recently discovered that restraint stress or a predatory odor engages asubpopulation of ZI-projecting substantia nigra (SN) non-DA neurons, which weremostly GABAergic neurons confirmed by RNAscope in situ hybridization. This findingsuggests that a subpopulation of ZI neurons is required to be inactive to cope withthreatening conditions. We further showed that GABAergic neurons accounted for~60% of the SN-innervated ZI cells. Moreover, by employing an intersectionalanterograde tracing approach to target SN-innervated ZI cells, we mapped the EYFP-expressing axonal projections of SN-innervated ZI cells and detected prominentinnervations in several regions, including lateral habenula (LHb) and lateralperiaqueductal gray (lPAG), which are well known for their roles in regulatingdefensive behaviors. These results suggest that SN-to-ZI input may be likely through adisinhibiting mechanism to engage neural substrates of defense for coping withthreatening situations.Previous study has shown that chronic pain impairs defensive responses. Through theZI afferent mapping analysis described above, we have also detected many ZI-projecting cells in the anterior part of anterior cingulate cortex (aACC), which is wellknown for its critical role in pain chronification. Whether aACC-to-ZI input is involvedin the regulation of defensive behavior is poorly understood. Therefore, as the first step,we will employ a mouse model of neuropathic pain to investigate whether aACC-to-ZIinput is involved during pain chronification.

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Poster

PSTR295: Avoidance and Other Defensive Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR295.14/M3

Topic: G.01. Fear and Aversive Learning and Memory

Support: NINDS Grant R21-NS1260628
NIMH Grant R21-MH101696

Title: Chemogenetic activation of CRF-expressing lateral Habenula neurons alters threat-evoked defensive behaviors in mice

Authors: *W. J. FLERLAGE[1,2], E. THOMAS[2], S. GOUTY[2], S. C. SIMMONS[2], B. M. COX[2], T.-Y. J. WU[3], M. C. TSUDA[4], F. S. NUGENT[2];
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Abstract: The lateral habenula (LHb) is a diencephalic brain region associated with value-based decision making and stress evasion through its modulation of monoaminergic systems. Specifically, increased activity of the LHb is associated with drug addiction, and stress-related disorders such as depression, anxiety and PTSD. Previously, we have shown that the LHb is highly responsive to the neuromodulatory actions of corticotropin-releasing factor (CRF) but it is yet to be known which brain regions provide CRF inputs to the LHb. In order to identify CRF projections to LHb, we performed retrograde and anterograde tracing in CRF-Cre male and female mice and identified several brain regions including a subpopulation of LHb expressing CRF (LHbCRF neurons) that could play a role in LHb-related motivated behaviors. We then used Gq-DREADD chemogenetic activation of LHbCRF neurons and tested the behavioral effects of LHbCRF neurons on a battery of behavioral tests of motivation. We found that while chemogenetic activation of these neurons did not affect mouse behavior in the open field test, light-dark box test, elevated zero maze and Y-maze, it increased the percentage of active escape behavior in the visual looming shadow task in both male and female mice (although with different effects on the latency to escape behavior). Overall, we have identified a new subpopulation of LHb neurons that promote active defensive behaviors in response to threat in both male and female mice. Our future studies will further explore the physiological and behavioral effects of LHbCRF projections in motivated behavior.

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Poster

PSTR295: Avoidance and Other Defensive Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR295.15/M4

Topic: G.04. Emotion

Support: NIH Grant 5R01MH119384-04
NIH Grant 5R01MH123634-04
NIH Grant 1R21MH109722-01A1
NIH Grant 1R21HM113103-01A1

Title: Machine Learning Based Neural Decoding Model for Predicting Defensive Behaviors to Improve Targeted Closed Loop Deep Brain Stimulation

Authors: *S. M. HU[1], J. MORROW[1], S. NAGRALE[1], R. L. YOUNK[1], M. SHOARAN[2], A. S. WIDGE[1];

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Abstract: Defensive behaviors are essential evolutionary mechanisms for survival in response to threatening stimuli across different species. However, excessive expression of these behaviors manifest themselves as symptoms in psychiatric disorders. Recent studies have implicated frontal-amygdala circuits, particularly communication between the prelimbic cortex (PL) and basolateral amygdala (BLA), in defensive behaviors in both humans and rodents. Previous studies have shown that features like power, coherence, and phase amplitude coupling can quantify communication between regions of the brain. These features that arise from the local field potential (LFP) are particularly important because they can also be chronically measured and tracked in humans to build closed-loop deep brain stimulation treatments for psychiatric disorders. However, the inherent variability within and between individuals with psychiatric disorders poses a challenge in constructing closed-loop systems that can effectively target symptoms on an individualized basis. Decoding plays a vital role in developing individualized stimulation therapies by using neural activity to predict behavior. Because decoding relies on predictive modeling, machine learning has emerged as a powerful tool for deciphering the link between complex neural activity and specific behaviors or symptoms.
15 male adult Long-Evans rats were subjected to a traditional fear conditioning paradigm, during which LFP was recorded using microwire bundles implanted in the PL and BLA regions of the brain. Features from the neural data in the time, spectral, and connectivity domains were analyzed and used to predict defensive behavior. Defensive behaviors (freezing, darting, and bar press suppression), were captured using headstage accelerometry, video recording, and bar press rate. Our findings show that these methods are able to sufficiently capture and quantify the behaviors we seek to decode. This analysis is an exploratory study that provides a machine learning based decoding model that uses neural features to predict moment-to-moment behavior. Additionally, this study shows the exact features that are heavily leveraged when predicting those behaviors. This predictive modeling will aid in the understanding of the neural circuitry associated with psychiatric disorders and can be used to identify and improve targets for stimulation.

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Poster

PSTR295: Avoidance and Other Defensive Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR295.16/M5

Topic: G.01. Fear and Aversive Learning and Memory

Support: International Society of Neurochemistry Career Development Grant (CJP)
ARC Discovery Project Grant DP210102672 (CJP, AJL, JHK)
Macquarie University Research Excellence Scholarship 20224425 (BKR)

Title: Efferent connectivity and neurochemical phenotype of topographically distinct lateral hypothalamus and zona incerta RXFP3+ cells

Authors: *B. K. RICHARDS[1], A. I. J. KILBY[1], J. L. CORNISH[1], J. KIM[2], A. J. LAWRENCE[3], C. J. PERRY[1];
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Abstract: RXFP3 is a ligand-activated G-protein coupled receptor and the cognate receptor for the conserved neuropeptide relaxin-3. We recently showed that activating an RXFP3-expressing (RXFP3+) population in the lateral hypothalamus (LH) and zona incerta (ZI) induced striking escape-like jumping behaviour that was only apparent in a subset of experimental mice. Given the known anatomical and functional diversity of these nuclei, we posited that activating RXFP3+ LH/ZI cells with varying neurochemistry and hodology between mice may have generated these bimodal phenotypes. Therefore, this study aimed to characterise the neurochemical phenotype and downstream connectivity of these cells. All experiments used RXFP3-Cre transgenic mice. The neurochemical phenotype of RXFP3+ LH/ZI cells was characterised with RNAscope *in situ* hybridisation (n = 4 mice). We used probes that allowed us to detect the co-localisation of *Rxfp3* mRNA with *GAD1*, *vGlut2*, *Sst*, *Pvalb*, and *TH* transcripts. Most *Rxfp3*+ ZI cells were GABAergic (*GAD1*+; M = 77.1%, SEM = 2.4%), though 11.5% (± 1.1%) were glutamatergic (*vGlut2*+). Furthermore, large proportions of neurochemically distinct GABAergic *Rxfp3*+ clusters populated topographically distinct areas of the ZI, with 77.0% (± 2.9%) of A13 *Rxfp3*+ cells expressing *TH*, 58.6% (± 2.2%) of rostral ZI *Rxfp3*+ cells expressing *Sst*, and 67.8% (± 3.9%) of dorsal/ventral ZI *Rxfp3*+ cells expressing *Pvalb*+. In contrast, the LH primarily comprised a heterogeneous *Rxfp3*+ population of intermingled GABAergic and glutamatergic cells, with 33.4% (± 2.4%) and 39.3% (± 2.7%) of *Rxfp3*+ LH cells expressing *GAD1* and *vGlut2*, respectively. To identify downstream targets of these neurons, unilateral injections (25 nl) of a Cre-dependent anterograde viral tracer were made at systematically varying areas across the rostrocaudal extent of the ZI and LH (n = 4 mice per area, 4 areas). Emerging data revealed marked differences in efferent projections to nuclei involved in mediating defensive behaviours. Notably, the ventrolateral periaqueductal gray received the most input from dorsal/ventral ZI RXFP3+ cells, while the lateral habenula and dorsal premammillary nucleus received most input from rostral LH RXFP3+ cells. Collectively, these results indicate that RXFP3+ LH/ZI cells are a heterogeneous population where topographical location dictates neurochemical composition and efferent output. These findings inform future functional interrogation studies to address the involvement of identified RXFP3+ LH/ZI subpopulations and their downstream targets in threat-related behaviours.

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Poster

PSTR295: Avoidance and Other Defensive Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR295.17/M6

Topic: G.01. Fear and Aversive Learning and Memory

Title: Control of defensive behavior by the nucleus of Darkschewitsch GABAergic neurons

Authors: *Y. SHAO[1], Y. YU[2], S. DUAN[3];
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Abstract: Control of defensive behavior by the nucleus of Darkschewitsch GABAergic neurons
Shao Yujin, Huiying Zhao, Jinrong Liu, Hongbin Yang, Shumin Duan, and Yan-qin Yu*
The Sch. of Brain Sci. and Brain Med., Hangzhou Zhejiang, China
The nucleus of Darkschewitsch (ND), mainly composed of GABAergic neurons, is widely recognized as a component of the eye-movement controlling system. However, the functional contribution of ND GABAergic neurons (ND_{GABA}) in animal behavior is largely unknown. Using activity-dependent labeling, we found have identified ND_{GABA} neurons were selectively activated by different types of fear stimuli, such as predator odor and foot shock. Optogenetic activation of ND_{GABA} strongly promoted freezing behavior, accompanied by reduced heart rates and dilated pupils. Conversely, inactivation of these neurons largely blocked freezing behavior induced by fear stimuli. Moreover, using neuroanatomical tracing methods, we identified an excitatory pathway from the lateral periaqueductal gray (lPAG) to the ND that induces freezing by exciting ND inhibitory outputs to the motor-related gigantocellular reticular nucleus, ventral part (GiV).we discovered that these GABAergic neurons integrated wide-range inputs and innervated multiple motor-related brain regions. Collectively, these findings suggest the ND_{GABA} population serves as a novel hub for controlling defensive responses, a mechanism critical for understanding how the freezing behavior is encoded in the mammalian brain.

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Poster

PSTR295: Avoidance and Other Defensive Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR295.18/M7

Topic: G.01. Fear and Aversive Learning and Memory

Support: NSERC Discovery Grant RGPIN-2024-04133
NSERC CGS M (KSB)
NSERC CGS D (PM)
NSERC USRA (JPL)
I-CUREUS (JPL)

Title: Zona incerta neurons release GABA and dopamine in the superior colliculus to mediate fear responses during hunger

Authors: *P. MILLER[1], J. PHY-LIM[2], K. BAKER[3], D. FUSCA[4], R. CHEE[5], H. FENSELAU[6], P. KLOPPENBURG[7], M. J. CHEE[1];
[2]Neurosci., [1]Carleton Univ., Ottawa, ON, Canada; [3]Univ. of Toronto, Toronto, ON, Canada; [4]Univ. of Cologne, Biocenter, Cologne, Germany; [5]Univ. of Alberta, Edmonton, AB, Canada; [6]Max Planck Inst. For Metabolism Res., Koeln, Germany; [7]Univ. of Cologne, Cologne, Germany

Abstract: A hungry animal adapts rapidly to environmental threats and feeds only when if it is safe. The zona incerta (ZI) is a GABAergic region that suppresses fear and promotes feeding. A subset of ZI neurons also express tyrosine hydroxylase (TH) and produce dopamine (DA). These ZI DA cells project prominently to midbrain motor regions, including to the motor-related superior colliculus (SCm), which promotes escape behaviors, but it is not known if GABAergic ZI DA cells release GABA and/or DA and whether they regulate escape behaviors via the SCm. We transduced *Th-cre* ZI cells with a Cre-dependent virus encoding the excitatory DREADD hM3(Dq) and implanted a guide cannula at the midline above the intermediate and deep SCm layers. A food pellet was placed beneath an overhead looming dot, and intra-SCm infusion of Compound 21 (C21; 1 ng) increased the latency for fasted mice to escape into their shelter (vehicle: 2.3 ± 0.3 s; C21: 7.7 ± 4.3 s; n = 3); sated mice did not exhibit altered escape latency. To determine the mechanisms of neurotransmission underlying ZI *Th-cre* projections, we transduced ZI *Th-cre* cells with a Cre-dependent virus encoding channelrhodopsin and found that 470-nm photostimulation of channelrhodopsin-expressing fibers in the SCm elicited an optogenetically-evoked inhibitory postsynaptic current (oIPSC) in one-third of SCm patch-clamp recordings (n = 29/87 cells). These oIPSCs persisted in tetrodotoxin but were abolished in bicuculline thus indicating monosynaptic GABA release. To assess DA release, we transduced ZI *Th-cre* cells with the red-shifted opsin ChrimsonR and expressed the dopamine sensor dLight1.1 in the SCm. Red light 610-nm photostimulation (0.5 Hz train of 20 Hz pulses) of ChrimsonR-expressing *Th-cre* fibers in the SCm elicited dLight1.1 fluorescence that peaked within 5 minutes (n = 7; N = 4) and gradually returned to baseline over 20 min thus indicating DA presence. The change in dLight fluorescence in the SCm slice was site-specific and matched the spatial distribution of ZI *Th-cre* projections. In sum, ZI projections to the SCm integrate fear and hunger signals, and ZI *Th-cre* cells can release GABA and DA for acute or prolonged regulation of SCm cells to decrease fear responses during calorie restriction.

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Poster

PSTR295: Avoidance and Other Defensive Behaviors

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR295.19/M8

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Self-supervised behavior modeling with dense keypoint tracking

Authors: Y. YU[1], ***J. LI**[2], A. BOWEN[3], C. A. CAMPOS[1];
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Abstract: Behavior is the final substrate of neural computation. Accordingly, rich descriptions of behavior are essential for brain decoding efforts, allowing us to understand action generation and distinguish between motor and cognitive processes. We hypothesized that leveraging a rich postural representation of the body would allow us to learn more precise, transferable representations of behavior from video. Here, we utilize a near-continuous 3D map of the mouse body consisting of over 30,000 points to extrapolate posture from 2D video data. Our goals were to optimally process this high-dimensional spatial data in both space and time to 1) identify discrete postures and 2) parse sequences of postures to segment behavior. To address these challenges we embed frame-wise spatial data in latent space and compress latent representations into posture tokens. Subsequently, posture tokens are used to train a transformer-based sequence prediction model. Initial efforts demonstrate this multistep approach is adept at learning temporal representations up to 4 s in duration, which enables one-shot behavioral sequence retrieval that bypasses the annotation and training steps previously required for supervised behavioral analysis. Our ongoing studies focus on segmenting behavior and making these resources readily available to support research in neuroscience and related fields.

Disclosures: Y. Yu: None. **J. Li:** None. **A. Bowen:** None. **C.A. Campos:** None.

Poster

PSTR296: Motivated Behavior: Behavior, Circuits, and Pharmacology

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR296.01/M9

Topic: G.03. Motivation

Support: National Institutes of Health Grant AA030931
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National Institutes of Health Grant 5T32MH065215-18
Brain and Behavior Research Foundation
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Title: Food restriction level and reinforcement schedule differentially influence behavior during acquisition and devaluation procedures in mice

Authors: *C. J. KIM[1,2], M. CHEVEE[2], N. CROW[2], E. G. FOLLMAN[2], M. Z. LEONARD[2], E. S. CALIPARI[2];
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Abstract: Behavioral strategies are often classified based on whether reinforcer value controls reinforcement. Value-sensitive behaviors, where animals update their actions when reinforcer value is changed, are classified as goal-directed; value-insensitive actions, where behavior remains consistent when the reinforcer is removed/devalued, are considered habitual. Basic reinforcement principles help bias behavior toward either process: random ratio (RR) schedules are thought to promote goal-directed behaviors while random intervals (RI) foster habitual control. Although they are commonly used, how external factors change the ability to produce habits or goal-directed actions has not been well characterized. In rodent research, caloric restriction is often performed to enhance animal's task engagement. Thus, we altered food restriction levels and asked how they change behavior during the acquisition of RI/RR tasks as well as during the devaluation procedures that are used to determine the sensitivity of behavior to value. We used a total of 43 male and 44 female C57BL/6J mice, and all animals were first trained on fixed ratio 1. We then trained mice on two schedules with commonly used intervals/ratios (RI30/RI60, RR10/RR20) and found that RI30/RI60 was more effective than RR10/RR20 at increasing response rates, indicating that the intended differences in sensitivity to value were confounded with differences in response rates. To address this, we adjusted the number of responses required in RR to match the actions-per-outcome achieved on RI. The first set of micewas classified into 3 different food restriction levels (no/mild/strong restriction) and trained on RI30/RI60. Another set of mice was then trained on RR with ratios that matched the actions-per-outcome of each RI group. All mice underwent devaluation sessions thereafter. When the responses-per-reinforcer ratios were matched, we find that food restriction and schedule independently influence reinforcer delivery and response rate, with more restricted mice and RR group achieving higher rates than less restricted mice and RI group, respectively. We also find a combinatorial effect of restriction and task schedule where food restriction increases reinforcer delivery rates and response rates more effectively for RR group than RI group. Moreover, food restriction decreased the response rate during sequential devaluation sessions that reflected the effect of extinction rather than devaluation. Together, our results

suggest that external factors such as food restriction must be accounted for, together with reinforcement schedules, to appropriately interpret the cognitive basis of behavior.

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Poster

PSTR296: Motivated Behavior: Behavior, Circuits, and Pharmacology

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR296.02/M10

Topic: G.03. Motivation

Support: KAKENHI 23H04673 (HN)
JST SPRING JPMJSP2145 (TO)

Title: Dorsomedial prefrontal cortex controls the timing of locomotion and anticipatory licking during goal-directed behavior in mice

Authors: *H. NISHIMARU[1,2], J. JUNQUEIRA[3], T. OTSUKI[4], J. MATSUMOTO[1,2], T. SETOGAWA[1,2];
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Abstract: It has been shown that reward information processing is crucial in motor selection and performance during motivated behaviors. However, neuronal mechanisms of how it is integrated with motor control signals in the brain to generate and coordinate appropriate motor behaviors remains largely unknown. In rodents, locomotion is one of the most studied natural motor repertoires and is known to be essential for seeking and obtaining rewards (For recent review, see Nishimaru et al. Neurosci Res. 189:83-9, 2023). Licking behavior is also widely studied in rodents as a behavioral signal of reward expectation and consumption as well as in the context of motor control (Inagaki et al. Annu Rev Neurosci 45:249-271, 2022). In rodents, dorsomedial prefrontal cortex (dmPFC) which includes the anterior cingulate cortex (ACC) and secondary motor cortex (M2) has been shown to encode and integrate sensory information and motor control during the decision-making processes (Barthas and Kwan, Trends Neurosci. 40:181-193. 2012). In a previous study, we showed that ACC neurons dynamically encode reward information during locomotor behavior (Sachuriga et al. Front Sys Neurosci 15:655110, 2021). In the present study, to examine the role of these prefrontal regions in producing effectively timed licking responses and coordinated locomotor patterns, we developed a self-paced, locomotor-based behavioral task in which the mice learned to spontaneously initiate trials, and to maintain their speed until cue presentation which signaled upcoming reward delivery. They also

learned to start licking just after the cue presentation, before the reward delivery. We then examined the functional role of dmPFC in generating these learned motor behaviors by infusing muscimol, a $GABA_A$ receptor agonist to pharmacologically inactivate this brain region. Here, we show that 1) the temporal control of locomotion and licking in this behavioral task is shaped and coordinated by both implicit and explicit reward information and 2) pharmacological inactivation of dmPFC disrupted the coordination of these motor behaviors leading to an increased number of failed trials. Our results suggest that dmPFC plays an important role in controlling the motor behavior based on reward expectations.

Disclosures: H. Nishimaru: None. **J. Junqueira:** None. **T. Otsuki:** None. **J. Matsumoto:** None. **T. Setogawa:** None.

Poster

PSTR296: Motivated Behavior: Behavior, Circuits, and Pharmacology

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Program #/Poster #: PSTR296.03/M11

Topic: G.03. Motivation

Support: NSERC Award RGPAS-2020-00030
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Title: What predicts the choice to expend effort for reward? A translational study

Authors: *B. J. FORYS[1], C. A. WINSTANLEY[1,2], A. KINGSTONE[1], R. M. TODD[1,2]; [1]Psychology, [2]Djavad Mowafaghian Ctr. for Brain Hlth., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: We must often decide how much effort to expend for everyday rewards – but we do not fully understand how constraints on our ability to exert effort and our willingness to seek rewards impact effort decisions. Past rodent work suggests that willingness to deploy cognitive effort can be driven by individual differences in perceived reward value, anhedonia-like behaviour, or chronic stress. However, many factors driving cognitive effort deployment cannot easily be captured in rodents. Furthermore, we do not fully understand how individual differences in short-term memory ability, depression, chronic stress, and reward sensitivity impact cognitive effort for reward in humans. In this translational study, we adapted an effort choice paradigm used in rodents to examine whether these factors predict cognitive effort deployment for higher reward in humans using an online visual short-term memory task. Undergraduate participants were grouped into high and low effort groups ($n_{High\ Effort}$ = 348, $n_{Low\ Effort}$ = 81; n_{Female} = 332, n_{Male} = 92, M_{Age} = 20.37, $Range_{Age}$ = 16-42) based on decisions in this task. After doing a monetary incentive task to measure reward sensitivity, participants completed short-term memory task trials where they could choose to encode either fewer (low

effort/reward) or more (high effort/reward) squares before reporting whether the colour of a target square matched the square previously in that location. We found that only greater short-term memory ability predicted whether participants chose a much higher proportion of high vs. low effort trials. Drift diffusion modeling showed that high effort group participants were more biased than low effort group participants towards selecting high effort trials. Next, we asked whether short-term memory ability explains shifts in trade-offs between effort and reward when effort options and rewards are varied. To address this question, in a follow-up study we parametrically varied effort options and rewards to evaluate whether motivation for effort systematically differs with effort demands and available reward. We also asked participants to describe their effort deployment experiences and used qualitative thematic analysis approaches – evaluating patterns of meaning in subjective experiences – to explore themes underpinning effort decisions that may be unique to human experiences of these decisions. Our findings highlight the role of individual differences in cognitive effort ability in explaining cognitive effort deployment choices.

Disclosures: B.J. Forys: None. **C.A. Winstanley:** None. **A. Kingstone:** None. **R.M. Todd:** None.

Poster

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Topic: G.03. Motivation

Support: National Institute of General Medical Sciences: T34GM145384

Title: Effects of a High Fat Diet on Reward Maximization Utilizing a Sequential Patch Depletion Paradigm in Rats

Authors: *B. REED[1], K. BANDUCCI[1], B. KRIEG[1], N. ALAMMARI[1], I. GARCIA[1], G. DEGANTE[1], T. MORENO[1], I. SINGH[2], I. C. SUMAYA[1], A. M. GANCARZ-KAUSCH[1]; [1]Dept. of Psychology, California State University, Bakersfield, Bakersfield, CA; [2]Stockdale High Sch., Bakersfield, CA

Abstract: In the United States, about two-thirds of adults ages 20 and older consume more than the recommended 10% of fat from daily caloric intake. It is well known that chronic intake of a diet high in fat and sucrose in humans has been linked to various negative health outcomes including obesity, diabetes, and cardiovascular disease. However, less known are the adverse effects on the brain and behaviors including reward-seeking and choice. We investigated the effects of diet (Western, high fat diet: 45% fat and 6% sucrose) on reward maximization. To test this, rodents were randomly assigned to either high fat diet or standard chow for 60 days.

Subsequently, rats were tested on a sequential patch depletion paradigm where they were offered a concurrent choice between two water "patches" and could elect to "stay" in the current patch or "leave" for an alternative patch. Staying in the current patch resulted in decreasing subsequent reward magnitudes, whereas the choice to leave a patch was followed by a delay and a resetting to the maximum reward magnitude. Preliminary data indicate rats exposed to a high fat diet stay in the patch significantly longer than controls and make significantly fewer patch changes. This pattern of choice resulted in rats exposed to a high fat diet earning fewer rewards and consuming less total water per session compared to rats exposed to standard chow. These data indicate high fat diet produced deleterious effects and resulted in less reward maximization compared to controls. These data have grave implications for humans, suggesting a link between chronic high fat Western diet and altered reward maximization and choice.

Disclosures: B. Reed: None. **K. Banducci:** None. **B. Krieg:** None. **N. Alammari:** None. **I. Garcia:** None. **G. Degante:** None. **T. Moreno:** None. **I. Singh:** None. **I.C. Sumaya:** None. **A.M. Gancarz-Kausch:** None.

Poster

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Program #/Poster #: PSTR296.05/

Topic: G.03. Motivation

Title: Identification and Prioritization of Food Intake Targets Through Human Genetics and Mouse Behavioral Transcriptomics Profiling

Authors: *J. YANG[1], T. WANG[1], L. CAI[2], P. LI[1], J. DUAN[1], L. JIANG[1], A. KENNEDY[1]; [1]Novo Nordisk, Beijing, China; [2]Novo Nordisk, Oxford, United Kingdom

Abstract: Obesity, a condition with significant health implications and a growing global prevalence, is linked in part to dysregulated eating behavior, stemming from the widespread availability of highly palatable foods. This availability contributes to an increase in hedonic feeding. Human genetics serves as a potent approach for identifying disease causal genes. However, many changes, particularly those long-term diseases like obesity, may develop over an extended period and may not be immediately relevant for acute therapeutic modulation. Therefore, integrating an appropriate behavioural model with pronounced acute effects enables the prioritization of genes that are acutely modulated in feeding responses. In this study, we initially identified genes potentially play a causal role in obesity by applying Mendelian randomization and colocalization analysis on genetic association studies from large-scale human cohorts and expression quantitative trait loci (eQTL) data or alternative splicing pattern (sQTLs) from human brain bulk transcriptomics. The hedonic feeding mouse model (male, n=8) was performed providing restricted access to a palatable high-fat diet (HFD) for 2 hours (h) per day,

while normal chow was available for the remaining 22 h. Caloric intake was monitored. When mice were subjected to scheduled HFD for 2 h per day, they swiftly adjusted their feeding behaviour, displaying voluntary hyperphagia by consuming over 70% of their daily caloric intake. In contrast, the control group (male, n=8) with continuous access to standard chow exhibited the majority of calorie intake during the remaining 22 h, with only 16% occurring during the 2-h scheduled feeding. We performed bulk RNA-seq experiments on the hypothalamus collected 1 h after HFD on day 15, followed by DESeq2 and LeafCutter analyses to identify the differentially expressed genes (DEGs) and genes showing alternative isoform usage (AIUs) between the hedonic feeding and control groups. Combing the human genetic analysis, we identified and prioritized 20 genes with evidence of eQTL colocalization and another 15 genes supported by sQTL colocalization. Notably, *one DEG, Ache and one AIU, Nisch* were among the prioritized list, both with approved drugs and with previously reported effects on body weight and food intake. Reverse translation of the target identification paradigm was obtained by applying these drugs to the hyperphagia seen in the hedonic feeding model. In summary, combining evidence from human obesity genetics and transcriptomics of hedonic feeding model, we successfully identified and prioritized targets with therapeutic potential directed at modulating food intake and obesity.

Disclosures: J. Yang: A. Employment/Salary (full or part-time):; Novo Nordisk. **T. Wang:** A. Employment/Salary (full or part-time):; Novo Nordisk. **L. Cai:** A. Employment/Salary (full or part-time):; Novo Nordisk. **P. Li:** A. Employment/Salary (full or part-time):; Novo Nordisk. **J. Duan:** A. Employment/Salary (full or part-time):; Novo Nordisk. **L. Jiang:** A. Employment/Salary (full or part-time):; Novo Nordisk. **A. Kennedy:** A. Employment/Salary (full or part-time):; Novo Nordisk.

Poster

PSTR296: Motivated Behavior: Behavior, Circuits, and Pharmacology

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Program #/Poster #: PSTR296.06/M13

Topic: G.03. Motivation

Support: INSERM Starting package grant

Title: On the causal role of orbitofrontal cortex theta activity in effort-based decision-making

Authors: E. LOPEZ-BRAVO[1], N. TRINH[2], P. VASSILIADIS[3], E. KOUN[1], A. FONCELLE[1], M. VERNET[1], ***G. DEROSIERE**[4,5];
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Abstract: From rodents to humans, animals must constantly decide whether to engage (or not) in physical efforts to reach rewarding goals. This key process - usually referred to as effort-based decision-making - has been linked to neural activity changes within a key fronto-striatal network, encompassing the orbitofrontal cortex (OFC), the supplementary motor area (SMA), the dorsal anterior cingulate cortex (dACC), and the ventral striatum. Previous research has demonstrated that OFC activity preferentially scales with reward magnitude, while SMA activity is associated with prospective effort, suggesting these areas estimate reward and effort values, respectively. Additionally, both reward- and effort-dependent activity changes have been observed in the dACC and ventral striatum, indicating a role in balancing reward and effort. Interestingly, reward valuation in the OFC has been linked to oscillatory responses in specific frequency bands, particularly the theta band (4 to 8 Hz). However, these studies were correlational, and the causal role of OFC theta activity in reward valuation, if any, remains untested. Our study addresses this gap. We employed a randomized, double-blind, sham-controlled design involving 20 healthy participants. Participants attended three experimental sessions, each involving an effort-based decision-making task with concurrent High-Density transcranial Alternating Current Stimulation (HD-tACS) of the OFC. The sessions included one of three HD-tACS conditions: sham, active control (20 Hz), and theta stimulation (6 Hz). To mitigate potential learning effects on participants' behavior, sessions were spaced one week apart. Electroencephalography (EEG) was recorded pre- and post-tACS application in each session. Statistical analysis of visual analogue scales and questionnaires confirmed that participants could not distinguish between the three HD-tACS conditions. EEG analyses revealed significant entrainment of theta oscillatory activity over frontal electrodes, specifically following theta stimulation. Behavioral data and computational modeling analyses are currently underway to quantify the impact of OFC theta stimulation on acceptance rates and reward valuation.

Disclosures: E. Lopez-Bravo: None. **N. Trinh:** None. **P. Vassiliadis:** None. **E. Koun:** None. **A. Foncelle:** None. **M. Vernet:** None. **G. Derosiere:** None.

Poster

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Location: MCP Hall A

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Program #/Poster #: PSTR296.07/M14

Topic: G.03. Motivation

Title: Learning Low Dimension Embedding Of Dopamine Travelling Waves in Striatum

Authors: *H. ZHANG, A. HAMID;
Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Learning Low Dimension Embedding Of Dopamine Travelling Waves in Striatum Haowei Zhang and Arif HamidDepartment of Neuroscience, University of

MinnesotaDopamine (DA) is important for reward learning and motivated behaviors, yet the temporalproperties, anatomical distribution, and specific decision signals encoded are not fully understood.Our group recently discovered wave-like DA activation patterns, characterized by directionalmotifs, that vectorize the timing of regional DA dynamics to coordinate activity across functionallyrelated striatal territories. We posit that these patterns hold computational significance inreinforcement learning (RL) by enabling differential spatiotemporal credit assignment to striatalsubregions. Here, our studies aim to dissect the characteristics of these DA waves, addressingthe dimensional complexity of the diverse spatial patterns and temporal scales across animalsand experimental conditions. To achieve these goals, we employ low-dimensional embeddings tovisualize and interpret the dynamics by capturing their geometric properties. We apply thesemethods to DA dynamics (axonal GCAMP activity or concentration via dLight imaging) that arerecorded as time-series videos via chronic imaging windows over the striatum. To determineproper linear projections and denoise the video data, we first apply principal component analysis(PCA), which maximizes the explained variance while reducing dimensions. We observe that thepatterns of principal components, which represent key DA wave features, are consistently presentwithin trials and show strong similarity across hundreds of sessions and animals. This consistencysuggests a spatiotemporal activity rule for these waves. We extend our analysis to additional non-linear methods to discover activation rules that may serve computational functions during briefbehavioral moments during task performance. This approach not only advances ourunderstanding of DA functions but also facilitates a more structured interpretation of neuralcomputations in behaviorally relevant contexts.

Disclosures: H. Zhang: None. **A. Hamid:** None.

Poster

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Topic: G.03. Motivation

Support: NIH Grant R03DA054461

Title: A comparative analysis of the current punishment model and a new quantitative punishment model for sucrose self-administration in male and female rats

Authors: *I. MADHURANTHAKAM[1], M. JOB[2];
[1]Chem. and Biochem., Rowan Univ., Glassboro, NJ; [2]Biomed. Sci., Cooper Med. Sch. of Rowan Univ., Camden, NJ

Abstract: Introduction
To address the limitations of the current punishment model in differentiating shock-resistant

versus shock-sensitive (SR vs. SS) subjects, we propose a Quantitative Punishment Model with Clustering (QPMC). QPMC utilizes clustering of several behavioral variables and eliminates the subjective endpoints the current model uses. We present evidence demonstrating significant differences between groups identified by QPMC and the current model in subjects self-administering sucrose pellets as a natural reward.

Methodology

Male (n = 10) and female (n = 12) adult Long Evans rats were subjected to a hotplate test to determine baseline pain sensitivity. Subsequently, the animals were allowed to self-administer (SA) sucrose pellets at fixed-ratio 1 schedule for 6 hours daily for 3 weeks, followed by a week of similar SA sessions structured into eleven components of 30 mins each with 3-min intervals. A second hotplate test was conducted to evaluate the impact of prolonged sucrose intake on pain sensitivity. The rats were then subjected to a punishment regimen of foot shock intensities increasing from 0 to 1.0 mA at 0.1 mA increments. Following a week of recovery, the animals were tested for pain sensitivity a third time to assess the impact of the overall procedure. Rats were then perfused 19 to 25 days later. For the current model, we employed a 30% suppression in sucrose SA levels at 0.4 mA as the separation criteria. For QPMC, we clustered several variables collected throughout the study period. We compared the groups identified by the current model to that of the QPMC model.

Results

There were no sex differences in all the variables assessed. For males, the current punishment model identified one group (SR=10), whereas for females, it identified two groups (SS n=3, SR n=9; P=0.0294) at a suppression threshold of 30% and foot shock intensity of 0.4 mA. The QPMC detected one cluster for both males (n=10) and females (n=12). For the current model, there was no relationship between the average sucrose intake and the percentage suppression threshold for both males (R^2<0.01) and females (R^2<0.01). For the QPMC, there was an inverted U-shaped relationship between the baseline sucrose intake and the suppression index for males (R^2=0.4) but not for females (R^2=0.09).

Conclusion/Future Directions

Our findings indicate that QPMC is a more conservative identification tool than the current model. Next, we will employ immunohistochemical methods to validate our new model. We are currently comparing QPMC and the current punishment model for methamphetamine user typology.

Disclosures: I. Madhuranthakam: None. **M. Job:** None.

Poster

PSTR296: Motivated Behavior: Behavior, Circuits, and Pharmacology

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Topic: G.03. Motivation

Support: R01NS111028
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Title: Mesoscale calcium dynamics observed across the cortex of freely moving mice identify brain states during the state of thirst

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Abstract: Title: Mesoscale calcium dynamics observed across the cortex of freely moving mice identifybrain states during the state of thirst.
Authors: Eunsong Ko[1], Daniel Surinach[1], Mathew Rynes[2], Ihor Hryb[1,3], Kapil Saxena[1], TrishaSamba[4], Amira Sinclair[4], Suhasa B Kodandaramaiah[1,2,3]
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Abstract: Hunger and thirst result in dramatic changes in behavioral states expressed by animals as compared to satiated brains. The neural activity corresponding to these behavioral states is potentially different at the brain-wide level, but this is yet to be investigated. In this work, we recorded cortex-wide calcium dynamics in Thy1-GCamp6f mice (n= 12) using a head-mounted microscope, the mini-mScope (Rynes*, Surinach* et al. 2021) when they were navigating a modified elevated plus maze to receive a water reward. The elevated maze consisted of a single arm, with the ends covering tall walls and a central portion without walls. Mice introduced at one end were required to traverse the exposed mid-section of the maze to receive water reward at the other end. Mice learned to successfully navigate to the water rewards as trials progressed (n= 9 trials). Thirsty mice spent significantly more time licking at the water reward port (60+/-10 s/day) compared to control mice that were not water deprived (1+/- 1 s/day). We evaluated the mesoscale calcium dynamics during licking behaviors in thirsty mice. The approach to the water port was preceded by robust and prolonged activation of the frontal regions of the cortex, with licking onset resulting in posteriorization of cortical activation. Disengagement from licking resulted in stereotyped activation of the medial regions of the cortex. Analysis and comparison of Ca^{2+} dynamics during approaches to and away from the water port further revealed significant differences in the dynamical activation of the cortex between the thirsty and control mice. We conclude that a state of thirt results in a significant alteration of motivated water reward-seeking behavior, which is associated with large-scale changes in cortical activity patterns.

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Poster

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Title: Analysis of monosynaptic inputs to neurons in the shell of the nucleus accumbens that project to the ventral pallidum

Authors: S. H. LI, *S. LI, G. J. KIROUAC;
Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: The ventral striatopallidal system is conceptualized as a basal forebrain macrosystem involved in regulating behavioral responses associated with reward and motivation. The nucleus accumbens, which forms a large part of the ventral striatum, integrates signals from the prefrontal cortex, hippocampus, basal lateral amygdala and thalamus to regulate behavior via projections to the ventral pallidum (VP). Excitation of medium spiny neurons in the shell of the nucleus accumbens (NAcSh) inhibits tonically active neurons in the VP which lead to disinhibition of dopamine neurons in the ventral tegmental area (VTA). The present investigation applied an intersectional monosynaptic rabies tracing approach to quantify the brain-wide sources of afferent inputs to NAcSh neurons that project to the VP in male and female rats. The tracing experiments specifically targeted the rabies virus to dorsomedial NAcSh (dmNAcSh) neurons that project to the medial VP (mVP) which densely innervates the VTA. The ventral subiculum of the hippocampus (vSub) is the largest source of input cells to the NAcSh-mVP projecting neurons. Other strong sources of input cells include the anterior part of the paraventricular nucleus of the thalamus (aPVT), VTA, VP, lateral preoptic area, lateral hypothalamus and other regions of the hypothalamus. Moderate sources of input cells comprise the dorsolateral bed nucleus of the stria terminalis, lateral central nucleus of the amygdala, and the basolateral nucleus of the amygdala whereas minor sources included the prefrontal cortex and other regions of the brain. The experiments are ongoing but the data collected to date indicate a lack of sex differences in the inputs to the projection neurons studied. In summary, the vSub and the aPVT are major sources of cortical and thalamic monosynaptic inputs to the dmNAcSh-mVP projecting neurons. These anatomical findings support experimental data indicating that vSub and aPVT afferent inputs converge in the NAcSh to regulate dopamine neuron activity and behavior.

Disclosures: S.H. Li: None. **S. Li:** None. **G.J. Kirouac:** None.

Poster

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Horizon Europe Research & Innovation Programme under Grant agreement N. 101092612 (Social and hUman ceNtered XR (SUN) project)

Title: Non-invasive temporal interference stimulation of the striatum to investigate mechanisms of motivated behavior in healthy humans

Authors: *P. VASSILIADIS[1], E. BEANATO[1], S. LOSACCO[1], F. WINDEL[1], T. POPA[1], T. MORISHITA[1], E. NEUFELD[2], J. DUQUE[3], G. DEROSIERE[4], M. J. WESSEL[1], F. C. HUMMEL[1];
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Abstract: A crucial function of the striatum is the ability to incentivize behavior based on reward. Yet, because of its deep localization in the brain, it has so far not been possible to investigate the causal role of the striatum in reward processing non-invasively during healthy behavior. Here, we leveraged a new non-invasive brain stimulation technology, namely transcranial temporal interference stimulation (tTIS, Grossman *et al.,* 2017; Wessel *et al.* 2023, Nat Neurosci) to target the striatum during reinforcement learning of a motor skill. In this randomized, sham-controlled, double-blind study, we applied tTIS while 24 participants were practicing a force-tracking task with or without real-time reward feedback and with concurrent functional magnetic resonance imaging (fMRI). Based on previous studies showing reinforcement-dependent, time-locked bursts of high gamma striatal activity following reward delivery (Berke 2009, EJN), we compared the behavioral and neurophysiological effects of 80Hz tTIS ($tTIS_{80Hz}$) with those of 20Hz tTIS (TIS_{20Hz}) and sham stimulation ($tTIS_{sham}$). More specifically, we hypothesized that applying a constant, open-loop high gamma rhythm in the striatum would disturb the temporally precise and reinforcement-specific modulation of striatal high gamma activity and perturb reinforcement motor learning. As expected, participants learned more when reinforcement was provided in $tTIS_{sham}$. Consistent with our hypothesis, these benefits were abolished by $tTIS_{80Hz,}$ but not by $tTIS_{20Hz}$. Moreover, in line with a role of striatal beta oscillations in motor function, motor learning without reinforcement was reduced with

$tTIS_{20Hz}$, but not with $tTIS_{80Hz}$. We also find that the impairment of reinforcement-related benefits in motor learning with $tTIS_{80Hz}$ was associated to individual changes of neural activity in the putamen and caudate nucleus, suggesting that the observed behavioral effects were indeed related to neuromodulation of striatal activity. By combining these data with other ongoing studies employing tTIS (total of >250 sessions), we further show that the stimulation is safe, generally perceived as "mild" and compatible with efficient blinding. Finally, we will show how the technology can be applied in other forms of motivated behavior such as in the context of effort-based decision-making for reward. Put together, our work suggests that striatal tTIS is a promising tool to investigate fundamental mechanisms of motivated behavior paving the way for their modulation in populations of patients affected by disorders of motivation.

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Poster

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Title: Monkeys' Preference for free choice opportunities reflects a balance of intrinsic and extrinsic rewards

Authors: *E. OWCZAREK, E. PETROPOULOU, J. MUNUERA, B. LAU;
Paris Brain Inst., Paris, France

Abstract: Decisions are frequently explained as the balancing of potential extrinsic rewards (ER) and punishments, such as food or lost time. However, rational models of decision making based on this idea frequently fail to accurately predict behavior when humans and other animals base decisions on value attribution that is not clearly linked to extrinsic outcomes. Indeed, some situations appear to be sought for themselves and are therefore defined as intrinsically rewarding (IR). For example, there is strong evidence that humans prefer opportunities to choose, even when these imply receiving less future ERs (Munuera et al., PLoS Comp Biol, 2023). We aimed to better characterize the behavioral mechanisms involved in the encoding of IR in non-human primates (NHP). Two rhesus macaques performed a two-step task allowing temporal dissociation of IRs and ERs. During step 1, NHPs are given the choice between 2 abstract fractal images associated respectively to a 'free choice condition' or a 'forced condition'. If they selected the

free condition, they then had to choose during step 2 between two different abstract images, one of which was rewarded more than the other. In the forced condition, only one (different) abstract image could be selected during step 2, which rewarded as much as the one that was most rewarded in the free condition. Given that ER is the same in both conditions, preferences at the step 1 can only be explained by a preference for one of the two conditions that is unrelated to extrinsic reward. We reversed the association between fractal images and condition in step 1, and used multiple image sets to confirm condition preference. We analyzed choice and video-based analysis of facial movements to show that NHPs expressed an intrinsic preference for choice opportunities - even though selecting the free choice option at step 1 could lead to loss of ERs for incorrect target selection at step 2. However, free choice preference was balanced by 1. the preference of NHPs for certain abstract images themselves, 2. a constant side bias towards contralateral targets, 3. the satiety for ER over the course of a session. Intersession analyses showed a stable combination of choice, side, and image preferences in step 1 choice behavior. On the other hand, intrasession analyses showed that each monkey exhibited an idiosyncratic balance of IRs and ERs that was modified over the course of a session. We will investigate those dynamics at the neural level by asking whether the dopaminergic system is involved in the encoding of such hierarchical preference.

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Poster

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Program #/Poster #: PSTR296.13/M20

Topic: G.03. Motivation

Support: NIH Grant DA019473
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Title: Effect of stimulating dopamine D1- or D2-receptor-expressing neurons of the nucleus accumbens on cue-triggered reward seeking

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Abstract: Animals learn to locate primary rewards via reward-associated environmental cues. Reward-predictive cues increase the activity of a large number of nucleus accumbens (NAc) neurons. This neuronal response correlates with reward seeking and is thought to mediate that behavior. The NAc is predominantly (~95%) composed of GABAergic projection neurons that express either the dopamine D1 or D2 receptor. To this day, the respective contribution of D1

and D2 neurons in cue-guided reward seeking remains largely unknown. As a first step to resolve this issue, we examined the role of D1 and D2 neuronal activity in the response to reward cues by combining optogenetic stimulations with electrophysiological unit recordings. We used D1- and A2A-cre rats (females and males) to selectively drive viral-mediated expression of ChR2 in D1 and D2 neurons, respectively. Rats were initially food restricted and trained to discriminate cues (sound or lights) that either predicts a sucrose reward (reward stimulus, S^+) or no rewards (no-reward stimulus, S^-). After acquisition, we examined the effect of stimulating D1 or D2 neurons (single pulse of 10, 100 or 500 ms, 1 mW) on S^+-induced neuronal excitations in the NAc and reward seeking. This was examined while rats were food restricted to produce high reward seeking and while they were not restricted to produce lower responding. We found that stimulating D1 neurons alone increases NAc neuronal activity. When given at S^+ onset, D1 stimulation potentiated cue-induced NAc neuronal excitations, especially when S^+ responding was low. In contrast, activation of D2 neurons alone decreased the activity of a subset of NAc neurons. When given at S^+ onset, stimulation of D2 neurons either increased or decreased cue-induced NAc neuronal excitations. At the behavioral level, neither D1 nor D2 neuronal stimulation altered S^+-evoked reward seeking. We also examined the effect of D1 or D2 neuronal stimulation (100 ms) on reversal learning, where the former S^+ is no longer predictive of the reward (new S^-), and the former S^- is now the S^+. We found that light activation of D2 neurons caused rats to discriminate their response (i.e., higher response to the S^+ than S^-) earlier than control rats, whereas light activation of D1 neurons did not. All the above observations were found in males and females. Together, these results suggest that while activating D1 or D2 neurons is insufficient to alter the probability of already acquired cue-triggered reward seeking, D2 but not D1 stimulation is sufficient to accelerate new reward-cue associations when the predictive value of environmental cues changes.

Disclosures: A. Servonnet: None. **S.M. Nicola:** None.

Poster

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Topic: G.03. Motivation

Support: PI's Start Up Funds

Title: Comparative Study of the Role of Dorso-Medial Striatum (DMS), Dorso-Lateral Striatum (DLS) and Nucleus Accumbens (NAc) in Reinforcement Learning and Decision Making

Authors: *A. BASAK, E. GOODIN, I. OZDEN;
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Abstract: Reinforcement learning (RL) lies at the center of our ability to adapt to a dynamically changing world. It is a process guided by reward and punishment to identify optimal actions and decisions by trial and error. In the brain, the neural circuitry of RL has been associated with the basal ganglia system, particularly the striatum, which receives reward-related information as dopaminergic projections from dopamine centers. Past research has suggested that different parts of the striatum play different functional roles in reward-driven behavior: The dorsomedial striatum (DMS) is involved in learning/selecting appropriate actions based on the expected outcomes. The lateral striatum (LS) is associated with habit formation and translating motor commands into specific actions. And the nucleus accumbens (NAc) is involved in reward expectation and pleasure-seeking. Our lab research focuses on goal-directed action selection, which is mainly associated with the DMS. However, recent studies have shown that other parts of the striatum also play a role in goal-directed behaviors and are modulated by dopaminergic projections (REFS). However, which aspects of the learning process each striatal region specifically contributes is still under debate (REFS). A better understanding of how different parts of the striatum affects basic behaviors might provide insight into their contributions to more complex behaviors. Accordingly, here we report different behavioral consequences of optogenetic stimulation of the direct pathway medium spiny neurons (dMSNs) in different striatal regions in three behavioral paradigms: (1) free behavior under unilateral stimulation of dMSNs; (2) field-preference under bilateral stimulation of the dMSNs; (3) an odor-cued Go-NoGo task under bilateral stimulation of dMSNs during feedback-period. Our results show that unilateral stimulation of the dMSNs of the DMS (n=5) and LS (n=2) led to rotational behavior in mice ($p<0.005$), whereas stimulation of dMSNs in NAc (n=3) did not. In the field-preference test, all mice showed a strong field preference in response to optogenetic stimulus ($p<0.005$). In the Go-NoGo task, we did not see any improvement in learning performance in response to optogenetic stimulation of dMSNs in DMS, however, mice performed the next trial after stimulus faster. These results are consistent with the idea that the DMS and LS associate dopamine input with motor movement, action selection, or motivation rather than with a pure reward signal (REFS). On the other hand, in NAc the effect of dopamine input is more consistent with reward (REFS). Currently, we are analyzing the data for mice where we stimulated dMSNs of the LS and NAc.

Disclosures: A. Basak: None. **E. Goodin:** None. **I. Ozden:** None.

Poster

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Topic: G.03. Motivation

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Title: The role of extended amygdala CRF neurons in incentive motivation

Authors: *V. KNAPP[1], R. THAKRAR[1,2], L. J. TITTLE[4], A. RAMASWAMI[1,2], K. EMERY[1,3], K. C. BERRIDGE[1,3];
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Abstract: Corticotropin-releasing factor (CRF) neurons are traditionally assumed to generate aversive stress states, particularly during withdrawal from drugs of abuse (George et al., 2012). However, other evidence shows that CRF signaling in nucleus accumbens (NAc) can generate positively valenced incentive motivation to pursue and consume rewards (Lemos et al., 2012; Peciña et al., 2006). Additionally, optogenetic laser stimulation of CRF neurons in the central amygdala (CeA) and NAc of *crh*-Cre rats intensifies and focuses pursuit of a laser-paired sucrose or cocaine reward over an equal reward without laser stimulation. Optogenetic excitation of CRF neurons also supports laser self-stimulation indicating positive valence of CRF neuronal excitation in CeA and NAc (Baumgartner et al., 2021, 2022). However, CRF neurons co-release other neurotransmitters such as GABA which may be more likely to account for these incentive effects (Pomrenze et al., 2015). Thus, it is unknown whether CRF itself versus other neurotransmitters mediate this positively-valenced motivation. To specifically test the role of CRF receptor activation in CeA and NAc CRF neuronal incentive motivation, we administered i.c.v. microinjections of a global CRF antagonist or vehicle prior to laser self-stimulation by *crh*-Cre rats, or prior to 2-choice tasks in which rats could choose to earn either laser-paired sucrose reward or identical sucrose reward without laser. We found that CRF receptor blockade reduces laser self-stimulation of CeA and NAc CRF neurons and eliminates focused and intensified incentive motivation for laser-paired sucrose rewards in the two-choice sucrose task. This work builds on recent evidence that CRF neurons are capable of generating incentive motivation without distress and supports an alternative role for CRF in reward salience without necessitating anxiety.

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Poster

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Program #/Poster #: PSTR296.16/M23

Topic: G.03. Motivation

Title: Dynamics of Brain Activity During Sexual Experience: Role of the mPFC and NAcc

Authors: *A. GÓMEZ MÉNDEZ[1], C. DOMÍNGUEZ-ESTRADA[1], C. SOTELO-TAPIA[2], P. CORTES ESPARZA[3], M. HERNANDEZ[1], M. A. GUEVARA[1];
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Abstract: For this study, sexual experience in male rats is defined as the occurrence of sexual interactions until the subject reaches ejaculation. To determine sexual experience, some authors rely on copulatory efficiency, which is characterized by decreased latencies and frequencies of copulatory parameters (mount and intromission) as the male rat gains more sexual experience. Additionally, it has been shown that sexual experience is associated with neuro-morphophysiological changes such as an increase in dendritic spine density in specific brain areas related to the expression of sexual behavior, including the nucleus accumbens (NAcc) and the medial prefrontal cortex (mPFC). However, the impacts of sexual experience on the functioning of these brain areas are not fully understood. This study aims to understand the neural processes associated to sexual experience and its relation with reward processes. Thus, the effect of sexual experience on EEG activity in the mPFC and NAcc of male rats as they acquire sexual experience in the presence of a receptive female is investigated. EEG activity was recorded in nine male Wistar rats while they remained attentive to a receptive female, although without direct contact with her. Subsequently, the rats underwent a sexual experience acquisition process. The study was designed with four experimental conditions, alternating EEG recordings and sexual experience acquisition sessions every two days. The results reveal that mPFC activity decreases in response to sexual stimuli as experience increases, suggesting a possible adaptation process to the stimuli emitted for the female. On the other hand, NAcc activity shows an initial decrease in low-frequency activity after sexual experience, followed by an increase in high-frequency activity as experience is consolidated, indicating a shift in stimulus processing towards precise modulation of behavioral responses. These findings contribute to understanding the neural mechanisms underlying the behavioral changes observed with sexual experience.

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Poster

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Program #/Poster #: PSTR296.17/M24

Topic: G.03. Motivation

Title: Caffeine's Influence on Post-Intromission Response to Sexual Stimuli in Male Rats: an EEG study

Authors: *C. DOMÍNGUEZ-ESTRADA[1], A. GÓMEZ MÉNDEZ[1], A. C. MEDINA[2], M. A. GUEVARA[1], M. HERNANDEZ[1];
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Abstract: It has been described that caffeine produces an increase in the general activation of the organism. This activation facilitates the deployment of various behaviors, including sexual behavior. In this context, it has been reported that after the male rat performs the intromission behavior, his attention and motor activation towards the stimuli of the sexual partner increases, a state that has been associated with an increase in sexual motivation and arousal. Sexual arousal is closely related to sexual motivation whose induction and maintenance depends on the functioning of several structures of the mesocorticolimbic dopaminergic system, particularly the medial Prefrontal Cortex and the Nucleus Accumbens, structures that in turn are sensitive to the effects of caffeine.The aim of this study was to evaluate the effect of general activation, associated with acute caffeine administration, on the response to sexual stimuli post-intromission using electroencephalographic (EEG) and behavioral parameters in the male rat. Two independent variables were considered in two levels, one for caffeine and other for the post-intromission state. Also, behaviors shown in the sexual incentive arena were measured such as preference for a receptive female over a not receptive one, nose pokes and movement. No significant differences were found for the behavioral parameters. However, the left prefrontal-accumbens EEG phase correlation in the beta and gamma bands was higher in the "caffeine with intromission" group with respect to the "no caffeine with intromission" group. In addition, the inter-accumbens EEG phase correlation in theta1, beta and gamma bands were higher in the "caffeine with intromission" group with respect to the "no caffeine with intromission" group.These results shown that acute caffeine administration increase the degree of interaction between the prefrontal and accumbens regions as well as between the accumbens nuclei, which probably is necessary to the male rat assign an adequate incentive value to sexual stimuli emitted for the female. Further research is warranted to elucidate the precise mechanisms underlying caffeine's effects on sexual arousal and its neural correlates.

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Poster

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Topic: G.03. Motivation

Support: This work was supported by the Ministry of Science and Higher Education (Poland) as a project under the program Excellence Initiative – Research University (2020-2026) no. BOB-IDUB-622-28/2023 (IV.4.1.).

Title: Exploring circadian rhythm distinctness: A role in reward and punishment processing

Authors: *P. SCISLEWSKA[1,2], M. ZAREBA[3], J. LENGIER[1], A. E. SCHIRMER[4], P. BEBAS[1], I. SZATKOWSKA[5];
[1]Fac. of Biol., Univ. of Warsaw, Poland, Warsaw, Poland; [2]Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland; [3]Univ. Jaume I, Castellon de la Plana, Spain; [4]Biol., Northeastern Illinois Univ., Chicago, IL; [5]Nencki Inst. of Exptl. Biol., Polish Acad. of Sci., Warsaw, Poland

Abstract: Introduction: Chronobiologists have been fascinated with studying human chronotypes for a long time, but is looking at a chronotype alone enough to fully grasp circadian rhythms? This study delves into the intricate interplay between reward and punishment processing, considering not only chronotype (circadian phase) but also the distinctness of the circadian rhythm (subjective amplitude). **Methods:** We examined 37 healthy participants (aged 20-30) using functional magnetic resonance imaging (fMRI) during the Monetary Incentive Delay Task, which is a common method of assessing motivated behavior. Circadian rhythmicity characteristics were measured using the Morningness-Eveningness Stability-Scale improved (MESSi) questionnaire. We also administered questionnaires assessing personality (NEO-Five Factor Inventory; NEO-FFI) and reinforcement processing: the Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ), and the Positive and Negative Affect Schedule (PANAS). Statistical analyses were conducted utilizing the FSL neuroimaging program and Python-based statistical packages such as Pandas, NumPy, SciPy. **Results:** Among individuals with pronounced eveningness, fMRI data revealed a negative correlation in Occipital Pole activity during the anticipation phase of reward trials. However, when eveningness was controlled for distinctness, the observed effects dissipated. Instead, during the anticipation phase of 'punishment' trials, a positive correlation between the distinctness value and activity in the bilateral Superior Frontal Gyrus and Supplementary Motor Cortex (BA 8, 6) was revealed. Furthermore, a positive association between a value of distinctness and neuroticism levels, sensitivity to punishment, and negative affect was observed, with no corresponding effects found for eveningness. **Conclusions:** These findings underscore the multifaceted nature of circadian rhythmicity, indicating that dimensions beyond chronotype significantly shape human behavior. The results emphasize the negative emotionality aspect of high-distinctness people and suggest the association between a higher value of distinctness and enhanced neural processing of punishment-related information.

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Poster

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Topic: G.03. Motivation

Support: NIH Grant R15MH125282

Title: Using RNAScope to co-localize estrogen receptors andΔFosB in the nucleus accumbens and ventral tegmental area in the mouse brain

Authors: A. BRAVACOS, C. MURRELL, R. ZHANG, *L. BEEN;
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Abstract: During pregnancy, estrogen levels increase rapidly and peak just before birth. After parturition, estrogen levels quickly drop to pre-gravid levels and remain suppressed until ovulation resumes. The mesolimbic pathway, including the ventral tegmental area (VTA) and nucleus accumbens (NAc), contains estrogen receptors (ERs). Further, this pathway is impacted by the peripartum estrogen fluctuations including altered dopamine dynamics and increased ΔFosB, a transcription factor associated with long-term neuroplasticity. It is unknown, however, how these dramatic peripartum estrogen fluctuations change ER expression in the brain. Further, the lack of reliable antibodies has hindered characterization of ERs in the brain. We therefore used RNAScope to co-localize ERs and ΔFosB in the mesolimbic pathway in adult female C57BL/6 mice. Specifically, we simultaneously visualized Esr1 and Esr2, the genes which encode ERα and ERβ respectively, along with FosB in the NAc. Further, we simultaneously visualized Esr1 and Esr2 in the VTA. In the NAc, we found that FosB tends to co-localize exclusively with Esr1, not Esr2. In the VTA, we found that Esr1 and Esr2 rarely co-localize; VTA cells tend to express solely Esr1 or Esr2. These results provide important data about baseline expression of ERs in the mesolimbic pathway. Future experiments will assess changes in these patterns in response to peripartum estrogen fluctuations.

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Poster

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Support: NIH Grant K99 MH132772

Title: Calcium signaling in the nucleus accumbens during social interactions in male and female Syrian hamsters

Authors: *J. M. BORLAND[1], P. E. ROTHWELL[2], R. L. MEISEL[3];
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Abstract: The rewarding properties of social interactions are critical for the expression of adaptive social behaviors, and the development and maintenance of beneficial social relationships. The nucleus accumbens (NAc) encodes reward for social behaviors and calcium is a biomarker of neuronal activity. In the following study, I investigated changes in calcium signaling in the NAc in male and female Syrian hamsters with repeat social experience, i.e. the effects of social experience on calcium activity. I predict that with repeat social experience there is a potentiation in calcium signaling in the NAc. Four adult male and four adult female Syrian hamsters were single housed and injected with a virus containing a calcium indicator (AAV5-hSyn-Soma-jGCaMP8f) and a fiber optic cannula into the NAc. Four weeks later, males and females underwent four days of testing in which they experienced a 10-min interaction with a smaller stimulus hamster. Females were tested during diestrus. Both male and female Syrian hamsters naturally establish dominant-subordinate relationships. With repeat social experience, there is a decrease in the latency to initiate an aggressive interaction ($p < 0.050$), a social behavior shown to be rewarding. There is also an increase in the area under the curve (AUC) and peak height in calcium for the initiation of a social interaction, compared to just before the interaction, in male and female Syrian hamsters ($p < 0.050$). As hypothesized, with repeat social experience there is a further potentiation in calcium signaling for the initiation of a social interaction compared to signaling during prior tests ($p < 0.050$). These studies support that social experience results in a potentiation in synaptic communication between neurons in the NAc in males and females. Future studies will increase sample sizes to allow analysis of sex as a factor, investigate the role of glutamate from the prefrontal cortex on this calcium signaling and social reward, and dissociate the role of the direct versus indirect circuit in the NAc in males and females. Advancing understanding of sex differences in social reward will advance understanding of sex differences in the susceptibility of psychiatric disorders.

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Poster

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Army Research Office 78259-NS-MUR

Title: Why did we evolve to seek information? Testing two computational theories

Authors: *E. BROMBERG-MARTIN[1], J. MEREL[2], I. E. MONOSOV[3,4,5,6,7];
[1]Washington Univ., Saint Louis, MO; [2]Fauna Robotics, New York, NY; [3]Neurosci., Washington Univ. Sch. of Med., St Louis, MO; [4]Bioengineering, Washington University, St Louis, MO; [5]Neurosurgery, Washington University School of Medicine, St Louis, MO; [6]Electrical Engineering, Washington University, St Louis, MO; [7]Pain Center, Washington University School of Medicine, St Louis, MO

Abstract: We constantly seek information about the world around us. Many of our information seeking actions are clearly adaptive and provide us with obvious, objective benefits. Remarkably, however, humans and several animal species can persistently seek information about uncertain future rewards even when this information has no apparent objective value for controlling future outcomes. Thus, in this form of information seeking, organisms appear to endow information with subjective value of its own. We recently found that key features of these information value computations appear strikingly conserved in humans and monkeys (Bromberg-Martin and Feng et al, 2024). This raises a fundamental, outstanding question: why did we evolve to seek this form of information? One hypothesis is that it has no adaptive purpose: that the subjective value we place on information is an accident of evolution, a *bug* in neural value computations. However, we and others have proposed that the subjective value of information evolved as a *feature*, with an important adaptive purpose: as an estimate of the benefits information would provide organisms in natural environments (Bromberg-Martin and Monosov, 2020). If so, we should be able to use considerations of natural environments to build principled theories that explain and predict how organisms value information in experiments. Here we present preliminary work modeling and testing two theories that explain how this form of information seeking could help organisms maximize reward in natural environments. The first theory, based on reinforcement learning, proposes that organisms value information because it helps solve the temporal credit assignment problem, by narrowing down which states and actions deserve the credit for obtaining rewards (Bromberg-Martin and Hikosaka, 2009). The second theory, based on optimal foraging, proposes that organisms value information because it helps them decide when it is more profitable to pursue a potential reward or abandon it to search elsewhere (Vasconcelos et al, 2015). We find that both theories correctly predict several key features of information seeking behavior in our data. That is, several parameters that govern the objective value of information for agents in computational models embodying these theoretical environments, also govern the subjective value that real humans and monkeys place on information in experiments. However, neither theory alone appears to fully explain all features of information seeking behavior. Thus, our work provides evidence that information seeking evolved as a feature rather than a bug, and sheds light on its potential adaptive functions in natural environments.

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Poster

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Program #/Poster #: PSTR296.22/M29

Topic: G.03. Motivation

Title: Orexinergic input to the Supramammillary nucleus modulates seeking behavior in mice

Authors: *B. GETACHEW[1], J. MENDOZA[3], Y. ARIMA[2], S. IKEMOTO[4];
[1]Neurocircuitry of Motivation, [2]Neurocircuitry of Motivation Section, NIDA/NIH, Baltimore, MD; [3]Neurocircuitry of Motivation, Natl. Inst. of Drug Abuse, Baltimore, MD; [4]Behav Neurosci Br, NIDA / NIH, Bel Air, MD

Abstract: Stimulation of the orexinergic terminal at the supramammillary nucleus reinforces seeking behavior in mice
AuthorsB.Getachew, J.Mendoza, Y.Arima, S.Ikemoto Neurocircuitry of Motivation Section, National Institute of Drug Abuse**Disclosures: B.Getachew:** None, **J.Mendoza:** None**, Y. Arima:** None**, S.Ikemoto:** None **Abstract**
Hypothalamic orexinergic (ORX) neurons are crucial for arousal and reward-seeking behavior, with specific ORX projections to the ventral tegmental area heavily implicated in these processes. Recent studies suggest that the hypothalamic supramammillary region (SuM) plays a role not only in arousal but also in seeking behavior, and ORX neurons provide significant inputs to the SuM. This led us to hypothesize that ORX neurons provide excitatory inputs to SuM neurons, thereby reinforcing seeking behavior. To test this hypothesis, we first investigated whether ORX terminals form synaptic contacts with SuM neurons. To achieve this, we injected AAV1-SYN1-FLEX-mGFP-2A-SYP-mRuby into the hypothalamic ORX field of ORX-Cre mice. The results showed a high density of ORX fibers and numerous synaptic terminals in the SuM. Next, we examined whether SuM neurons express ORX receptors. Using RNAscope analysis, we found mRNA expression for both ORX receptors 1 and 2 in SuM neurons. Lastly, we assessed whether activation of ORX terminals in the SuM reinforces behavioral responses. We injected AAV9-EF1a-DIO-ChR2 into the ORX field of ORX-Cre mice to express the excitatory opsin ChR2 on ORX terminals. We then performed optogenetic intracranial self-stimulation and real-time preference procedures. The results indicated that mice quickly learned to respond to a lever delivering photostimulation to the SuM, and they spent more time in the compartment where they received photostimulation of the ORX-SuM terminals. In summary, our data suggest that ORX neurons innervate the SuM, activating its neurons to reinforce seeking behaviors. Future studies will explore whether ORX terminal afferents to the SuM play a broader role in various seeking behaviors, including exploratory behavior. Additionally, given that ORX

neurons can release both orexins and glutamate from their terminals, we aim to discern the relative contributions of these two neurotransmitters in the context of seeking behavior.

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Title: Classification of Sign-Tracking and Goal-Tracking: Introducing k-Means and Derivative Approaches

Authors: *C. GODIN[1], F. HUPPÉ-GOURGUES[2];
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Abstract: When organisms like rodents undergo Pavlovian conditioning, different types of behaviors arise within groups. Some subjects tend to interact with the cue that precedes a biologically potent stimulus, whereas others tend to orient directly towards the stimulus. The cue approach style is usually quantified with a PCA Index score, and classified as Sign-Tracking (ST), Goal-Tracking (GT) or Intermediate (IN) (Meyer et al., 2012). However, the cutoff values used to separate scores in the three groups are often arbitrary and inconsistent. We present two methods of PCA Index score classification: the k-Means classifier and the derivative method. Both methods extract cutoff values based on the distribution of PCA Index scores in the sample. Overall, our results suggest that these classification methods, particularly the derivative method based on mean scores of the last two days of conditioning, are useful tools for identifying STs and GTs, even within relatively small or skewed samples. Implementing these classification techniques will improve the precision of subject classification, thereby enhancing transparency and replicability of results based on the ST-GT model.

Disclosures: C. Godin: None. **F. Huppé-Gourgues:** None.

Poster

PSTR296: Motivated Behavior: Behavior, Circuits, and Pharmacology

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR296.24/M31

Topic: G.03. Motivation

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Title: Medial septum glutamate neurons and interregional theta oscillations during goal-directed behaviors

Authors: *C. DARDEN[1], A. KESNER[2];
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Abstract: Cognitive flexibility allows both humans and animals to adapt their thinking and behavior to changes in both external environment and internal motivation states. While this ability is advantageous, evidence suggests that this mechanism is altered during addiction and is involved in generating and upholding maladaptive behavioral patterns. Research implicates midbrain dopamine (DA) neuron activity in driving reward-seeking behavior, and these cells have been shown to encode real-time behavioral feedback such as reward prediction errors. Like the nucleus accumbens and other midbrain DA neurons targets, the medial septum (MS) plays a role in reward seeking and learning. Previous experiments from our lab shows that a subpopulation of MS neurons, medal septum glutamate neurons (MS^{GLU}), are reinforcing when excited optogenetically and that chemogenetic modulation of MS^{GLU} alters nucleus accumbens dopamine (NAc^{DA}) signaling when retrieving an unexpected award. Moreover, chemogenetic excitation of MS^{GLU} enhances the speed at which animals learn to associate new cue-reward pairings, and these changes are encoded by NAC^{DA} signaling over days. While NAc^{DA} plays a role in these strategy switching processes, other processes related to MS^{GLU} neuron activity affect these behaviors. For example, MS^{GLU} neurons play a canonical role in hippocampal theta oscillations, a pattern of neuronal synchrony important for cognitive processes (strategy switching). Coordination between hippocampal and medial prefrontal (mPFC) theta processes is also important for cognitive flexibility. Since MS^{GLU} neurons project to both hippocampus and PFC, we used a chemogenetic approach along with local field potential recording (LFP) to investigate how modulation of MS^{GLU} alters hippocampal-mPFC theta oscillatory properties (power, coherence, etc). Preliminary analyses indicate that, while chemogenetic modulation of MS^{GLU} neurons has minimal effects on theta power or hippocampal-mPFC coherence during foraging, in general MS theta activity is tightly coupled to that of mPFC when animals are engaged in appetitive behaviors. Additionally, new data suggest that chemogenetic modulation of MS^{GLU} neurons alters mPFC, MS, and hippocampal theta power after, but not before, lever presses that were previously reward-bearing during a strategy switching task. While much remains to be learned about MS^{GLU} neuron involvement in cognitive flexibility and strategy switching, elucidating these mechanisms may provide possible therapeutic targets for psychiatric disorders stemming from maladaptation in these processes.

Disclosures: C. Darden: None. **A. Kesner:** None.

Poster

PSTR296: Motivated Behavior: Behavior, Circuits, and Pharmacology

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Program #/Poster #: PSTR296.25/M32

Topic: G.03. Motivation

Support: Len Blavatnik STEM Research Fellowship Fall 2023
FCRH Undergraduate Research Grant Summer 2023

Title: Activity of ventral pallidum cholinergic neurons increases in response to reward and is modulated by reward value

Authors: *A. GANNON, R. SETARA, R. SALAZAR, C. MAYSONET, D. ZUELKE, E. F. GALLO;
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Abstract: The ventral pallidum (VP), a major output nucleus of the mesolimbic system, has been implicated in motivated behavior. VP activity encodes the value of primary rewards and incentive cues and promotes reward-seeking actions. A mechanistic understanding of the VP's role in motivation remains elusive, in part due to the region's cellular heterogeneity. While most VP functions are classically assumed to be mediated by GABAergic projection neurons, the VP also contains a subpopulation of cholinergic projection neurons (VP-CPNs), whose role in reward processing is still unknown. Here, we used *in vivo* fiber photometry to investigate VP-CPN activity in the mouse VP during a simple instrumental task. We delivered an adeno-associated virus expressing a calcium sensor (FLEX-jGCaMP7f) followed by implantation of an optic fiber into the VP of adult ChAT-IRES-Cre mice. Mice were trained on a continuous reinforcement (CRF) schedule with 60 trials per session. Each trial began with extension of a lever, which when pressed yielded a milk reward. VP-CPN Ca^{2+} signals were analyzed as time-locked events to the onset of the cue (lever extension), lever press, or the reward presentation. We found that VP-CPN activity consistently increased above baseline following both cue presentation and lever presses. However, the largest increase in activity was observed in response to reward presentation. We showed that this response was specific to retrieved rewards. To further understand the VP-CPN response to reward, mice were pre-fed the reward for 30 minutes before CRF to induce reward devaluation. Indeed, pre-feeding led to fewer rewards retrieved, longer press latency, and longer session duration. Contrary to our prediction, pre-feeding did not alter the peak amplitude in reward-evoked VP-CPN activity and significantly prolonged the response compared to the non-pre-fed state. To determine if the reward-evoked response was modulated by reward palatability, we then diluted the milk reward with water (100, 50, 25, 10, 2, and 0%), each dilution presented separately over 6 recording sessions in a pseudo-

randomized order. Increasing reward dilution significantly decreased the magnitude of the VP-CPN response to reward. Together, these results indicate that VP-CPNs robustly and transiently increase their activity in response to reward retrieval. Moreover, reward-evoked activity is modulated by satiety state as well as by reward palatability. These findings suggest a novel role for VP-CPNs in processing reward value, providing new insight into the cellular mechanisms by which the VP contributes to reward-related behavior.

Disclosures: A. Gannon: None. **R. Setara:** None. **R. Salazar:** None. **C. Maysonet:** None. **D. Zuelke:** None. **E.F. Gallo:** None.

Poster

PSTR296: Motivated Behavior: Behavior, Circuits, and Pharmacology

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Topic: G.03. Motivation

Support: NSF IOS 1557987
NIH 1R01DA044199

Title: Peripubertal social adversity alters motivated behaviors across the lifespan

Authors: *C. NEMESKAL, J. CAVANAUGH, K. S. SMITH;
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Abstract: While positive social connections protect physical and mental wellbeing, social adversity promotes vulnerability to chronic stress and disease. Specifically, social disruption during the critical period of adolescence increases susceptibility to prevalent neuropsychiatric disorders, including anxiety and major depression. Though these disorders are often associated with anhedonia and avolition, a gap remains in understanding the impact of social disruption on motivated behaviors across the lifespan. This project identified the lasting impacts of social disruption during puberty and early adulthood on motivated and decision-making behaviors. Wild-type rats were assigned to one of three pubertal conditions [chronic isolation (CI), recurrent social disruption (RSD), or pair housing (PH)] and were trained to self-administer social and food rewards in an automated operant assay. Following puberty, animals were re-assigned to one of three adult conditions [CI; RSD; PH], creating nine factorial conditions. Incentive values of social and food rewards and reward preferences were assessed both prior to and following the condition switch. In adolescence, pubertal RSD maladaptively influenced both social and non-social functioning, reducing motivation for social and food rewards. Alternatively, pubertal CI enhanced social motivation. RSD-induced reductions in reward incentive value that arose during puberty persisted into early adulthood, even after animals were reassigned to PH or CI, demonstrating the lasting effects of RSD on motivated behavior. In contrast, pubertal PH

phenotypes were protective against the impact of social adversity during early adulthood, suggesting that stable and normative social environments during the critical period of adolescence are necessary to prevent lasting motivational damage in adulthood. Moreover, the data suggest that reassignment to PH in adulthood may confer protective benefits to animals that experienced social adversity in adolescence. These findings illuminate both the lasting impacts of adolescent social disruption on motivated behaviors across the lifespan and the potential for rescue of maladaptive phenotypes, highlighting the complex development of the social decision-making network. Ultimately, these data provide a framework for the development of targeted therapeutics to ameliorate social and motivational dysfunction in humans.

Disclosures: C. Nemeskal: None. **J. Cavanaugh:** None. **K.S. Smith:** None.

Poster

PSTR296: Motivated Behavior: Behavior, Circuits, and Pharmacology

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Program #/Poster #: PSTR296.27/M34

Topic: G.03. Motivation

Support: CNHS Summer Research Fellowship 2020 & 2024
NIDA Drug Supply Program

Title: Chemogenetic modulation of projections from the ventral pallidum to the nucleus accumbens affects sucrose consumption but not motivation to work for sucrose in female rats.

Authors: M. PEROUTKA[1], Z. N. WILSON[2], ***I. RIVERO-COVELO**[3];
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Abstract: Our goal was to disentangle how specific brain circuits contribute to food intake in a non-deprived status. Specifically, we assessed how chemogenetic activation or inactivation of the projections from the ventral pallidum (VP) to the shell of the nucleus accumbens (AcbSh) affected motivation to work for sucrose and consume a 20% sucrose solution in non-food deprived female rats.
Here, we used a dual vector approach with either excitatory or inhibitory designer receptors exclusively activated by designer drugs (DREADD) to target specific projections from the VP to the AcbSh. This specificity was achieved by injecting inhibitory or excitatory Cre dependent adeno-associated viruses (AAV) in the VP, and retrograde AAV Cre in the AcbSh. Intraperitoneal injections of clozapine-N-oxide (CNO) resulted in no changes in performance in a progressive ratio task where the rats pressed a lever for banana flavored pellets. In contrast,

chemogenetic inhibition of projections from the VP to the AcbSh resulted in a decrease in 20% sucrose consumption. Inversely, chemogenetic excitation resulted in an increase in sucrose consumption compared with vehicle injections.
Overall, our results indicated that the selective excitation or inhibition of projections from VP to the AcbSh did not have a significant effect on the motivation to work for food, suggesting that these projections might not mediate this behavior. Interestingly, excitation or inhibition of this pathway modulated sucrose consumption, implicating this circuit in the control of food intake under non-deprived conditions. Taken together, these results suggest that projections from the VP to the AcbSh modulate food consumption but not motivation to work for food.

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Poster

PSTR296: Motivated Behavior: Behavior, Circuits, and Pharmacology

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR296.28/M35

Topic: G.03. Motivation

Support: NIDA NRSA F31 DA054781

Title: Allopregnanolone regulation of phasic dopamine release and motivated behavior

Authors: *M. H. MCFARLAND[1], C. HUANG REN[2], L. MELTONLANE[2], A. MORROW[3], D. L. ROBINSON[4];
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Abstract: Neurosteroids are compounds that are synthesized de novo in the brain and influence neuronal activity. Allopregnanolone, a neurosteroid that is a potent, positive allosteric modulator of gamma-aminobutyric acid type A (GABA-A) receptors, has emerged as a drug with considerable potential in the treatment of mental and affective disorders, including substance use disorders, postpartum depression, and premenstrual dysphoric disorder. Moreover, allopregnanolone is considered to have a better safety profile than other drugs that target GABAA receptors, such as benzodiazepines. Prior research in our lab has shown that allopregnanolone dose- and sex-dependently reduces electrically-evoked dopamine release in the nucleus accumbens (NAc) in anesthetized male and female rats. However, it is possible that the dopamine measurements were impacted by anesthesia in addition to allopregnanolone. Thus, these experiments systematically tested the effects of allopregnanolone on various behavioral and electrochemical measures in awake rats. Given the allopregnanolone-induced reduction in dopamine release observed in our previous study, a key question that arose was whether

allopregnanolone is considered aversive to rats. Thus, we first performed conditioned place preference and found that 15 mg/kg allopregnanolone produced a robust place preference ($P<0.05$) in both males and females, indicating that allopregnanolone's subjective effects are not aversive but rewarding. Next, using fast-scan cyclic voltammetry, we extended our previous work by assessing the effect of allopregnanolone on spontaneous dopamine transients in awake rats. We found that 15 mg/kg allopregnanolone reduces phasic dopamine transient frequency ($P<0.05$) and amplitude (marginal) in the NAc of freely-moving female, but not male rats. Finally, we trained animals to a self-administer sucrose to assess whether allopregnanolone would alter motivation for a natural reward and cue-evoked dopamine release. We found that allopregnanolone (7.5, 15 mg/kg) did not significantly alter time to complete the session, inter-trial and inter-press intervals, rate of pressing, or trial latency in either sex ($P's>0.05$), suggesting allopregnanolone does not alter sucrose reward, motivation, or fast motor actions. Moreover, 15 mg/kg allopregnanolone did not alter cue-evoked dopamine transient amplitude ($P>0.05$). Together the results from this study clarify the regulation of dopamine neurotransmission and motivated behavior by allopregnanolone,
which has clinical implications for its use as an alternative therapeutic to benzodiazepines to treat various psychiatric disorders.

Disclosures: M.H. McFarland: None. **A. Morrow:** None. **D.L. Robinson:** None.

Poster

PSTR296: Motivated Behavior: Behavior, Circuits, and Pharmacology

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR296.29/M36

Topic: G.03. Motivation

Title: Characterizing dopamine transmission and reward-based behaviors in mice with low striatal dopamine D2 receptors.

Authors: *S. E. CERVENY[1], L. M. AMARANTE[2], E. MURRAY[3], R. BOCK[1], V. A. ALVAREZ[4];
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Abstract: Disruptions in dopamine (DA) signaling within the basal ganglia are implicated in multiple neuropsychiatric disorders, from substance use disorders to anxiety and depression. These disorders are characterized by alterations in risk-taking behaviors. Similary in rodents, genetic elimination of DA D2 receptors (D2Rs) located on striatal indirect-pathway projection medium spiny neurons (iMSNs) increases avoidance behaviors, while reductions in striatal D2Rs located on dopaminergic terminals correlate with increased novelty-seeking. The present study

aims to further characterize the effect of low striatal D2R expression on reward-based behaviors and dopamine transmission with the goal of understanding how striatal D2R expression impacts responses to risk. We used littermate male and female C57Bl6/J mice with D2R knockdowns on iMSNs, on dopaminergic terminals, on both iMSNs and dopaminergic terminals, and control animals. To assess risk-avoidance in these mice, we used the elevated zero maze (EZM) and light-dark box (LDB) tasks. To study reward seeking, reversal learning, and punishment resistance, mice completed operant responding tasks for sucrose and water during social housing conditions in Intellicages. We also conducted ex-vivo fast-scan cyclic voltammetry (FSCV) recordings to measure electrically evoked DA signals in the dorsomedial and dorsolateral striatum of these mice. Pharmacology was used to test the regulation by nicotinic acetylcholine receptors of the evoked dopamine signals between genotypes. Mice with reduced striatal D2R expression on their dopaminergic terminals moved significantly faster than mice with reduced iMSN D2R expression and mice with reduced D2R expression on both cell types ($p<0.05$). Mice with reduced iMSN D2R expression displayed a risk-aversion phenotype and spent less time in the LDB open zone ($p<0.05$). Intellicage data show that, regardless of D2R expression, mice can learn reversals of probabilistic contingencies and will pursue sucrose rewards paired with air puff punishments over unpunished water rewards. Ex-vivo FSCV data do not reveal significant differences in DA signals between groups in artificial cerebrospinal fluid nor after application of the nicotinic acetylcholine receptor antagonist Dihydro-β-erythroidine hydrobromide (DHBE). These results suggest that reduced striatal D2R expression yields phenotypes that vary significantly in exploration and avoidance behaviors. This should be considered in future research aiming to explore the neural underpinnings and the factors that impact behavioral responses to “risky” choices.

Disclosures: S.E. Cerveny: A. Employment/Salary (full or part-time):; NIMH. **L.M. Amarante:** A. Employment/Salary (full or part-time):; NIMH. **E. Murray:** None. **R. Bock:** A. Employment/Salary (full or part-time):; NIMH. **V.A. Alvarez:** A. Employment/Salary (full or part-time):; NIMH.

Poster

PSTR296: Motivated Behavior: Behavior, Circuits, and Pharmacology

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR296.30/M37

Topic: G.03. Motivation

Title: The function of the nucleus accumbens to ventral pallidum pathway in food and socially motivated behaviors

Authors: *S. AGRAWAL[1], J. CAVANAUGH[2], K. S. SMITH[1];
[1]Psychological and Brain Sci., [2]Dept. of Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: The function of the NA to VP pathway in food and socially motivated behaviorsUnderstanding the neural mechanisms underlying decision-making related to social interactions iscrucial for addressing deficits associated with neuropsychiatric disorders. This study focuses on apathway connecting the nucleus accumbens (NA) and ventral pallidum (VP). The hypothesis wasthat this connection is important for regulating instrumental learning to seek social behavior withinthe broader Social Decision-Making Network (SDMN), as well as in regulating the instrumentalmotivation to seek food in rats. Employing an automated operant assay, 50 Wild Type Long-Evansrats were trained to press a lever for food or a different lever for social interaction. This setupallowed for a precise examination of motivational differences and the impact of neural pathwaymanipulation. Initial experiments investigated sex differences across multiple motivational tests,including fixed-ratio, discrete choice, and progressive ratio sessions. There were no sexdifferences. Subjects consistently displayed a strong preference for food rewards over socialrewards or a choice to pursue no rewards. Quantitatively, the behavior directed to food rewardswere higher by approximately 50% compared to behavior to social rewards. Subsequent testsinvolved the application of DREADDs (Designer Receptors Exclusively Activated by DesignerDrugs) to inhibit the NA-to-VP pathway. Contrary to expectations, this targeted inhibition did notsignificantly alter our main measures of instrumental reward seeking; there appeared to be nodiscernible effect on the baseline preferences for either food or social rewards. Nevertheless, adeeper analysis revealed distinct behavioral subgroups of animals with different forms offood/social motivations, with implications for the NA-VP pathway in driving these motivations.This research aids our understanding of how specific neural pathways contribute to social andnonsocial instrumental reward seeking by highlighting a possible lack of critical NAc-VP role inthis decision process, but also highlights the potential for individual differences to be a key factorin resolving the neural underpinnings of both types of reward seeking.
Keywords: social decision-making network (SDMN), reward motivation, incentive value, designerreceptor exclusively activated by designer drugs (DREADDs), nucleus accumbens, ventralpallidum, social rewards

Disclosures: S. Agrawal: None. **J. Cavanaugh:** None. **K.S. Smith:** None.

Poster

PSTR297: Circuits and Neurotransmitters Regulating Social Behavior

Location: MCP Hall A

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Program #/Poster #: PSTR297.01/M38

Topic: G.04. Emotion

Support: NIH Grant 1R01DA055849-02
NASEM Ford Foundation Fellowship to EMR

Title: Reunited and it Feels so Good: VP GABA Neurons in Social Reward, Interaction, and Communication

Authors: *E. M. RAMIREZ[1], S. WALAWALKAR[2], R. ROKERYA[3], Y. XIE[1], S. V. MAHLER[1];
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Abstract: During COVID-19 lockdowns and the countless Zoom get-togethers that came with it, people across the world were desperate for social contact and yet the brain mechanisms underlying this basic human craving to be together are not well understood. The ventral pallidum (VP) is an intriguing target for this research due to its role in motivated responses to both food and drug rewards but do VP GABA neurons and circuits also participate in social reward, motivation, or communication?Here we investigate the role of GABAergic VP neurons in social reward in adult male and female GADiCre transgenic rats. We targeted inhibitory and excitatory DREADDs (hM4Di and hM3Dq) to VP GABA neurons, allowing bidirectional control of them during behavior. Wildtype and DREADD-expressing animals were socially isolated from their same-sex, littermate for one week before being undergoing assessments of 1) social conditioned place preference (CPP), 2) social play and investigation, and 3) social communication via ultrasonic vocalizations (USVs). First, rats were trained to pair one chamber with their former cagemate, and another with no rat. On test day, in the absence of other rats, preference for the rat-paired side was determined following stimulation (hM3Dq+CNO), inhibition (hM4Di+CNO) or no manipulation (CNO only) of VP GABA neurons. Next, rats remained socially isolated until again being reunited with their cagemate twice more, in 60 min social interaction tests, where investigation, play and USV emission was quantified following counterbalanced CNO and vehicle. Rats were again isolated for a week, then neural activity (c-Fos) resulting from after a final social reunion session, or a similar test without a social partner, was quantified. To determine specificity of VP GABA manipulation effects to USVs emitted during social communication, we also tested effects of VP GABA neuron inhibition on USVs elicited by systemic amphetamine administration (1mg/kg or 2 mg/kg), tested in both solitary, and social conditions.We found that chemogenetic manipulations of VP GABA neurons bidirectionally regulate social reward, social behavior, and USV production, with some sex-specific patterns. Results clearly show that VP plays an unappreciated role in social reward, interaction, and communication of rats, and may thus contribute to social as well as non-social aspects of reward-related psychiatric disorders like addiction.

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Poster

PSTR297: Circuits and Neurotransmitters Regulating Social Behavior

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Topic: G.04. Emotion

Support: NSF GRFP DGE-1839285
P50 DA044118

Title: Persistent effects of adolescent THC exposure on aversive motivational learning

Authors: *M. X. MARTINEZ, G. O. BRAVO, S. V. MAHLER;
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Abstract: Adolescent use of Δ^9-tetrahydrocannabinol (THC) is linked to later-life changes in cognition, memory, and susceptibility to drug use. Animal models suggest this could be a causal effect of THC influencing adolescent circuit development, perhaps especially in the prefrontal cortex and interconnected circuits like the amygdala. These regions are involved in cognition, stress, and addiction, and undergo developmental change during adolescence and thus may be sensitive to persistent disruption by THC. Here, we employed our well-characterized adolescent THC exposure model in rats to examine long-term effects on stress-related behaviors and brain activity, and their relationship to addiction-relevant opioid drug seeking. Specifically, we exposed female and male Long-Evans rats to THC (5mg/kg i.p.) or vehicle daily during adolescence, from postnatal day 30 to 43, followed by a washout period in which rats grew to adulthood (PD70+). They were then behaviorally tested for their responses to a localized aversive stimulus (a shock-delivering rod), and to a diffuse aversive stimulus (a foot shock) to assess defensive and avoidant behaviors. Conditioned responses to both the shock rod and the contextual and temporal cues predicting foot shock were also quantified. Subsequently, rats were also trained to self-administer the rewarding opioid drug remifentanil, and behavioral economic demand elasticity was quantified to reveal hedonic set point and motivation to pursue drug. We then asked how stress (footshock or the pharmacological stressor yohimbine) alters these opioid seeking parameters in rats with or without adolescent THC history. Results will lend insight into the impact of adolescent THC exposure on stress and addiction, with implications for potential long-lasting negative effects of teenage cannabis use in humans.

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Poster

PSTR297: Circuits and Neurotransmitters Regulating Social Behavior

Location: MCP Hall A

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Topic: G.04. Emotion

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NIH Grant P50 DA044118
NIH Grant R01 DA055849

Title: Does acute psychedelic "therapy" in rats persistently reverse stress-induced behavioral abnormalities?

Authors: *K. LAWSON[1], C. RUIZ[2], H. VU[3,1], A. DHAMI[4], M. T. BIRNIE[5], T. Z. BARAM[6], S. V. MAHLER[7];
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Abstract: Depression, PTSD, and addiction are common mental health disorders that affect millions of people worldwide. Risk of developing these disorders is increased in those with a history of stress during childhood, or trauma as an adult. To determine the causal mechanisms of adversity-induced brain changes with relevance to psychiatric disorders, we have established robust rat models of early-life adversity (ELA) and adulthood trauma that robustly alter reward seeking and emotional learning, allowing us to test novel treatment strategies. In the last few years, psychedelic drugs like LSD, psilocybin, and DMT have re-emerged in psychiatry, with apparent potential for treating depression, addiction, and other psychiatric disorders. These promising initial clinical findings inspired us to develop a new rat model of “psychedelic therapy”, allowing mechanistic investigations into their therapeutic actions in the brain. Our preliminary data provides evidence that a single dose of the psychedelic serotonin 2A (5-HT2A) receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) persistently reverses ELA-induced anhedonia in male rats, and also trauma-induced fear hypersensitivity in both sexes. We find evidence for both “set and setting” influencing the therapeutic effects of the psychedelic, and use brain-wide c-Fos expression analyses in cleared rat brains to determine the brain circuits involved. Results advance the modeling of psychedelic therapy in rodents, identify novel neural substrates of psychedelic drug effects, and thus provide new insights that could be capitalized upon when developing maximally effective, but minimally disruptive new therapeutic strategies in humans.

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Poster

PSTR297: Circuits and Neurotransmitters Regulating Social Behavior

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Topic: G.04. Emotion

Support: CONACHYT CF-2023-G-206
UNAM-DGAPA IN204824, IN203518
CONACHYT scholarship 958647

Title: Novel social approach model for assessing the display of prosocial behavior in prairie voles

Authors: *M. AQUINO[1], J. VELAZQUEZ-MOCTEZUMA[2], R. G. PAREDES[3], F. CAMACHO[4], R. MERCADILLO[5], W. PORTILLO[6];
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Abstract: Prosocial behaviors, including altruism, empathic and sympathetic processes, have been observed in non-human primates and some rodents. These behaviors, which involve actions aimed at helping another conspecific without expecting external compensation, are crucial for understanding the neurobiological bases of neuropsychiatric disorders. While prosocial studies have been conducted in rats or mice, these animals exhibit limited social behaviors. In contrast, prairie voles, which form lifelong bonds showing a social preference for each other over a possible new mating partner.The present work aims to implement the social approximation test to evaluate the social approach behavior that is generated in prairie voles when they recognize a nociceptive state. **Methodology:** male and female voles (n=20 for each sex) were used as experimental animals E, and 25 males and females as stimulus S. The social approximation test was performed in a three-compartment arena connected with a central area. A stimuli vole inside an acrylic box with small holes was placed in each compartment and the experimental vole was placed in the central area. The time that the experimental vole spent in each compartment was recorded. Male and female voles were tested under three conditions: Sexually naïve experimental voles were exposed to one vole that was previously administered with 1% formalin on its hind leg to induce pain and two voles without nociception. Subsequently, experimental voles cohabitated for 24h with an opposite-sex stimulus animal to pair bond and a partner preference test was performed, in this condition the experimental animals chose between their sexual partner, an animal that was administered formalin and a stimulus prairie vole without nociception. **Results:** Sexually naïve male and female voles spend more time in the compartment where a conspecific is experiencing nociception, which means that higher prosocial behaviors are being displayed. Pair-bonded voles spend more time with their sexual partners independently if their partners experience nociception. **Conclusion:** Sexually naïve prairie voles can interpret the signals that a foreign prairie vole is in nociception. When voles establish a pair bond, they spend more time with their sexual partner than with a foreign conspecific in pain. Social approximation tests allow us to evaluate prosocial behaviors in prairie voles.

Disclosures: M. Aquino: None. **R.G. Paredes:** None. **F. Camacho:** None. **R. Mercadillo:** None. **W. Portillo:** None.

Poster

PSTR297: Circuits and Neurotransmitters Regulating Social Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR297.05/N2

Topic: G.04. Emotion

Support: SNF 310030E-173565
SNF CRSK-3_190779
Synapsis Foundation No. 2020-PI02

Title: Parvocellular starter oxytocin neurons synchronize bursting activity of magnocellular oxytocin neurons in hypothalamic nuclei of lactating rats

Authors: *R. STOOP[1], Y. TANG[2], R. NIU[2], E. VAN DEN BURG[2], Q. KRABICHLER[3], V. GRINEVICH[3];
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Abstract: Lactation is a fundamental instinct in mammals for raising offspring that is initiated and maintained by the hypothalamic neuropeptide oxytocin (OT) released by magnocellular neurons in the paraventricular and supraoptic nuclei (PVN and SON). These cells exhibit unique synchronous pulsatile bursting activity, triggering the release of OT into the bloodstream to induce milk let-down from the mammary glands. However, the mechanism that triggers this their activation remains largely unknown. **Methods:** Building on our previous findings that parvocellular OT neurons gate somatosensory and algesic inputs onto magnocellular OT neurons in virgin rats, we employed newly developed viral vectors for opto- and chemo-genetic activation and silencing, combined with in vivo multi-unit opto-electrode recordings, in lactating rats, to explore the role of parvocellular OT neurons during lactation. **Results:** Pup suckling triggered bursting of magnocellular OT neurons that was highly (sub-millisecond) synchronous across the PVN and SON, as revealed by dual simultaneous recordings. Chemo-genetic inhibition of parvocellular OT cell activity abolished magnocellular bursting activity induced by pup suckling, whereas optogenetic activation of opto-tagged parvocellular cells induced bursting that was less synchronized than that induced by pup suckling. Finally, we show that the synchronized bursting activity of magnocellular OT cells is followed by massive OT release within the PVN, as revealed by a newly developed oxytocin sensor. **Conclusion:** Our work demonstrates that a small number of starter cells can induce large scale neuronal activity, even across brain nuclei, that underlies physiology and behavior. This novel principle of neuronal regulation could shed light on the distinct roles that oxytocin may play in feeding, social

behavior and emotion, and could provide further insights into how behaviors are directed by the brain.

Disclosures: R. Stoop: None. **Y. tang:** None. **R. Niu:** None. **E. Van den Burg:** None. **Q. Krabichler:** None. **V. Grinevich:** None.

Poster

PSTR297: Circuits and Neurotransmitters Regulating Social Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR297.06/N3

Topic: G.05. Mood Disorders

Support: NEDO STS 2020
Tsubota Lab

Title: The effect of violet light treatment on social behavior

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Abstract: External light stimuli can act as both visual and non-visual signals through the photoreceptor opsin. In mammals, opsin-5, also known as OPN5 or neuropsin, is expressed in the retinal ganglion cells (RGCs) and hypothalamus; it is activated by violet light (VL) with a wavelength of 360-400 nm, and has been linked to regulation of the circadian cycle, thermogenesis, and myopia. However, the specific functions of VL and OPN5 in the brain remain to be fully elucidated. In the current study, we confirmed that VL, as an external stimulus, plays a role in mood regulation. Through the social defeat stress model, our research suggests that VL improves depression-like behaviors in an OPN5-dependent manner and increases neural activity as well as oligodendrogenesis in the prefrontal cortex and nucleus accumbens. The signal received by OPN5-positive RGCs is transmitted to the habenular region of the brain. Our findings suggest that VL plays a role in the maintenance of neural networks through the induction of oligodendrogenesis. In conclusion, VL may have a therapeutic role in clinical depression, although further studies are required to confirm our preliminary findings.

Disclosures: M. Hayano: A. Employment/Salary (full or part-time):; Tsubota Lab. 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.; Tsubota Lab. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Tsubota Lab. **Y. Noda:** None. **K. Tsubota:** A. Employment/Salary

(full or part-time):; Tsubota Lab. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Tsubota Lab.

Poster

PSTR297: Circuits and Neurotransmitters Regulating Social Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR297.07/N4

Topic: G.05. Mood Disorders

Support: F31MH133309
R00DA045795
R01DA058958

Title: A Novel Role for Transcriptional Regulators of Transposable Elements in Social Behaviors

Authors: *N. L. TRUBY[1], R. K. KIM[2], G. SILVA[1], J. PICONE[3], R. L. NEVE[4], P. J. HAMILTON[5];
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Abstract: Social behaviors are central to the health of society and the individual and are disrupted in a number of psychiatric illnesses. However, the neurobiological origins of complex social behaviors are incompletely understood. The Zfp189 gene product is a KRAB zinc finger transcription factor whose expression and function in the rodent prefrontal cortex (PFC) was previously determined to be protective against stress-induced social deficits. To interrogate the function and gene targets of ZFP189, we reprogrammed the endogenous ZFP189WT by replacing the repressive KRAB domain with an enhanced transcriptional activation domain (VP64-p65-Rta (ZFP189VPR)) or by removing the functional moiety entirely (ZFP189NFD). Upon packaging these ZFP189 variant constructs in viral vectors and delivering to mouse PFC, we interrogated the transcriptional and behavioral adaptations mediated by these synthetic ZFP189 transcription factors. We observed that dysregulation of ZFP189-mediated transcription in this brain area, achieved by delivery of synthetic ZFP189VPR, precipitates social behavioral deficits in terms of social interaction, motivation, and the cognition necessary for the maintenance of social hierarchy, without other observable behavioral deficits. By performing RNA sequencing in virally manipulated prefrontal cortex tissues, we discovered that ZFP189 transcription factors of opposing regulatory function have opposite influence on the expression of genetic transposable elements as well as genes that participate in immune functions. Collectively, this work indicates that ZFP189 function in the prefrontal cortex coordinates

transcriptional neuroadaptations necessary for social behaviors by directly binding transposable element-rich regions of DNA to regulate immune-related genes. Given the evidence for a co-evolution of social behavior and the brain immune response, we posit that ZFP189 may have evolved to augment brain transposon-associated immune function as a way of enhancing an animal's capacity for functioning in social groups.

Disclosures: N.L. Truby: None. **R.K. Kim:** None. **G. Silva:** None. **J. Picone:** None. **R.L. Neve:** None. **P.J. Hamilton:** None.

Poster

PSTR297: Circuits and Neurotransmitters Regulating Social Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR297.08/N5

Topic: G.04. Emotion

Title: The Response of the Gut Microbiome to the Social Transfer of Pain in Mice

Authors: *P. REZAIE BOROON[1], M. DOTHARD[2], K. CONWAY[2], S. C. TUY[3], J. ZHANG[4], J. GILBERT[2], M. L. SMITH[5];
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Abstract: Gut microbiota can induce host production of metabolites and neurotransmitters that mediate gut-brain signaling and neuronal pathways that physically link the gut and the brain, so-called the "gut-brain axis." A growing body of evidence indicates that alterations in the gut microbiome can impact mood, pain and socio-emotional functioning. However, the extent to which the gut-brain axis might contribute to social communication or complex social behaviors like empathy remains largely unexplored. Empathy is the ability to recognize and respond to another's sensory or emotional state and is an essential social behavior in humans and animals. We recently developed the "social transfer of pain" model of empathy-like behavior in the mouse where 'bystander' mice demonstrate pain and negative affective states after a social interaction with a partner experiencing inflammatory pain (due to injection of Complete Freund's Adjuvant, CFA). In this study, we report robust microbial community functional and compositional changes in bystander mice comparable to CFA mice following the social transfer of pain. Specifically, both groups showed an increase in short chain fatty (SCFA) acid-producing microbes as well as an increase in SCFA levels, which regulate the immune system and inflammatory responses. Moreover, ~70 genera were found to be significantly differentially abundant in bystanders but not CFA-injected mice, (e.g. Eubacteria, Ruminococcus, Clostridium, and Butyrovibrio genera) and bystander and CFA between-group microbial diversity (i.e. beta diversity) was significantly altered over time compared to control mice. Future work aims to

determine whether the microbiome modulates the social transfer of pain via microbial immunomodulatory mechanisms, as well as if antibiotic depletion impacts pain and empathy-like behaviors. These novel findings suggest a putative role for the gut-brain axis in modulating the social transfer of pain. Investigating how the gut might influence such a complex social phenomenon could lead to discoveries that impact everything from mental health treatments to enhancing social bonding and empathy in various populations.

Disclosures: P. Rezaie Boroon: None. **M. Dothard:** None. **K. Conway:** None. **S.C. Tuy:** None. **J. Zhang:** None. **J. Gilbert:** None. **M.L. Smith:** None.

Poster

PSTR297: Circuits and Neurotransmitters Regulating Social Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR297.09/N6

Topic: G.04. Emotion

Title: Oxytocin Modulation of the Social Transfer of Pain

Authors: *J. ZHANG[1], M. L. SMITH[2];
[1]UCSD, La Jolla, CA; [2]Psychological Sci., UCSD Dept. of Neurosciences, La Jolla, CA

Abstract: Empathy is the ability to understand the emotional and sensory states of others and is crucial for the well-being and survival of social animals. We established the “social transfer of pain” paradigm in mice, where a ‘bystander’ (BY) mouse acquires the sensory and emotional state of a social partner in pain. Following a 1-hr interaction with a familiar social partner experiencing inflammatory pain (Pain; induced by an injection of complete Freund’s adjuvant; CFA), BY mice demonstrate hypersensitivity and negative affective behaviors comparable to Pain mice. Acquisition of pain in BY requires the anterior cingulate cortex (ACC). However it is unknown which inputs into the ACC are necessary for these empathy-like behaviors or which subpopulations of cells within this circuit are involved in producing pain and affective changes. The ACC receives inputs from diverse brain regions, including the paraventricular nucleus of the hypothalamus (PVN) which is the primary source of oxytocin (OT) in the brain. OT is critical for human empathy, and both the neuropeptide and its receptor (OTr) are integral to mouse models of empathy-like behaviors, including observational fear and consolation. In these studies, we first genetically labeled activated neurons by utilizing TRAP2 (FosCreERT2) mice crossed with the Ai14-TdTomato reporter line. We observed enriched OT and Fos double-positive neurons in Pain and BY mice compared to controls following 4-hydroxytamoxifen (4-OHT) administration. Moreover, acute intranasal oxytocin (20 mg/kg) enhances and prolongs pain in BY following only a 10 min social interaction, which is not long enough for saline-treated BY to acquire the social transfer of pain. Retrograde tracing from ACC does not reveal any labeling in the PVN, suggesting a potential transsynaptic connection through the thalamus. We are currently

determining whether inhibiting OT release using chemogenetic or pharmacological approaches is sufficient to prevent the social transfer of pain. Our studies will be foundational for the understanding of neural mechanisms underlying empathy and explore a role for oxytocin as a potential therapeutic target for empathy deficits in many psychiatric disorders.

Disclosures: J. Zhang: None. **M.L. Smith:** None.

Poster

PSTR297: Circuits and Neurotransmitters Regulating Social Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR297.10/N7

Topic: G.05. Mood Disorders

Support: UNAM-PAPIIT-IA202724

Title: Potentiated vasopressin ascending system in the C58J mice: is there a relationship between vasopressin system abnormality and autistic phenotype?

Authors: *O. HERNANDEZ PEREZ[1], E. CALLADO[2];
[1]Fac. of Med., UNAM, México city, Mexico; [2]Physiol., Fac. of Med., Univ. Nacional Autonoma de Mexico, CDMX, Mexico

Abstract: The neuropeptide vasopressin influences different aspects of behavior, like emotions, anxiety, depression, memory, etc. Previous reports have suggested the participation of the vasopressinergic system in social behavior through the innervation of various nuclei of the brain, signaling through the V1a and V1b receptors. One of the mental disorders that sociability is affected is ASD, and some years ago, it was described as the participation of the vasopressin system in this disorder. The C58/J inbred mouse strain has a behavioral profile that reflects the core symptoms of autism, including deficits in sociability, impaired communication, and overt motor stereotypies. However, little is known about any anomaly in neuropeptide circuitry in these animals. To evaluate vasopressin's anatomic influence on ASD, we study the C58J strain, an animal model of ASD.
Using immunohistochemistry to detect AVP, we found potentiated peptide and atypical innervation patterns in different nuclei, including the nucleus basalis of Meynert (NBM), the bed nucleus of stria terminals, and the medial amygdala. We used i.p. administration of a hypertonic saline solution (900 mM) and micro-positron emission tomography (micro-PET) technique to assess the functional consequence of this observed AVP system's potentiation. We found an increase in standardized uptake value (SUV) in the thalamus, hypothalamus, hippocampus, brain stem, central gray, superior colliculi, inferior colliculi, and midbrain after salt loading during which the hypothalamic AVP system is potently upregulated. Finally, we observed changes in the morphology and number of dendritic spines in the C58J strain compared to C57BL.

Disclosures: O. Hernandez Perez: None. **E. Callado:** None.

Poster

PSTR298: Chronic Stress and Anxiety: Genetics to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR298.01/N8

Topic: G.04. Emotion

Support: UNAM-PAPIIT-IA202724

Title: Evaluation of anxiety behavior in mice C58/J using hole board test.

Authors: *E. LUNA CASTAÑÓN[1], O. HERNANDEZ PEREZ[2];
[1]Facultad de Medicina UNAM, CDMX, Mexico; [2]Fisiology, Fac. of Med., UNAM, México city, Mexico

Abstract: Evaluation of anxiety behavior in mice C58/J compared to C57/BL using hole board test.Luna-Castañón E. Oscar R. Hernandez-PerezAutism spectrum disorder (ASD) is defined as a diminution of a person's socializationabilities. ASD has been recognized with three parameters: aberrant socioemotionalbehavior, diminution of nonverbal communication, and interpersonal relationship deficiency.The C58/J mice have been identified as a model of autism spectrum disorder (ASD) becauseof their physiological and contextual characteristics without being genetically modified. Thehole–board test is a behavioral test used to evaluate different aspects of cognitive abilitiesand emotions in rodents. It has recently been used to assess behavioral characteristics thatare supposed to reflect an animal model of ASD. We used the hole board test to evaluateanxiety levels *in mice C58/J* compared to C57/BL as a control for both males and females.We measured main behavior parameters: latency to the first nose-poke, the number of nose-pokes, the time spent in nose-pokes, the time in the periphery of the box, the time in thecenter of the box, and locomotion measures (traveler distance and speed). We foundsignificant differences in females mice in number (C57/BL: 35.83±3.85 vs. C58/J:18±6.94,P>0.05) and time of nose-poke (487.2 ± 22.99 vs. 534±17.96, P>0.01). To compare malesvs. females in the two strains (C57/BL vs. C58/J) using two-way ANOVA (sex and strainfactors), we found that only the sex factor showed a significant difference in nose-pokelatency (C57/BL: 9.72 vs. 31.31; C58/J: 7.85 vs. 28.61, respectively; P>0.01). No significantdifferences were found in male mice C58/J compared to C57/BL using t-test in anyparameter evaluated; however, a tendency to decrease the number and time of nose-pokeswas observed. The observed changes allow us to infer a decrease in exploratory behaviorsin the hole board that can be attributed to the strain of mice and more clearly detected infemales. Anxiety behaviors are not affected in C58/J mice, which could indicate that higher-intensity stimuli are necessary to trigger significant levels of anxiety.

Disclosures: E. Luna Castañón: None. **O. Hernandez Perez:** None.

Poster

PSTR298: Chronic Stress and Anxiety: Genetics to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR298.02/N9

Topic: G.04. Emotion

Title: Unraveling the Role of Rtl1 in Anxiety

Authors: *P.-Y. CHEN[1], H.-S. HUANG[2];
[1]Grad. Inst. Brain and Mind Sci., Natl. Taiwan Univ., Taipei, Taiwan; [2]Grad. Inst. of Brain and Mind Sci., Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Anxiety disorders have garnered increasing attention due to their prevalence and impact on mental health. Understanding the multifaceted etiology of anxiety disorders is crucial for effective intervention. Recent research has delved into the realm of epigenetics, uncovering the influence of imprinting genes on the brain. Surprisingly, we have unveiled a significant association between retrotransposon-like 1 (*Rtl1*) and anxiety disorders. Notably, *Rtl1* exhibits exclusive expression in the placenta, brain, and adrenal gland of adult mice, with particularly high levels observed in Tyrosine Hydroxylase (TH) positive cells of locus coeruleus (LC).The locus coeruleus (LC), widely throughout the brain and involved in various regulatory mechanisms, has long been associated with stress-induced anxiety. Building upon these findings, our study uncovered that LC neurons from paternal *Rtl1* knockout mice displayed delayed onset times of action potentials and inward currents, coupled with reduced neuronal excitability. Such results highlight the significance of imprinted genes in the brain's response to stress, particularly in the LC.To investigate specific pathway of LC engrams output, involve *Rtl1* causal related anxiety phenotype. We employed chemogenetic techniques in our studies. Moreover, by selectively manipulating *Rtl1* expression in LC with CRISPR, our study aims to delineate the contribution of *Rtl1* to anxiety pathogenesis.We seek to unravel the intricate mechanisms through which imprinting genes influence the brain's response. This research represents a significant step towards comprehending the molecular underpinnings of anxiety disorders and may pave the way for the development of targeted therapeutic interventions.

Disclosures: P. Chen: None. **H. Huang:** None.

Poster

PSTR298: Chronic Stress and Anxiety: Genetics to Behavior

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Support: NSERC Discovery Grant RGPIN-2017-06272
NSERC Canadian Graduate Scholarship – Doctoral program
NSERC Canadian Graduate Scholarship – Master's program

Title: Deletion of beta-klotho in the mouse ventral subiculum is anxiolytic

Authors: *B. S. BONO, M. J. CHEE;
Neurosci., Carleton Univ., Ottawa, ON, Canada

Abstract: Anxiety is an emotionally adaptive response to perceived threats and is a major contributor to the experience of stress. Beta-klotho (KLB) is an obligate co-receptor required for the central actions of fibroblast growth factor (FGF) 19 and FGF21 that mount a stress response via the paraventricular hypothalamus (PVH). However, it is not known if *Klb* expression regulates anxiety-related stress responses, and as *Klb* hybridization is sparse within the PVH, we hypothesized that *Klb* regulates the stress axis upstream to the PVH. One candidate region is the ventral subiculum (SUBv), which regulates the stress axis biphasically. We performed *in situ* hybridization to map the spatial distribution and abundance (low, medium, high) of *Klb* mRNA in the SUBv of male and female wildtype brain tissue. The SUBv of females had considerably more *Klb* cells but were mostly within the anterior SUBv. Meanwhile, *Klb* hybridization in the male SUBv was evenly spread and peaked posteriorly. The abundance of *Klb* hybridization in the SUBv suggested that it may be functionally relevant, thus to determine if SUBv *Klb* regulated anxiety responses, we delivered an adeno-associated virus encoding Cre recombinase-EGFP (or EGFP in control littermates) into the SUBv of male and female *Klb-flox/flox* mice to delete *Klb* expression. After four weeks for *Klb* deletion, we assessed anxiety-like and memory-related behaviours. First, the loss of SUBv *Klb* did not alter exploratory behaviour in an open field arena, but when presented with a palatable food reward, all SUBv *Klb* deleted mice spent more time in the center of the open field. Interestingly, males and females differed in their approach or avoidance behaviours when presented with a food reward, as female mice following the loss of SUBv *Klb* made less entries into the center. Furthermore, SUBv *Klb* deletion also increased time and entry into the open arms of an elevated plus maze and support the anxiolytic effect of SUBv *Klb* deletion. Finally, we performed the object location task to confirm that anxiety-related behaviors were uniquely impacted by *Klb* deletion in the SUBv. Importantly, there were no differences in spatial memory, as all mice spent more time interacting with the moved object during testing. However, during training mice with SUBv *Klb* deletion were less likely to interact with the foreign objects. Collectively, these results indicated that SUBv *Klb* promoted anxiogenesis, but the differential abundance of *Klb* between sexes was not related to the expression of anxiety-related behaviors.

Disclosures: B.S. Bono: None. **M.J. Chee:** None.

Poster

PSTR298: Chronic Stress and Anxiety: Genetics to Behavior

Location: MCP Hall A

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Program #/Poster #: PSTR298.04/N11

Topic: G.04. Emotion

Title: Serotonergic inputs to the ventral hippocampus underlie sex differences in anxiety

Authors: *L. PIZZOCCARO[1], A.-S. SIMARD[2], S. VAN DER VELDT[3], G. DUCHARME[5], B. AMILHON[4];
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Abstract: Anxiety disorders are among the most common psychiatric conditions worldwide, with women being almost twice more likely than men to be diagnosed with an anxiety disorder throughouttheir lifetime. Serotonergic (5-HT) neurons from the raphe nuclei are heavily involved in the regulation of mood and anxiety, yet the neural substrates underlying sex-related differences in anxiety remain largely unknown. The ventral hippocampus (vHP) acts as a major modulator of anxiety and receives dense 5-HT inputs. We hypothesize that serotonin release in the vHP is crucial in controlling anxiety, and that this process is influenced by sex. All experiments were carried out on male and female SERT-Cre mice, which expresses the Cre recombinase in 5-HT neurons. A retrograde viral vector carrying the fluorescent protein eYFP was injected into the vHP, allowing us to target exclusively the raphe-vHP serotonergic neurons. vHP-projecting raphe neurons were located in various raphe sub-regions, including the median raphe (MnR) and B9. Pathway specific optogenetic activation of 5-HT neurons projecting to the vHP was achieved using the excitatory opsin ChETA. Activating vHP-projecting 5-HT neurons robustly increased anxiety levels in a sex-dependent manner, with female mice showing increased anxiety in a battery of previously validated anxiety tests. Local field potential recordings in the ventral HP showed that this sex-dependent modulation of anxiety is accompanied by female-specific alterations of vHP oscillations, including theta rhythm. To investigate the cellular mechanism underlying the sex-differences in anxiety behavior, we performed whole-cell patch-clamp to record, ex vivo, the electrical properties of vHP-projecting 5-HT neurons. Strikingly, 5-HT neurons from female mice have a more depolarized resting membrane potential compared to male mice. Moreover, MnR-vHP serotonergic neurons from female mice show a significantly higher firing rate compared to male mice, with female, but not male, mice also showing spontaneous firing. Together, these results provide a novel mechanistic insight into a previously under-investigated sexual dimorphism in the raphe-ventral hippocampus serotonergic pathway, thereby paving the way for new therapeutic avenues for the treatment of anxiety disorders in females.

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Poster

PSTR298: Chronic Stress and Anxiety: Genetics to Behavior

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Topic: G.05. Mood Disorders

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Office of Naval Research (ONR) N00014-19-1-2149
The Hope for Depression Research Foundation
Pritzker Neuropsychiatric Research Consortium

Title: A neural signature of temperament: unique and shared features in the hippocampus and nucleus accumbens of selectively bred rats that differ in exploration, anxiety, and addiction

Authors: *E. K. HEBDA-BAUER[1], M. H. HAGENAUER[1], H. KHALIL[1], S. J. WATSON, Jr[1], A. A. PALMER[2], J. LI[1], H. AKIL[1];
[1]Univ. of Michigan, Ann Arbor, MI; [2]UCSD, La Jolla, CA

Abstract: Selective breeding of rats for high and low propensity to explore a novel environment has produced two rat lines with highly divergent behaviors: bred Low Responders (bLR) are extremely inhibited and anxious in a novel environment, whereas bred High Responders (bHR) are exploratory and sensation-seeking. These traits map onto temperament extremes predictive of externalizing and internalizing psychiatric disorders and model two paths to drug use. Examining gene regulation in hippocampus (HPC) and nucleus accumbens (NAcc)--two brain areas involved in temperament, stress reactivity, and reward processing--in the bHR/bLR model will help elucidate molecular correlates of individual differences in vulnerability to psychiatric and addictive behaviors. We performed RNAseq/ATACseq in NAcc (N=40) and RNAseq in HPC (N=24) from two generations of adult male and female bHR and bLR animals. Selective breeding produced a robust molecular phenotype, showing greater differential gene expression associated with bHR/bLR lineage than with sex. This differential gene expression is more pronounced in NAcc (N=1,820 genes, FDR<0.05) obtained from a later generation than HPC (N=144 genes, FDR<0.05). Gene set enrichment analysis revealed both unique and common gene sets between brain areas when comparing bHRs to bLRs. In bHRs, neurons are enriched in HPC, while oligodendrocytes are enriched in NAcc; growth/proliferation pathways are upregulated in HPC, while phospholipid metabolic processes are upregulated in NAcc. In bLRs, microglia-related pathways are enriched in both brain areas, plus energy regulation, mitochondria, and immune-related pathways. In contrast, metabolism pathways are upregulated in HPC, while secretory pathways are upregulated in NAcc of bLRs. Regions of differentially

accessible chromatin between bHRs and bLRs are being identified in NAcc. Notably, increased gene expression of synuclein gamma (Sncg) in bLRs vs bHRs (FDR=0.017) is coupled with increased chromatin accessibility ($p<0.006$) in NAcc. The correlation between Sncg gene expression and the ATAC-Seq peak height ($R^2=0.61$, $p=4.688e-09$) reveals a dichotomy among the bLRs such that half show similar gene regulation to that of the bHRs while the other half show a higher gene expression and chromatin accessibility. Implications of this bimodal distribution of Sncg among the bLRs will be explored further. In addition to gene regulation differences in the NAcc, our findings elucidate common and unique gene expression patterns in two brain areas involved in shaping temperament differences contributing to externalizing and internalizing behaviors inherent in psychiatric disorders.

Disclosures: E.K. Hebda-Bauer: None. **M.H. Hagenauer:** None. **H. Khalil:** None. **S.J. Watson:** None. **A.A. Palmer:** None. **J. Li:** None. **H. Akil:** None.

Poster

PSTR298: Chronic Stress and Anxiety: Genetics to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR298.06/N13

Topic: G.05. Mood Disorders

Support: Emmanuel College

Title: Stress-susceptible Ank3+/- mice exhibit lower glucocorticoid receptor protein expression in hippocampus and frontal cortex

Authors: *Z. PALERMO, I. CRAUS, A. DRILLEN, A. GEMOS, M. P. LEUSSIS; Psychology & Neurosci., Emmanuel Col., Boston, MA

Abstract: Ankyrin 3 (*ANK3*) is a gene that has been linked to increased risk of multiple psychiatric diseases including bipolar disorder, schizophrenia, and post traumatic stress disorder. These and other psychiatric disorders are often considered stress-related disorders. Research in multiple mouse models with reduced *Ank3* expression has shown increased stress susceptibility yet the mechanisms underlying the observed changes in stress regulation have not been examined. Glucocorticoid receptors (GRs), particularly in the hippocampus are known to moderate the stress response. Thus, the goal of these experiments was to compare GR levels in adult male wild-type (*Ank3 +/+*) and heterozygous mice with reduced *ANK3* exon 1b expression (*Ank3 +/-)*. Western blots were conducted to assess levels of GR expression in the hippocampus and frontal cortex, with normalization to total protein levels. In the hippocampus, *Ank3 +/-* had 45 percent lower GR expression compared to WT mice ($p<0.05$). Similarly, in the frontal cortex, *Ank3 +/-* had 30 percent lower glucocorticoid receptor expression compared to WT mice ($p<0.01$). Prior to western blotting, we also tested behavior to confirm the mice expressed the

known phenotype. Behaviorally, adult male *ANK3*+/- displayed more impulsivity and less anxiety-like behavior compared to wild type in behavioral tests including the elevated plus maze and novelty suppressed feeding, as previously reported. Due to its involvement in modulating glucocorticoid signaling and the stress response, FK506 Binding Protein 51 (FKBP5) protein levels will also be assessed. Overall, decreased GR expression levels, especially in the hippocampus, could represent one mechanism by which mice with reduced Ank3 expression are more susceptible to stress.

Disclosures: Z. Palermo: None. **I. Craus:** None. **A. Drillen:** None. **A. Gemos:** None. **M.P. Leussis:** None.

Poster

PSTR298: Chronic Stress and Anxiety: Genetics to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR298.07/Web Only

Topic: G.05. Mood Disorders

Support: Universidad Iberoamericana. Ciudad de México
Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT)

Title: Prolonged periods of maternal separation do not induce high voluntary ethanol intake and cognitive impairment in Wistar rats.

Authors: *S. MU?OZ SANCHEZ[1], L. RODRIGUEZ SERRANO[2], O. R. GALICIA, Sr.[3], M. H. BUENROSTRO-JAUREGUI[4];
[1]Univ. Iberoamericana. Ciudad de México, Mexico, Mexico; [2]Psicología., Univ. Anáhuac., Ciudad de mexico, Mexico; [3]Univ. Iberoamericana, Lomas de Santa Fe, Mexico; [4]Dept. of Psychology, Univ. Iberoamericana, Mexico City, Mexico

Abstract: Stress models during early developmental stages in animal studies are widely utilized across various neuroscience fields. Maternal separation (MS) in rodents serves as an early stress animal model, leading to neurochemical and behavioral alterations in offspring persisting into adulthood. We employed an MS model postnatally in male Wistar strain rats, comparing two temporary MS procedures: short-term (postnatal days 2 to 15, 180 minutes daily from 11:00 to 14:00) and long-term (postnatal days 2 to 21, 180 minutes daily from 11:00 to 14:00). At postnatal day 50, the alcohol test procedure commenced. During the habituation phase four days prior to the experiment, the water bottle (500 ml) was replaced in each cage with two smaller water bottles (100 ml each). Rats were then exposed to gradually increasing concentrations of alcohol solution, ranging from 2% to 8% v/v, over four consecutive days each, with free access provided. The positioning of water bottles and alcohol within the cage was daily alternated to

prevent place preference, and their volumes were weighed every 24 hours. Fresh alcohol concentrations were prepared daily by mixing the corresponding ethanol solution with tap water. Individual alcohol consumption was calculated in grams per kilogram, and the alcohol preference ratio was determined as alcohol intake versus water intake. Our findings revealed that animals exposed to three weeks of MS exhibited lower alcohol preference compared to those subjected to two weeks of MS. MS did not induce significant learning impairments in either the inhibitory avoidance task or the novel object recognition test (NORT), consistent with existing literature indicating changes related to separation duration. Despite the sparse conclusive data on the neurobiological consequences of MS, one of the future challenges lies in identifying and characterizing underlying neurobiological mechanisms, particularly at the individual animal level.

Disclosures: S. Mu?oz Sanchez: None. **L. Rodriguez Serrano:** None. **O.R. Galicia:** None. **M.H. Buenrostro-jauregui:** None.

Poster

PSTR298: Chronic Stress and Anxiety: Genetics to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR298.08/N14

Topic: G.05. Mood Disorders

Support: R01 DA048208

Title: Effects of Chronic Stress and Prefrontal Cortical REDD1 Overexpression on Attentional Set Shifting Behavior in Mice

Authors: *B. KURTOGLU[1], M. C. HEARING[2], J. R. MANTSCH[3];
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Abstract: Cognitive flexibility, the ability to adapt behaviors in response to changes in the environment is essential for everyday life, with deficits commonly observed in neuropsychiatric disease states and reducing resilience to negative life events. Deficits in cognitive flexibility are commonly observed in neuropsychiatric disease states including addiction and depression and are classified in the Research Domain Criteria matrix under the cognitive systems domain. The rodent prelimbic cortex (PrLC) plays a critical role in processing information necessary for optimal cognitive flexibility and is known to undergo structural and functional changes following prolonged stress exposure -- thus PrLC dysfunction represents a likely substrate for stress-induced deficits in cognitive control. We have recently shown that chronic unpredictable stress (CUS) produces an enduring dysfunction in PrLC physiology and impaired cognitive flexibility

using an operant-based attentional set shifting in male but not female mice. To gain more insight into this, our studies chose to focus on the protein REDD1 as it is increased in post-mortem dorsolateral prefrontal cortex tissue from individuals diagnosed with depression. In line with these findings, we find that there is an increase in REDD1 expression and a decrease in Raptor phosphorylation, one of the key elements of the mTORC1 complex, in the PrLC after CUS, suggesting disrupted mTORC1 function. To determine if REDD1 overexpression is sufficient to produce deficits in attentional set shifting, we used a viral vector to overexpress REDD1 in the PrLC of male mice. Relative to control mice, REDD1 mice required more trials to pass the extradimensional shift testing criterion equivalent to that produced by CUS. Notably, REDD1 overexpression did not impact acquisition of lever training, performance during a visual cue-based discriminative learning task, or measures of motivation for non-drug reward. Furthermore, we also examined the effects of REDD1 overexpression on PFC pyramidal neuron physiology and found that there is a reduction in miniature excitatory post synaptic current (mEPSC) signaling. The observation that CUS and REDD1 overexpression produce deficits in attentional set shifting in male mice likely has relevance for understanding stress-related disorders. Future research will assess the cell-type localization of REDD1 increases following stress in male mice, determine whether female mice are similarly affected by REDD1 overexpression and/or is upregulated in females following CUS, chronic CORT effects on attentional set shifting and examine the necessity of disrupted mTORC1 for stress effects

Disclosures: B. Kurtoglu: None. **M.C. Hearing:** None. **J.R. Mantsch:** None.

Poster

PSTR298: Chronic Stress and Anxiety: Genetics to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR298.09/N15

Topic: G.04. Emotion

Support: NIH Grant R01 DA032708
COBRE P30 CM140964
COBRE P20 GM148302
Center on Opioid and Cocaine Addiction P50 DA046373
SCTR pilot grant UL1 TR001450

Title: A novel long non-coding enhancer RNA (eRNA) forms R-loops to shape emotional experience induced behavioral adaptations

Authors: *R. AKIKI[1], A. GREIGE[2], D. J. WOOD[3], S. BERTO[4], C. W. COWAN[5], M. TANIGUCHI[6];
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[4]Neurosci., MUSC, Charleston, SC; [5]Neurosci., Med. Univ. of South Carolina, Charleston, SC; [6]Dept. of Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: Emotional experiences often lead to changes in neuronal activity and plasticity that supports adaptive changes in behavior, including maladaptive plasticity associated with mood and substance use disorders. We showed previously that the activity-regulated gene, *Npas4*, is rapidly and transiently induced by stress or cocaine conditioning in brain reward-related regions, and it's required for the development of several depression- and addiction-related behaviors. However, the mechanisms by which emotional stimuli rapidly activate *Npas4* expression remains unknown. In this study, we describe a novel non-annotated long non-coding enhancer RNA (eRNA) produced from a conserved genomic enhancer region of *Npas4* (*Npas4*^{eRNA}). Using viral-mediated approaches we show that the *Npas4*^{eRNA} is necessary and sufficient for the emotional stimilus-dependent expression of *Npas4*^{mRNA} in the brain's reward centers such as the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAc). We also discovered that this *Npas4*^{eRNA} forms RNA-DNA hybrids (R-loops) that could be constitutive or stimulus-dependent. Using viral-mediated sgRNA-targeted dCas9-RNaseH1, we show that the basal and activity-regulated R-loops at the *Npas4* enhancer are required for activity-dependent induction of *Npas4*^{mRNA} through the formation of a 3D chromatin-loop between the enhancer and proximal promoter of the *Npas4* gene. We also show that the *Npas4*^{eRNA} is necessary in the mPFC for the formation of Chronic Social Defeat Stress induced anhedonia-like behavior, and in the NAc for the formation of cocaine reward-context association. We also demonstrate that R-loops are dynamically regulated by cocaine conditioning at other immediate early gene enhancers transcribing eRNAs such as *Fos*. Our findings reveal a novel and unique epigenetic mechanism underlying activity-dependent gene expression through which a long-non-coding eRNA, produced from an activity-sensitive enhancer, forms an R-loop enable emotional stimulus-dependent induction of an essential immediate-early gene, *Npas4*, involved in maladaptive drug- and stress-induced plasticity.

Disclosures: R. Akiki: None. **A. Greige:** None. **D.J. Wood:** None. **S. Berto:** None. **C.W. Cowan:** None. **M. Taniguchi:** None.

Poster

PSTR298: Chronic Stress and Anxiety: Genetics to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR298.10/N16

Topic: G.05. Mood Disorders

Support: 30027171

Title: Profiling sex-specific behavioral patterns and nucleus accumbens neuronal subtype transcriptome networks in chronic social stress

Authors: *G. KUMAR[1], D. FRANCO[1], J. OLUSAKIN[1], R. R. CAMPBELL[1], M. BASU[2], S. A. AMENT[2], M. LOBO[1];
[1]Dept. of Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Dept. of Psychiatry and Inst. for Genome Sci., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: The neurobiological effects of chronic stress drive vulnerability for major depressive disorder (MDD). These effects in MDD-associated brain regions are sex-specific, suggesting a role in the disproportionately higher depression rates observed among females. One such region is the nucleus accumbens, (NAc), a hub in the reward circuitry that is altered in MDD subjects and mouse models. Chronic stress exposure induces divergent alterations in the two subtypes of the primary class of neuron found in the NAc - medium spiny neurons (MSNs). The exact nature of these alterations and their role in producing sex-specific behavioral outcomes is not yet well understood. Researchers use social stress models with high ecological validity to investigate the effects of chronic stress in animals. The chronic witness defeat stress paradigm (CWDS) is a recent development that permits the use of female mice in social stress studies. To examine the chronic social stress-induced alterations to the dopamine receptor 1 and 2 receptor medium spiny neurons (D1- and D2-MSNs) in the NAc, we employed CWDS with D1-Cre-RiboTag (RT) and A2A-Cre-RT female mice followed by RNA-seq profiling of ribosome-associated mRNA. The 3 Chamber Social Interaction test (3ChSI) was used to demonstrate a susceptible group that displays reduced social preference and a resilient group with social preference like controls. Weighted gene co-expression network analysis (WGCNA) using the female CWDS RNA-seq data with a publicly available dataset of MSN subtypes from socially stressed male mice identified subtype-specific modules differentially regulated across sex and stress groups that were involved in mitochondrial, synaptic and morphological adaptations. Recordings of 3ChSI were processed through DeepLabCut to obtain spatiotemporal data of mouse activity for a more granular characterization of the social behavior expressed in this test. Factor analysis using these variables identified latent factors driving the observed behavior during the social preference test including exploratory behavior, social preference and surveillance behavior. Through deconstruction of the MSN subtype gene expression and social interaction behavior profiles in the stress groups using dimension reduction techniques, we hope to enhance knowledge of the sex-specific alterations induced by chronic social stress and the impacted social behavior components. These studies can contribute to the development of targeted forms of treatment that address specific behavioral issues across vulnerable populations and simultaneously address mental health disparities observed in different population groups.

Disclosures: G. Kumar: None. **D. Franco:** None. **J. Olusakin:** None. **R.R. Campbell:** None. **M. Basu:** None. **S.A. Ament:** None. **M. Lobo:** None.

Poster

PSTR298: Chronic Stress and Anxiety: Genetics to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR298.11/N17

Topic: G.05. Mood Disorders

Support: NIMH R01 - 30006291

Title: The impact of chronic stress on RhoA expression in NAc D1-SPN region and projection-specific populations

Authors: *P. DAS[1], S. KHATRI KC[2], R. R. CAMPBELL[3], D. FRANCO[4], M. LOBO[5]; [1]Dept of Neurobio., Univ. of Maryland Baltimore, Baltimore, MD; [2]Dept. of Neurobio., Univ. of Baltimore, Baltimore, MD; [3]Anat. and Neurobio., Univ. of Maryland Sch. of Medicin Program In Neurosci., Baltimore, MD; [4]Neurosci., Univ. of Maryland, Baltimore, Baltimore, MD; [5]Anat. and Neurobio., Univ. of Maryland SOM, Baltimore, MD

Abstract: Title: <u>The impact of chronic stress on RhoA expression in NAc D1-SPN region and projection-specific populations</u> Authors: Payel Das[1], Smirti Khatri KC[1], Rianne R. Campbell[1], Daniela Franco[1], Mary Kay Lobo[1]. [1]Dept. of Neurobiology, University of Maryland, Baltimore, MD, USA **Abstract**: Major Depressive Disorder (MDD) is rising. One of the predominant risk factors of MDD is chronic stress (CS). In mice, in response to CS (chronic social defeat stress: CSDS; chronic witness defeat stress: CWDS), stress-susceptible mice show enhanced negative affective behavior in the behavioral tests, including social avoidance and anhedonia. CS affects the nucleus accumbens (NAc), a major reward and motivation brain nuclei in humans and rodents. The NAc has two subtypes of spiny projection neurons (SPNs): D1- and D2-SPNs, enriched with dopamine receptors 1 (drd1) and 2 (drd2), respectively. The D1-SPNs in different sub-regions of NAc play specific roles: activation of D1-SPNs in the NAc dorsal shell and ventral shell enhances reward and drives aversion, respectively. However, whether these subregion-specific D1-SPNs have differential responses to CS is unclear. Similarly, it is unclear if NAc-D1-SPN projections to the ventral tegmental area (VTA) or ventral pallidum (VP) have distinct responses to stress. To gain insight into this, we are examining Ras homolog family member A (RhoA) expression in D1-SPN populations to VTA vs VP across NAc subregions after CSDS in males and CWDS in females. Previously, our lab has shown that CSDS induces RhoA in NAc-D1-SPNs, resulting in their dendritic atrophy and stress susceptibility. C57 mice that received retrograde Cre into the VTA or VP mice underwent CS. After stress exposure, stress susceptibility and resiliency were detected by performing a three-chamber social interaction test. RNAscope on NAc slices with probes for D1, Cre, and RhoA to measure the RhoA-mRNA expression levels in D1-SPNs in NAc dorsal shell, ventral shell, and core that project to VTA showed that VTA projecting D1-SPNs in both dorsal and ventral shells of stressed groups have increased RhoA mRNA expression compared to the non-stressed controls. The susceptible group has a significantly higher RhoA mRNA expression compared to both the control and resilient groups. The analysis in VP D1-SPNs is in progress. These studies will determine if specific subpopulations of NAc D1-SPNs display typical stress response signatures of enhanced RhoA that underlies dendritic atrophy and stress-susceptible behavior. We can then use intersectional tools to target precise D1-SPN populations and provide a refined understanding of discreet brain cell types in stress response.

Disclosures: P. Das: None. **S. Khatri KC:** None. **R.R. Campbell:** None. **D. Franco:** None. **M. Lobo:** None.

Poster

PSTR298: Chronic Stress and Anxiety: Genetics to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR298.12/N18

Topic: G.05. Mood Disorders

Support: DSPAN 1F99NS135696-01

Title: Impact of social stress on microglia-neuron cytoarchitecture in the nucleus accumbens of male and female mice

Authors: *D. FRANCO[1], B. M. SIEMSEN[1], G. KUMAR[2], S. KEY[1], M. E. FOX[3], M. LOBO[4]; [1]Univ. of Maryland, Baltimore, Baltimore, MD; [2]Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD; [3]Pennsylvania State Univ. Col. of Med., Hershey, PA; [4]Univ. of Maryland SOM, Baltimore, MD

Abstract: Chronic stress is a known risk factor for neuropsychiatric disorders, and it has been shown to alter neuron and myeloid cell structure and function in brain reward areas. The nucleus accumbens (NAc), a hub for integrating reward and motivation, exhibits molecular and cellular alterations that are found in postmortem tissue of patients with major depressive disorder and drive motivational deficits in rodents. Moreover, exposure to chronic stress increases peripheral cytokines in individuals with post-traumatic stress, anxiety, and major depressive disorders and disrupts bidirectional CNS myeloid-neuronal communication in rodents exposed to social stress. Work from our lab has shown that exposure to chronic social defeat stress (CSDS), a validated animal paradigm of social stress, yields dendritic atrophy in NAc dopamine receptor-1 expressing medium spiny neuron (D1-MSNs) in mice that display negative affective behavior. Since microglia play a mediating role in regulating neuronal dendritic adaptations after social stress, we characterized microglia and D1-MSN interactions in the NAc after CSDS. While we observed a cell-subtype specific reduction in microglia-D1-MSN contact in the NAc after CSDS, recent preliminary evidence from the lab shows that mice that underwent Chronic Witness Defeat Stress (CWDS), a validated paradigm of vicarious social stress, show an increase in microglia-D1-MSN contact in the NAc. This suggests that chronic social stress, direct and indirect, may alter microglia-D1-MSN contacts in different, opposing ways. Ultimately, while it is evident that social stress alters microglia-D1-MSN contact, the molecular mechanisms driving these cell-subtype stress-induced changes in the NAc microenvironment remain unclear. Data from recent RNA-seq analyses from our lab using mice that underwent CWDS demonstrated enhanced Vtn (vitronectin) in D1-MSNs from mice associated with low social interaction. Specifically, vitronectin appears to play a driving role in a network of genes altered by chronic

stress. Given that vitronectin, an ECM glycoprotein, has been shown to induce microglia reactivity and increase inflammation-associated surface proteins, it is a promising molecular messenger mediating microglia-D1-MSN crosstalk. Ongoing work seeks to further characterize the role of vitronectin in neuron-microglia interactions in the context of social stress in mice subjected to both CSDS and CWDS. Identifying microglia mechanisms contributing to altered neuronal dendritic morphology and negative affective behaviors can pinpoint novel therapeutic targets for stress-related disorders and improve current treatments.

Disclosures: D. Franco: None. **B.M. Siemsen:** None. **G. Kumar:** None. **S. Key:** None. **M.E. Fox:** None. **M. Lobo:** None.

Poster

PSTR298: Chronic Stress and Anxiety: Genetics to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR298.13/N19

Topic: G.05. Mood Disorders

Title: Noradrenergic modulation of dopaminergic neurons in the dorsal raphe nucleus contributes to stress-induced anhedonia

Authors: *L. NAVA, L. BONTEMPI, C. BURTON, S. BARILE, G. ANGIUS, R. TONINI; Inst. Italiano di Tecnologia, Genova, Italy

Abstract: Major Depressive Disorder (MDD) is one of the most prevalent psychiatric disorders worldwide. Some possible factors leading to this pathology are stressful or traumatic life events. Anhedonia, the inability to experience pleasure, is both the most common symptom and the most difficult to treat. Anhedonia is characterized by dysregulation in monoaminergic transmission. The neuronal circuit substrates of this dysregulation are, however, still elusive. Among the monoaminergic regions of the brain, one of the most heterogeneous in terms of cell type composition is the dorsal raphe nucleus (DRN) which is involved in processing emotional state information. This brain region not only comprises serotonergic neurons ($DRN_{5\text{-}HT}$) but also dopaminergic cells (DRN_{DA}), which respond to both rewarding and aversive stimuli. While it is known that DRN_{DA} neurons process inputs about rewarding stimuli from the parabrachial nucleus (PBN), the source of aversive inputs is still unknown. Anatomical evidence suggests that the DRN receives inputs from the noradrenergic (NE) neurons of the locus coeruleus (LC), a brain region involved in the processing of arousal and stress responses. In this study, we investigated the role of LC projections to the DRN in anhedonia, hypothesizing that stress-induced NE release disrupts the DRN_{DA} neuron response to rewards through endocannabinoid signaling. By combining optogenetics, chemogenetics, and fiber photometry, we demonstrated that both activation of LC→DRN projections and repeated exposure to fear conditioning that induced anhedonia result in NE-mediated eCB release in the DRN. Furthermore, we found

decreased PBN→DRN_{DA} synaptic transmission following fear conditioning. In summary, our results demonstrate that activation of LC_{NE}→DRN input during fear conditioning induces depression of PBN→DRN_{DA} synapses, unveiling an unprecedented modulation of neuronal circuit involved in anhedonia.

Disclosures: L. Nava: None. **L. Bontempi:** None. **C. Burton:** None. **S. Barile:** None. **G. Angius:** None. **R. Tonini:** None.

Poster

PSTR298: Chronic Stress and Anxiety: Genetics to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR298.14/N20

Topic: G.05. Mood Disorders

Title: Postpartum Limited Bedding and Nesting Alters Maternal Defense and Habenular cFos Expression in Long Evans Rats

Authors: *S. A. KU[1], M. DUPUIS[2], D. A. BANGASSER[3];
[1]Neurosci. Inst., Georgia State Univ., Atlanta, GA; [2]Psychology, St. Joseph's Univ., Philadelphia, PA; [3]Dept. of Psychology and Neurosci., Temple Univ., Atlanta, GA

Abstract: Stress is a major risk factor for the development of postpartum disorders. We use a rat stress model of resource scarcity, the limited bedding and nesting (LBN) manipulation, where dams and pups are put in an environment with limited access to bedding and no enrichment from pups' postnatal days 2-10. Previously, we found that LBN increases dam's pup-directed behavior and decreases self-care: a phenotype which likely reflects hyperarousal. To determine if LBN impacts additional postpartum behaviors, we randomly assigned Long Evans dams to standard (n = 15) or LBN (n = 12) housing, then ran them through a resident-intruder task with a male adolescent intruder on PND 10 to elicit aggression. We found that dams have lower total attack durations (p =.01), which is driven by significant decreases in offensive (pin, wrestle, shove; p =.01), but not defensive (kick, box, bite; p =.36) durations. LBN consistently decreases attack duration across all offensive measures, though only pin (p = .008) and wrestle (p = .03) are significant (shove; p =.49). We conducted whole-brain cFos to determine regions underlying these behavioral shifts. Preliminary analyses point to a role for epithalamic habenular nuclei in our behavioral effects, as LBN dams show decreased cFos in the habenular commissure (p = .06), medial habenular nucleus (p = 0.04), and lateral habenular nucleus (p = .06). The habenula receives strong inputs from the medial prefrontal cortex (mPFC) and frontal association cortex (which includes mPFC) is significantly decreased in LBN dams (p =.001). These findings are the first to implicate habenular nuclei and their projections in stress-induced shifts in postpartum aggression. Thus, this work will reveal novel clinical targets for postpartum disorders, particularly those with symptoms involving to dysregulated affect and sociality.

Disclosures: S.A. Ku: None. **M. Dupuis:** None. **D.A. Bangasser:** None.

Poster

PSTR298: Chronic Stress and Anxiety: Genetics to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR298.15/N21

Topic: G.05. Mood Disorders

Title: Magnesium supplementation modulates expression of genes related to anxiety in Wistar rats: A microarray study

Authors: *M. MACÍAS-CARBALLO[1], G. CAMARGO HERNÁNDEZ[1], L. LOPEZ-MERAZ[2], C. A. PEREZ-ESTUDILLO[2], L. BELTRAN-PARRAZAL[2], C. MORGADO-VALLE[2];
[1]Univ. de Guadalajara, Tepatitlán de Morelos Jalisco, Mexico; [2]Inst. de Investigaciones Cerebrales, Univ. Veracruzana, Xalapa, Mexico

Abstract: Anxiety disorders are prevalent psychiatric conditions, highlighting the need for novel anxiolytic treatments with reduced dependence risk. Recent studies suggest magnesium (Mg2+) supplementation may have anxiolytic properties. We investigated the gene-modulating effects of chronic Mg2+ supplementation in rat brain using microarray analysis. Male rats (n=18) aged 3 months were divided into two groups: 1) Control (n=9), receiving water with 0.011 g/L MgCl2 for 4 months; 2) Chronic supplementation (n=9), receiving water with 0.011 g/L MgCl2 for 3 months followed by 5 g/L MgCl2 for 1 month. Following euthanasia, brains were removed, stored in RNAlater at 4°C, and processed for RNA extraction using TRIzol reagent and isopropanol precipitation. RNA samples underwent microarray analysis at the DNA Microarray Unit, Institute of Cell Physiology, UNAM. Gene expression was analyzed using t-tests to compare between groups, calculating fold changes to measure expression levels, and applying the False Discovery Rate (FDR) to control for false positives. The analysis of 5,000 genes revealed 161 overexpressed and 81 underexpressed genes, indicating no significant changes in glutamatergic receptor expression. Underexpressed genes included Htr6, Gsk3b, Arf2, and Cdkn2c, suggesting potential roles of serotonergic, glycogen synthase kinase-3β, and cell cycle regulation pathways in Mg2+'s anxiolytic effects. Overexpressed genes included Adora2a, Htr1d, Gabrd, Gabrp, Grik2, Chrna3, Apc, Ptpn11, and Ednra, implicating adenosine, serotonin, GABA, kainate, nicotinic, Wnt signaling, and endothelin pathways. These findings highlight differential gene regulation related to neurotransmission and cellular processes as potential mechanisms underlying Mg2+'s anxiolytic properties. However, further studies, including human trials, are necessary to confirm these findings and elucidate the precise anxiolytic mechanisms of Mg2+. All experiments adhered to Mexico's NOM-062-ZOO-1999, NIH Guide for the Care and Use of

Laboratory Animals, and ARRIVE guidelines for ethical conduct and reporting of animal research.

Disclosures: M. Macías-Carballo: None. **G. Camargo Hernández:** None. **L. Lopez-Meraz:** None. **C.A. Perez-Estudillo:** None. **L. Beltran-Parrazal:** None. **C. Morgado-Valle:** None.

Poster

PSTR298: Chronic Stress and Anxiety: Genetics to Behavior

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR298.16/N22

Topic: F.03. Stress and the Brain

Support: FRQS 352385

Title: Chronic stress alters presynaptic facilitation at mPFC terminals in the VTA and impairs defensive responses.

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Abstract: Cortically driven dysregulation of subcortical circuits can trigger depressive-like behaviours in humans and animals. Recent findings from our lab showed that chronic variable stress (CVS) induces functional and morphological changes to cortical neurons projecting to the VTA in a sex-dependent fashion. However, whether these modifications could affect the synaptic transmission to downstream regions remains unclear. The optogenetic stimulation of ChR2-expressing mPFC axons in live VTA slices revealed a short-term potentiation of glutamatergic transmission to connected cells, which was significantly impaired by 21 days of CVS in both male and female mice. Using a pharmacological approach, we discovered that this facilitation is mainly supported by presynaptic Ca^{2+}-interacting proteins. By selectively conducting RNA sequencing of PFC neurons projecting to VTA, we identified several key targets potentially involved in calcium-dependent plasticity at mPFC-VTA synapses. Notably, transcriptional patterns exhibit significant variation between male and female mice, as do the top dysregulated targets. In female mice, the CALHM1 gene (encoding a Ca2+ homeostatic protein) shows significant downregulation in samples from chronic stress conditions. Employing a CRISPR/dCas9 gene editing technique, we suppressed the CALHM1 gene in naïve females, leading to comparable plasticity deficits at the mPFC-VTA pathway, as seen in stressed females. Finally, we aimed to elucidate the potential role of this cortical impairment in the VTA region. To achieve this, we employed optogenetic techniques to silence the output from the mPFC to the VTA during aversive and salient behaviors, using the mosquito rhodopsin eOPN3. Precise and

timely inhibition of cortical axons in the VTA regions resulted in cognitive impairment in an operant aversive task and induced anxious-like behavior in an exploratory context. In summary, we delineated a novel form of Ca2+-dependent presynaptic plasticity at mPFC-VTA synapses, likely implicated in cognitive assessment and emotional processing in mice. Chronic stress induces a significant reorganization of key genes associated with cortical plasticity in the VTA, ultimately resulting in behavioral dysfunction. Gaining a deeper understanding of this intricate machinery will offer insights into the neuronal mechanisms driving behavioral adaptations to chronic stress.

Disclosures: L. Pancotti: None. **S. mansouri:** None. **C. Proulx:** None. **B. Labonté:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR299.01/N23

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA045764
NIH Grant U54MD007592-28

Title: Investigating the Relationship Between the Salience Network and Multiple Test of Drug Motivation

Authors: *N. DE AVILA[1], M. A. SMOAK[2], T. M. MOSCHAK[2], S. PATEL[3];
[2]Biol. Sci., [1]Univ. of Texas at El Paso, El Paso, TX; [3]Univ. of Arizona, Phoenix, AZ

Abstract: Substance use disorders (SUDs) present a major public health challenge, characterized by compulsive drug-seeking despite known risks. Understanding why occasional drug use evolves into problematic behaviors is a critical question in addiction research. The salience network (SN) has been linked to SUD and addiction, yet gaps remain in understanding its involvement across different stages of cocaine use. This investigation explores drug motivation using various tasks such as extinction, progressive ratio, self-administration, and histamine paired cocaine. Employing endoscopic calcium imaging, we anticipate observing reduced SN activity and resilience following cocaine exposure concurrent with heightened motivation. Overall, our preliminary data indicates that the salience network exhibits more excitation during extinction, progressive ratio, and histamine administration. However, no phasic activity was observed during self-administration. In sum, this suggests that the salience network responds differently across various behavioral paradigms, with heightened activity during certain tasks but not during others. Moving forward, we aim to include female subjects in our study to explore potential gender differences in network activity and behavior. Additionally, we plan to further

compare neural responses across different behaviors to gain a comprehensive understanding of the salience network's role in drug-seeking behavior.

Disclosures: N. De Avila: None. **M.A. Smoak:** None. **T.M. Moschak:** None. **S. Patel:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR299.02/N24

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA045764

Title: Optogenetic manipulation of INS-PrL connectivity: effects on cocaine self-administration and behavioral endophenotypes in male Long Evans rats

Authors: *R. SOSA JURADO[1], T. M. MOSCHAK[2];
[1]Univ. of Texas at El Paso, El Paso, TX; [2]Biol. Sci., Univ. of Texas at El Paso, El Paso, TX

Abstract: Drug abuse is characterized by compulsive consumption and craving escalation and includes key behavioral endophenotypes like distress tolerance (persistence in goal-directed behavior amid psychological distress) and impulsivity (lack of restraint and poor decision-making). These endophenotypes are driven by reward and decision-making circuitry, particularly the insula (INS) to prelimbic (PrL) pathway. In this study, we manipulated this pathway via in vivo excitatory optogenetic stimulation to observe effects on these behavioral endophenotypes. A p-AAV-CaMKIIa-mCherry-H134-ChR2 virus was stereotaxically implanted in the INS. Four groups of male Long Evans rats (ChR2/mCherry, and cocaine/control) were accustomed to the operant chambers before optical fiber and catheterization surgery while the virus expressed mCherry-ChR2 or mCherry-only proteins. The animals trained on a modified cue-titration paradigm (TT) to press a lever for a sugar reward, assessing impulsivity and distress tolerance (DT) across drug-naïve, short abstinence, and long abstinence timepoints (TT1,2,3, DT1,2,3). Baseline behaviors were stabilized before intrajugular catheterization and subsequent training to establish drug-naïve behavior scores. Two weeks of cocaine self-administration were followed by an extinction (EXT1) session with no drug in the operant chamber. The rats then returned to TT for short-abstinence behavioral metrics before a break and finally returned to self-administration to establish long-abstinence EXT2 scores to observe incubation of craving. Optogenetic stimulation was performed bilaterally at the PrL during the third DT and TT stages and EXT2. Two animals have undergone self-administration, showing consumption behavior, DT scores, and TT-derived impulsivity scores consistent with previous lab data. In future, these animals will undergo abstinence to establish behavioral changes, and optogenetic stimulation will occur at the third DT, TT stages, and EXT2, to observe behavior during neurocircuitry

manipulation. This design will help us establish the neuroetiology of these behaviors in relation to drug-seeking and drug-taking.

Disclosures: R. Sosa Jurado: None. **T.M. Moschak:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR299.03/N25

Topic: G.09. Drugs of Abuse and Addiction

Title: Altered sensitivity to cocaine in adolescent spontaneously hypertensive rats, a rodent model of attention-deficit/hyperactivity disorder

Authors: I. SCHOENBORN, A. RAMSDEN, S. ROONEY, T. MIKOLAJCZAK, *L. MARTINEZ;
Neurosci., Trinity Col., Hartford, CT

Abstract: Adolescents with attention-deficit/hyperactivity disorder (ADHD) are at greater risk for psychostimulant abuse compared to those without ADHD. This may be due to alterations in the mesolimbocortical dopamine system; indeed, psychostimulant-induced dopamine release is greater in adult spontaneously hypertensive rats (SHRs; a rodent model of ADHD) compared to reference animals. Here, we sought to determine the extent to which neurobehavioral responses to cocaine are altered in adolescent SHRs. To begin to address this question, adolescent male and female SHRs and Sprague Dawley (SD; a reference strain) rats were first assessed for behavioral signs of ADHD, including inattention (Y-maze test) and hyperactivity (open field test). Rats also underwent behavioral sensitization to cocaine (BSC) testing, which involved repeated cocaine (10 mg/kg) or saline (1mL/kg) injections and subsequent locomotor testing. Finally, immunohistochemistry for cFos was performed to evaluate the neural response to cocaine. We found that female SHRs exhibited enhanced BSC compared to female SD controls, whereas the opposite pattern of results was observed in males. Quantification of cFos expression is underway. These findings indicate that adolescent females with ADHD may be at an increased risk of developing substance use disorders to psychostimulant drugs like cocaine.

Disclosures: I. Schoenborn: None. **A. Ramsden:** None. **S. Rooney:** None. **T. Mikolajczak:** None. **L. Martinez:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR299.04/N26

Topic: G.09. Drugs of Abuse and Addiction

Support: R0045758

Title: Kcnt1 drives sex specific cocaine-seeking behavior on extinction day 1 in female rats

Authors: *A. COX[1], S. KELSEN[2], L. K. KACZMAREK[4], A. S. KOHTZ[3];
[2]Dept. of Psychiatry and Human Behavior, [3]Psychiatry and Human Behavior, [1]Univ. of Mississippi Med. Ctr., Jackson, MS; [4]Yale Univ. Sch. Med., New Haven, CT

Abstract: The inability to maintain abstinence is a hallmark of addiction, with cravings during initial abstinence predicting long-term relapse outcomes in humans and rodents. Promoting successful abstinence may be particularly complex in women, as psychological and biological responses to drugs of abuse differ between sexes. Several measures of cocaine dependence are greater in women, which can be paralleled in female rodents, yet the biological mechanisms underlying these sex differences remain unclear. Extinction day 1 (ED1) marks the initiation of abstinence when the expected drug is unavailable, representing a stressful time point where drug cravings increase. We have previously shown that the dorsal hippocampus plays a significant role in driving sex-specific engagement in cocaine-seeking behavior on ED1. Using whole-transcriptome sequencing (RNA-Seq) analysis, we identified sex-specific gene expression patterns in the dorsal hippocampus elicited by exposure to the cocaine self-administration context on ED1 that correlate with cocaine-seeking behavior. In females, we identified 101 transcripts with fold-change differences on withdrawal day 1 (WD1) compared to naïve rats, and 22 transcripts with fold-change differences on ED1 compared to WD1 controls. Remarkably, only three targets overlapped between the sexes. Furthermore, five genes identified in females significantly correlated to cocaine-seeking behavior on ED1 with R^2 values > 0.70. One of these targets, KCNT1, a potassium channel, negatively predicted cocaine-seeking behavior on ED1 in females. Inhibition of KCNT1 by PRX20 increased cocaine-seeking behavior on ED1, while agonism of KCNT1 with niclosamide decreased cocaine-seeking behavior on ED1 in females only. These findings suggest that sex-specific transcriptomic signatures in the dorsal hippocampus, particularly KCNT1, may play an important role in driving cocaine-seeking persistence during early abstinence. Targeting these molecular pathways could promote successful maintenance of abstinence, with potential implications for sex-specific addiction treatment strategies. This work was supported by R00-045758 to ASK.

Disclosures: A. Cox: None. **S. Kelsen:** None. **L.K. Kaczmarek:** None. **A.S. Kohtz:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR299.05/N27

Topic: G.09. Drugs of Abuse and Addiction

Support: R0045758

Title: Sex differences in the locus coeruleus drive cocaine-seeking behavior during extinction and withdrawal

Authors: *J. PERSON[1], E. J. VALLENDER[1], A. S. KOHTZ[2];
[1]Univ. of Mississippi Med. Ctr., Jackson, MS; [2]Psychiatry and Human Behavior, Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: Aim. The inability to maintain abstinence is a trademark of addiction yet effective maintenance therapies remain elusive. Furthermore, addiction treatment may be particularly complex in women; as psychological and biological responses to drugs of abuse differ in women compared to men. Several measures of cocaine dependence are greater in women, and can be paralleled in female rodents, yet the biological mechanisms for these sex differences remain unclear. Thus, understanding circuit and molecular signatures that drive increased drug-seeking among females is critical to the development of effective SUDs therapies. **Methods.** Herein, we tested sex differences in the role of locus coeruleus (LC) noradrenergic signaling to the dorsal hippocampus (dHPC) in driving operant cocaine memories cocaine-seeking persistence during extinction from self-administration (CSP) in male and female adult Sprague Dawley rats (n=6-8/group). Rats were implanted with PRSx8-HA-hM4Di or hM3Dq DREADDs to the locus coeruleus and guide cannulae were inserted to the dHPC. On extinction day 1, rats were infused with CNO and tested for cocaine seeking behavior. In a separate group, rats were similarly trained to self-administer cocaine, and euthanized naïve to cocaine, in 24 hr withdrawal, or in 24 hour withdrawal with cocaine-seeking. LC was fresh dissected and processed for RNA-Seq analyses. **Results.** Inhibition of LC-NE signaling to the dHPC using DREADDs attenuated CSP in females only, whereas excitation of LC-NE signaling to the dHPC increased CSP in males only. RNA-Seq analysis observations revealed notable gender-specific genetic changes in noradrenergic signaling pathways of the LC to the dHPC in rats. Also, transcriptomic alterations were discernible in gene expression profiles among control, extinction, and withdrawal rats. These changes were modulated by sex, with both universal and sex-specific changes observed. **Conclusions.** These studies show
the importance of the LC in SUDs, shedding light on potential sex-specific responses during the distinct behavioral phases of the addiction cycle. Furthermore, these effects may be driven by sex differences in adrenergic tone between the LC and dHPC. Thus, the substantial sex differences in the retrieval and retention of cocaine memories may be driven in part by the locus coeruleus.

Disclosures: J. Person: None. **E.J. Vallender:** None. **A.S. Kohtz:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR299.06/N28

Topic: G.09. Drugs of Abuse and Addiction

Support: DK111745

Title: Chemogenetic inhibition of the mesolimbic reward pathway disrupts mediated devaluation of cocaine reward

Authors: *B. MO[1], V. FEX[2], D. I. OLEKANMA[2], A. A. ARGUELLO[3], A. W. JOHNSON[3]; [1]Michigan State Univ., East Lansing, MI; [2]Neurosci., Michigan State Univ., East Lansing, MI; [3]Psychology, Michigan State Univ., East Lansing, MI

Abstract: Cocaine addiction is a major health concern in the US, yet there are no approved therapeutic targets for disrupting cocaine-seeking behaviors. In this series of studies, we describe a novel approach in which we attenuate cocaine-seeking by devaluing the memory of cocaine reward via the mesolimbic reward system. In rats, we used a dual-viral strategy to enable projection-specific hM4Di-DREADD inhibition of ventral tegmental area (VTA) cells projecting to the nucleus accumbens (NAc). Following recovery from surgery, rats underwent cocaine self-administration training, in which they responded for cocaine infusions paired with a tone-light cue. Next, rats received presentations of the cocaine-associated tone-light cue alone in a different context. This cue-evoked retrieval of cocaine reward was immediately paired with temporary gastric illness produced by lithium chloride (LiCl) injection, which served to promote memory devaluation of cocaine reward. This led to a subsequent reduction in cocaine seeking in rats that received the mCherry control virus. Notably, inactivation of the VTA→NAc pathway during memory devaluation prevented the disruption in cocaine seeking in the hM4Di group. Overall, these findings suggest that it is possible to attenuate cocaine-seeking using memory devaluation in a mesolimbic-dependent manner. Moreover, our novel approach can be leveraged to develop therapeutic tools for the treatment of cocaine use disorder.

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Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR299.07/N29

Topic: G.09. Drugs of Abuse and Addiction

Title: Inducible ablation and overexpression of dopamine D1 receptors and D1 receptor-selective neuronal circuit regulate extinction of cocaine reward memory

Authors: *Q. KONG, M. XU;
The Univ. of Chicago, CHICAGO, IL

Abstract: Formation and maintenance of cocaine-induced behaviors are tightly regulated by dopamine D1 receptors (D1Rs). Although stimulation of D1Rs is known to be involved in extinction of cocaine-induced behavior, the global effect of D1R stimulation or elimination is not well understood. In this study, mouse lines were engineered to express inducible D1Rs (iD1s) to create D1R ablation and overexpression models. We have found that in a cocaine conditioned place preference (CPP) model, while D1R overexpression after CPP acquisition attenuated CPP extinction, ablation of D1R accelerated it, suggesting that D1Rs act to maintain cocaine-induced behaviors. Furthermore, we ask whether D1R-specific neuronal circuits are involved in D1R-mediated extinction. Optogenetic and behavioral approaches were combined to examine the function of D1R-containing basolateral amygdala (BLA)- nucleus accumbens (NAc) microcircuit on cocaine CPP extinction. Intriguingly, while optogenetic inhibition of the BLA-NAc projection failed to induce a change in CPP extinction, optogenetic excitation effectively accelerated it. Taken together, these results suggest that D1R activation opposes its extinction of cocaine CPP, albeit in a manner dependent on the circuitry involved.

Disclosures: Q. Kong: None. **M. Xu:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR299.08/N30

Topic: G.09. Drugs of Abuse and Addiction

Title: Effects of increased endocannabinoid levels during adolescence on anxiety-related and cocaine-seeking behaviors in adulthood

Authors: *M. K. ESTES, ***M. ESTES**, V. S. GUDA, D. R. OLIVEIRA, C. J. HILLARD, J. R. MANTSCH;
Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Adolescence is a critical period of neural development. Both chronic stress and cannabinoid exposure during adolescence can lead to impaired emotional regulation, altered stress response, and an increased vulnerability for affective disorders in adulthood. Acute activation of the endocannabinoid (eCB) system can buffer some aspects of stress. However,

there is evidence that chronic stress can contribute to disruption of neurodevelopment and increase the risk for adulthood anxiety-related and substance use disorders. We aimed to investigate short- and long-term effects of elevating the two eCBs, N-arachidonoylethanolamine (AEA) or 2-arachidonoylglycerol (2-AG) during adolescence on anxiety-related behaviors. A second aim of our study was to investigate the effects of increased AEA and 2-AG during adolescence on drug-seeking behaviors in adulthood. Adolescent male Sprague Dawley rats were given daily intraperitoneal injections of either JZL184 (JZL;10 mg/kg), URB597 (URB; 0.4 mg/kg), or vehicle from PND 31-40. JZL and URB are inhibitors of enzymes that catabolize 2-AG and AEA, respectively. Behaviors were assessed twenty-four hours following the final injection on the elevated plus maze (EPM) and on PND65 in the open field. Following open field tests, venous catheters were implanted, and rats were trained to self-administer cocaine (0.5 mg/kg/inf, i.v.) during 2-h sessions. Both fixed ratio (FR) and progressive ratio (PR) schedule of reinforcements were utilized to analyze drug-seeking behavior. During adolescence, JZL-and URB-treated groups ($p = 0.02$ and $p < .01$, respectively) were significantly less active on the open arms of the EPM compared to vehicle-treated animals, suggesting potential increased anxiety. During adulthood, group differences in open field behavior were not evident. Acquisition of cocaine self-administration did not differ between groups. However, when the schedule of reinforcement changed from FR to PR, JZL and URB-treated rats had higher breaking points compared to VEH groups. Interestingly, JZL-treated rats also continued to press the lever more when returned to a FR schedule resulting in significantly higher cocaine infusions ($p = .05$) compared to VEH rats. During reinstatement testing, JZL-treated animals had reduced drug-seeking behavior following a priming injection of cocaine (10.0 mg/kg, i.p.). In conclusion, the data we gathered indicate that while effects on anxiety-related behaviors do not persist into adulthood, there is an increase in drug seeking in adults when eCB levels are increased during adolescence.

Disclosures: M.K. Estes: None. **M. Estes:** None. **V.S. Guda:** None. **D.R. Oliveira:** None. **C.J. Hillard:** None. **J.R. Mantsch:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR299.09/N31

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R01 DA035224 (H.E.M.)

Title: Nociceptin Signaling in the Nucleus Accumbens Shell Decreases Cocaine Sensitivity in a Sex-Dependent Manner and Acts Presynaptically on Dopamine Terminals

Authors: *N. BOLDEN, H. E. MELIKIAN;
Neurobio., UMASS Chan Med. Sch., Worcester, MA

Abstract: The initial stages of cocaine addiction are largely mediated by dopaminergic (DAergic) transmission at mesolimbic terminals in the nucleus accumbens (NAc), which signals for strong salient stimuli. Cocaine augments extracellular dopamine (DA) levels by directly inhibiting DA reuptake via the presynaptic DA transporter (DAT), and cocaine's actions at DATs are required for both contingent and non-contingent cocaine administration in mice. Despite decades of investigation aiming to unravel the mechanisms that impact cocaine reward, it is not well understood how reward perception is modulated in the NAc. Moreover, it is largely unknown whether regulatory mechanisms targeting presynaptic DAergic terminals impact the cocaine reward response. The neuropeptide nociceptin (N/OFQ) is a promising candidate as a potent reward modulator. N/OFQ is expressed throughout the CNS and signals via the $G_{i/o}$-coupled nociceptin receptor, NOPR, and previous studies demonstrated that N/OFQ signaling in NAc dampens cocaine-mediated increases in extracellular DA. However, it is unknown whether N/OFQ signaling in NAc similarly dampens cocaine reward, and whether N/OFQ may play a central role in gauging reward perception. Here, we leveraged pharmacological and genetic approaches to directly test whether N/OFQ signaling in NAc alters cocaine reward sensitivity in mice, and whether N/OFQ acts presynaptically on NOPRs in DAergic terminals. N/OFQ infusions into NAc shell (NAcSh) significantly diminished cocaine sensitivity in a conditioned place preference (CPP) assay in females, but had no effect in males. Conditional NOPR silencing in *NOPR*$^{fl/fl}$ DAergic neurons revealed that presynaptic NOPRs on DAergic terminals were required for cocaine sensitivity changes in response to N/OFQ infusion. *Ex vivo* slice biotinylation studies in NAc revealed that N/OFQ drove acute increases in DAT surface expression that were similarly dependent upon presynaptic DAergic NOPR expression. Ongoing studies will test whether presynaptic DAergic NOPRs can bi-directionally shift cocaine reward sensitivity and whether NOPR activation in NAcSh alters DA signaling during cocaine CPP, assessed using DA sensors and fiber photometry. Taken together, these findings indicate that N/OFQ robustly influences cocaine reward tone, primarily by acting presynaptically on DAergic terminals. Presynaptic NOPR-mediated DAT regulation further demonstrates that NOPRs on DAergic terminals in NAc are well-positioned to exert significant influence on DA signaling via presynaptic mechanisms.

Disclosures: N. Bolden: None. **H.E. Melikian:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR299.10/N32

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH DA036657

Title: The Role of D1 and D2 Medium Spiny Neurons During Hippocampal Replay Events in a Drug-Context Association Model

Authors: *A. FRYC[1], K. CLEMENZA[2], L. L. SJULSON[3];
[1]Albert Einstein Col. of Med., New York, NY; [2]Neurosci., Albert Einstein Col. of Med., Bronx, NY; [3]Neuroscience, Psychiatry, Albert Einstein Col. of Med., Bronx, NY

Abstract: Drug-context associations (DCAs) act as triggers for relapse in addiction, even after continued abstinence. DCAs are learned through consistent reinforcement of a drug in a particular context. Once learned, exposure to a DCA can trigger a cascade of events (i.e. drug seeking behavior) leading to relapse. Little is known about the mechanisms contributing to the acquisition and corresponding drug seeking behavior of DCAs. Here, we aim to elucidate the role of two regions that I hypothesize contribute to this phenomenon - the hippocampus and nucleus accumbens - during Conditioned Place Preference (CPP) conditioning. Specifically, we will perform simultaneous electrophysiology and calcium imaging recordings using custom made tetrode bundles and the Miniscope system in the hippocampal CA1 region and NAc, respectively. In the NAc, we are particularly interested in imaging D1 and D2 medium spiny neurons (MSNs), which are thought to be involved in updating and encoding the reward value associated with a context. We will investigate their involvement during CPP conditioning sessions, and during post hippocampal CA1 “replay” events, which are thought to encode spatial contexts. The results of this study can provide clarity on the progression of the reward value associated with an originally neutral spatial context, and distinguish between the roles of this circuit during learning and performance of reward related behavior.

Disclosures: A. Fryc: None. **K. Clemenza:** None. **L.L. Sjulson:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

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Program #/Poster #: PSTR299.11/N33

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 1R21DA058205

Title: Oxytocin attenuates cocaine conditioned place preference in male long-evans rats: role of oxytocin receptors in the ventral tegmental area

Authors: *I. MOOSA, A. LACKAN, T. YATES, K.-C. LEONG;
Trinity Univ., San Antonio, TX

Abstract: Cocaine addiction is a major global health concern characterized by high rates of relapse and limited pharmacological interventions, highlighting the need to find effective ways to combat maladaptive reward-seeking behavior. Current research suggests oxytocin (OXT) as a possible therapeutic intervention in addiction given its ability to modulate reward processing. The ventral tegmental area (VTA) is highly implicated in the reward pathway and its oxytocin receptors (OXTR) may be a necessary site of action for oxytocin attenuation of drug-seeking behaviors. The present study examined (1) the ability for OXT to attenuate the expression of cocaine-reward associations and (2) explored the role of oxytocin receptors (OXTRs) within the VTA in mediating this effect. Cocaine-preference behavior was established in male Long-Evans rats through a conditioned place preference (CPP) paradigm in which two chambers in an apparatus were either paired with cocaine (10 mg/kg; i.p.) or saline (VEH). In Experiment 1, OXT (1 and 3 mg/kg, i.p.) was systemically administered before test and found to successfully attenuate cocaine-associated place preference. In Experiment 2, using a shRNA-mediated OXTR knockout, we examined whether OXTR receptors within the VTA mediated this attenuating effect of OXT on cocaine preference behavior. These results illustrate that not only is OXT a relevant therapeutic agent for cocaine addiction-related behaviors but also provide insight into the role of VTA OXTRs in driving this effect thus shedding light on the neural mechanism underlying addiction and offering insights into potential avenues for intervention.

Disclosures: I. Moosa: None. **A. Lackan:** None. **T. Yates:** None. **K. Leong:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR299.12/N34

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA048055
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NIH Grant DA059983

Title: Optogenetic and chemogenetic inhibition of basolateral amygdala projections to medial prefrontal cortex subregions during cocaine extinction learning and reinstatement

Authors: *A. R. ZIMBELMAN[1], R. T. LALUMIERE[2,3];
[1]Psychological and Brain Sci., [2]Dept. of Psychological and Brain Sci., [3]Iowa Neurosci. Inst., Univ. of Iowa, Iowa City, IA

Abstract: Previous fear conditioning and drug seeking studies suggest that, often, the infralimbic cortex (IL) inhibits and the prelimbic cortex (PL) promotes such behaviors. Although downstream projections from these brain regions have been identified in the involvement of

regulating fear conditioning and drug seeking, it is unknown what inputs to these regions determine their influence over cocaine-seeking behavior. Evidence from fear conditioning studies suggests that separate subpopulations of basolateral amygdala (BLA) neurons project to the PL vs. IL and oppose each other for control over fear conditioning vs. the extinction thereof. These findings raise the possibility that the BLA is a key region influencing PL and IL activity and determining the degree of cocaine-seeking behavior. To address this question, female and male Sprague-Dawley rats received bilateral microinjections of an inhibitory opsin (eNpHR3.0) or eYFP-control into the BLA, bilateral fiber optics targeted at the IL or PL, and implantation of an intrajugular catheter. Rats then underwent at least 12 d of 2 h cocaine self-administration, in which an active lever press produced a cocaine infusion and light/tone cues. Rats then underwent 5 d of shortened (30 min) extinction training sessions, during which an active lever press resulted in no cocaine infusion or cues but initiated 20 s of optical inhibition of BLA terminals in the IL or PL. All rats then underwent 7 d of full-length (2 h) extinction training sessions with no inhibition to assess extinction retention. Immediate post lever press inhibition of BLA terminals in the IL or PL had no effect on active lever pressing during sessions with inhibition or the subsequent 7 d of full-length extinction sessions. These results suggest that BLA projections to the IL and PL are not involved in cocaine extinction learning. However, it is possible that these pathways are involved during other cocaine-seeking behaviors, as previous work found a role for BLA projections to the PL in the promotion of cocaine seeking during cued reinstatement. Ongoing experiments are examining the role of these pathways during cued and cocaine-primed reinstatement tests, via chemogenetic inhibition. Findings from these experiments will elucidate the role, if any, for these pathways in cocaine seeking during reinstatement.

Disclosures: A.R. Zimbelman: None. **R.T. LaLumiere:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR299.13/N35

Topic: G.09. Drugs of Abuse and Addiction

Support: MSU grant GE100332

Title: Analysis of operant self-administration behavioral with DeepLabCut:Protocol for video acquisition and DeepLabCut training

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Abstract: Analysis of operant self-administration behavioral with DeepLabCut
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Rationale: Substance use disorders (SUDs) are comprised of several complex behaviors. Patients diagnosed with SUDs can remain abstinent for extended periods, but exposure to drug-associated stimuli often triggers intense craving and a return to drug use. Operant self-administration is used to examine several behaviors in rodents: lever responding that results in 1) intravenous cocaine infusion paired with an explicit cue or environment: cocaine-taking, 2) no reward, cocaine-paired cue or context: extinction, and 3) presentation of the cocaine-paired cue or within a cocaine-paired context with no reward: cocaine-seeking. Supervised machine learning can be used to rapidly and efficiently analyze complex behavioral profiles, but currently there are limited protocols available for analysis of self-administration behaviors.
Objectives & Methods: We provide methodology to 1) collect high-quality videos of operant self-administration behaviors using Raspberry Pi microcomputers or GoPros, 2) obtain pose estimation data using the supervised machine learning software DeepLabCut and network training via a local high performance computer cluster, 3) proof of principle comparison of lever press data vs lever quadrant time generated from pose estimation data, and 4) visualizing complex behaviors in rodents using supervised machine-learning predictive classifiers (Simple Behavioral Analysis, SimBA).
Results & Conclusions: We find video acquisition to be simple and efficient with the largest amount of troubleshooting needed to set up network training conditions. Using pose estimation outputs from DeepLabCut, we recapitulated lever press behavior results with quadrant time. Future work aims to use behavioral segmentation software to probe for behavioral motifs that: precede or proceed cocaine intake or rewarded lever presses, are specific to early vs stable self-administration, correlate with magnitude of drug-seeking behavior.
Keywords: Video recording, operant, self-administration, relapse, rat, context, DeepLabCut, pose estimation, supervised machine learning, neuronal network

Disclosures: L. Pereira Sanabria: None. **L.S. Voutour:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR299.14/N36

Topic: G.09. Drugs of Abuse and Addiction

Title: Age- and sex-specific differences in projection inputs to the basolateral amygdala and dorsal hippocampus

Authors: *V. J. BRAMAN[1,2], L. PEREIRA SANABRIA[3], L. S. VOUTOUR[3], M. SINGH[4], A. A. ARGUELLO[5];
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Abstract: Rationale: Drug use during adolescence can permanently alter brain development and increase the risk of developing substance use disorders (**SUDs**). SUDs are characterized by cycles of use and relapse, with craving occurring up to 6 months after abstinence (i.e., incubation of craving). Using a rat model of cocaine self-administration, we found that male rats with a history of adolescent cocaine exposure displayed higher context-induced, cocaine-seeking behavior (responses on a lever previously paired with cocaine) after longer periods of abstinence, compared to adults. Higher activation of the prelimbic prefrontal cortex was also observed at timepoints where cocaine-seeking was highest in the adolescent group. To understand the potential effects of adolescent cocaine use on circuit development, we aimed to first examine for baseline age- and sex-dependent differences in projection inputs to the basolateral amygdala (a key target of the prelimbic cortex) and the dorsal hippocampus (a key region involved in contextual cocaine-seeking).
Objectives & Methods: We aimed to examine age- and sex-dependent differences in projection density to the dorsal hippocampus (DH) and basolateral amygdala (BLA) using fluorescent Choleratoxin B (CtB) retrograde tracers. Adolescent (P31) and adult (P60) male and female rats received stereotaxic infusion of CtB 488 into the BLA of one hemisphere and CtB 594 into the DH of the other hemisphere (0.4- 0.5 μL/side). After 7 days, rats were perfused, brains cryoprotected and sectioned at 40 μm. Tissue sections were slide mounted, dehydrated and photomicrographs were taken at 20X magnification from the following regions: PrL, infralimbic cortex (IL), orbitofrontal cortex (OFC), agranular insular cortex (AgI), nucleus accumbens (NAc) perirhinal cortex (PRh).
Results & Conclusions: Preliminary results show significantly higher CtB-positive cell counts in the PrL and IL of adolescent rats compared to adults, suggesting an age-dependent difference in PrL inputs to the BLA. An age-dependent difference in PRh inputs to the DH was also observed, with higher CtB-positive cells counts in the PRh of adolescent rats compared to adults. Future directions will examine for sex-dependent differences in inputs to the DH and BLA in adolescent and adult rats.

Disclosures: V.J. Braman: None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

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Topic: G.09. Drugs of Abuse and Addiction

Support: Internal UCSD Keck Grant 2034703 task 1

Title: Effects of Negative Feedback Chemogenetic in the Central Nucleus of the Amygdala on Cocaine-Related Behaviors

Authors: *S. L. PLASIL[1], S. ALLEY[2], J. QIAN[2], C. MAGNUS[2], S. M. STERNSON[3], O. GEORGE[4];
[1]Psychiatry, UCSD, San Diego, CA; [2]UCSD, San Diego, CA; [3]Neurosciences, UCSD, La Jolla, CA; [4]Dept. of psychiatry, Univ. Of California San Diego Neurosciences Grad. Program, La Jolla, CA

Abstract: Cocaine use disorder is a serious heath problem worldwide. Cocaine misuse is associated with cardiovascular and neurologic effects, and chronic repeated exposure leads to tolerance, and adverse psychological and behavioral effects. Acute rewarding effects act as reinforcing stimuli to sustain drug-seeking and drug-taking behavior and is linked to aversive effects such as anxiety and increased activity of stress-response systems.
The amygdala is linked to cocaine consumption, including mediating reward and drug craving. The central nucleus of the amygdala (CeA) is implicated in the stress response and effects of abusive substances. Corticotropin releasing factor (CRF) is a critical stress-related neuropeptide in major output pathways of the amygdala and is linked to anxiety, stress, fear, pain, motivation, and addiction behavior. The amygdalar CRF system is potently activated after cocaine administration, increasing expression and extracellular levels of CRF. Further, CRF stimulation in pre-exposed animals showed increased drug-seeking behavior. A synthetic physiology approach was applied that installs artificial chemical negative feedback loops, termed negative feedback chemogenetics (NFC). Protein engineering created an excitatory Cre-dependent cocaine-activated channel engaged only in Cre-expressing neurons and only during cocaine exposure. NFC allows control to evaluate CRF-expressing neurons for their ability to alter the chemical feedback responsible for the addictive reinforcement of cocaine. This approach ties the pharmacokinetic time-course of a self-administered cocaine to chemogenetic perturbation of the CeA CRF-system. The novel cocaine-activated channel encoded in an AAV virus (termed coca-5HT3-mCherry), or control virus, was stereotaxically injected into the CeA of adult CRF-Cre rats. Receptor expression, spread, and localization was confirmed and detailed in the CeA of a subset of injected rats via mCherry and alpha-bungarotoxin labeling. The virus activates CRF-neurons only when cocaine is ingested and is hypothesized to lead to aversive effects. We have piloted and begun to measure heart rate and respiration, anxiety-like behavior, locomotion, irritability-like behavior, mechanical stimulation, as well as dose response to cocaine IVSA, both short and long access cocaine intravenous cocaine self-administration (IVSA), IVSA progressive ratio, and willingness to overcome adverse consequences in coca-5HT3-expressing rats versus control. Data analysis is ongoing. We hypothesize observing heightened CRF signaling will alter CRF- and cocaine-related behavior.

Disclosures: S.L. Plasil: None. **S. Alley:** None. **J. Qian:** None. **C. Magnus:** None. **S.M. Sternson:** None. **O. George:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR299.16/N38

Topic: G.09. Drugs of Abuse and Addiction

Support: R01DA055008

Title: Vagus nerve stimulation modulates cocaine-induced synaptic plasticity between the prefrontal cortex and the basolateral amygdala and nucleus accumbens

Authors: R. AREZOOMANDAN, L. VU, C. DRISKILL, ***S. KROENER**;
Neurosci., Univ. of Texas at Dallas, Richardson, TX

Abstract: Cocaine use causes changes in the infralimbic cortex (IL), which mediates inhibitory control over cocaine-seeking, mainly via its projections to the nucleus accumbens shell (NAshell). Additionally, connections between the IL and the basolateral amygdala (BLA) play a role in extinction learning and relapse. We previously demonstrated that vagus nerve stimulation (VNS) can attenuate the reinstatement of cocaine-seeking following extinction, which is associated with changes in metaplasticity (the ability to evoke LTP or LTD) in the pathway from the IL to the BLA. In this study, we investigated synaptic changes in the reciprocal pathway from the BLA to the IL, as well as from the IL to the NAshell. Female (n=8) and male rats (n=21) self-administered cocaine or received yoked-saline infusions. Nineteen rats were sacrificed after the self-administration period to assess the effect of cocaine on synaptic plasticity in the two pathways. The remaining 10 rats underwent extinction training paired with VNS or sham stimulation for 12 days. Relapse to drug-seeking was assessed in a cued reinstatement session. In all rats evoked local field potentials (EFPs) were recorded in the IL and NAshell following either the last self-administration session or after cue-induced reinstatement. We measured changes in synaptic plasticity induced by high-frequency stimulation (HFS; two bursts at 50 Hz). In the IL to Nashell pathway HFS induced no change in yoked-saline, cocaine self-administering, and sham VNS-stimulated groups. However, compared to sham stimulation, VNS-treated rats exhibited long-term potentiation (LTP) in the NAshell following HFS in the IL ($p=0.02$). VNS also increased the current-voltage relationship of EFPs in this pathway. In the BLA-to-IL pathway, HFS induced LTP in both yoked-saline and cocaine-treated rats, but the magnitude of LTP was significantly larger in cocaine self-administering rats ($p=0.002$). Cocaine also altered the current-voltage relationship of EFPs, greatly increasing the baseline response in this pathway. Ongoing studies examine the effects of extinction and VNS on the connection between the BLA and IL. VNS facilitates extinction and reduces cocaine-seeking reinstatement. Strengthening the IL to NAshell pathway may contribute to these beneficial effects. Similarly, VNS may modify

the impact that cocaine-related signals from the BLA have on IL networks that regulate extinction and reinstatement.

Disclosures: R. Arezoomandan: None. **L. Vu:** None. **C. Driskill:** None. **S. Kroener:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

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Program #/Poster #: PSTR299.17/N39

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 5R01DA055008-03

Title: Vns induced changes in mpfc networks during reinstatement following extinction learning

Authors: *L. VU[1], C. DRISKILL[1], F. J. SALAZAR[3], S. KROENER[4], N. MOLIN[2], S. JALILVAND[1], L. WAYDICK[2], N. SUJI[2];
[1]Univ. of Texas at Dallas, Dallas, TX; [2]Univ. of Texas at Dallas, Richardson, TX; [3]Behavioral and Brain Sci., The Univ. of Texas at Dallas, Plano, TX; [4]Sch. of Behavioral and Brain Sci., Univ. of Texas at Dallas Sch. of Behavioral and Brain Sci., Richardson, TX

Abstract: In substance use disorder re-exposure to drug-associated cues and stress leads to drug cravings and relapse. Extinction learning forms new memories that compete with the drug-related memories to reduce craving. However, extinction learning alone is often insufficient to prevent relapse. Pairing non-contingent vagus nerve stimulation (VNS) during extinction reduces cue-induced reinstatement in a rodent model of cocaine self-administration. This VNS effect correlates with changes in the expression of immediate early genes in regions associated with reinstatement. The medial prefrontal cortex (mPFC) plays an important role in the regulation of drug-seeking behavior, with the prelimbic cortex (PL) driving drug-seeking behavior and the infralimbic cortex (IL) controlling the expression of extinction memories. Little is known about the networks that drive mPFC activity during cue-induced reinstatement, or how VNS alters these networks to reduce drug-seeking behavior.We infused a retrograde AAV expressing GFP into the IL or PL to label cells in regions that project to the mPFC and which are active during reinstatement, including the paraventricular nucleus of the thalamus (PVT), the basolateral amygdala (BLA), and the ventral hippocampus (vHipp). Rats self-administered cocaine for 15 days then underwent 10 days of extinction training paired with VNS or sham stimulation, followed by a cue-induced reinstatement session. Rats were sacrificed after reinstatement, and tissue from regions activated by drug seeking was stained for the immediate early gene cFos as a marker of neuronal activity. We then quantified VNS-induced relative changes in the number of cFos+ cells as well as the co-localization of GFP+ IL- or PL-projecting cells with cFos. VNS increased the overall number of cFos+ cells in the PVT following reinstatement, including

increased numbers of cFos+ cells projecting from the PVT to both the IL and PL. VNS caused no changes in the overall number of cFos+ cells in the vHipp, but a decrease in activity in IL-projecting cells. In the BLA VNS caused a relative decrease in overall cFos activity, as well as in IL-projecting cells; however, in contrast the number of cFos+ PL-projecting cells increased. We hypothesize that these VNS-induced changes in neuronal activity in projections to the mPFC contribute to suppression of drug-seeking behavior during cue-induced reinstatement. Our results provide a better mechanistic understanding of the networks through which VNS facilitates extinction learning from drug seeking behavior.

Disclosures: L. Vu: None. **C. Driskill:** None. **F.J. Salazar:** None. **S. Kroener:** None. **N. Molin:** None. **S. Jalilvand:** None. **L. Waydick:** None. **N. Suji:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH (DA044980)
T32 HL 149646

Title: Circadian disruption during involuntary abstinence increases cue-induced cocaine reinstatement in females but not males

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Abstract: Circadian rhythms, supported by clock genes, synchronize with predictable temporal changes in the environment and permit routine biological functions related to sleep, feeding, and other vital functions. However, under some circumstances, the coupling between external cues and internal biological clocks can be disrupted and become misaligned, such as rapidly shifting time zones (jetlag). Prior work demonstrated that even temporary misalignment is associated with negative changes in physical and mental health. Two places where circadian misalignment and mental health intersect are in stress and drug use. Circadian disruption is an intensely negative experience, and prior work has suggested that experiencing acute or chronic stressors during drug abstinence can potentiate seeking and taking. For example, repeated social defeat stress potently increases cocaine-seeking behavior in a manner that scales with the degree of defeat. While less is known about whether circadian misalignment would be similar, evidence suggests that this stressor functionally alter many of the same brain circuits in the prefrontal cortex affected by other stressors. In this study, we trained rats to self-administer cocaine (0.75mg/kg/infusion intravenous; 2h/d for 14d) during their active (dark) phase (~ ZT14-18)

where nosepokes delivered the drug along with a 20sec audiovisual cue (tone+light). On the first day of abstinence, rats experienced a brief extinction session (no drug or cue following nosepoke) to assess baseline seeking. Then, rats were assigned to a Control condition (continued 12:12 light-dark schedule), or a circadian disrupted schedule (10:10 light-dark; "T20") during a 30d period of involuntary abstinence. Subsequently, all rats were returned to the standard 12:12 light-dark schedule, where the experienced (1) two consecutive days of extinction, and (2) one day of cue-induced reinstatement. Female rats who experienced the T20 circadian misalignment showed significantly more cue-induced reinstatement than both control females and T20 males. These data suggest that circadian disruption may have differential effects in males and females on drug-seeking actions, and may indicate a sex-related role in stress-drug interactions.

Disclosures: J. Torres: None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR299.19/O1

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA14328

Title: Intake-dependent plasticity in nucleus accumbens group 1 metabotropic glutamate receptors following cocaine self-administration diferentially regulates drug seeking during abstinence.

Authors: *M. GHASEMZADEH[1], L. METKO[2], T. MAXIM[2], N. ABRAHAM[2], C. JOHNSTON[2];
[1]Marquette Univ., Milwaukee, WI; [2]Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: A major obstacle in the treatment of addiction is the propensity to relapse, often mediated by drug-associated cues, even after prolonged period of abstinence from drug use. Repeated exposure to cocaine leads to enduring alterations in glutamatergic signaling in the brain reward circuit that play an important role in long-lasting molecular, cellular, and behavioral neuroadaptations. Therefore, glutamate signaling has been investigated as a target for the treatment for addiction. Recent studies suggest that group I metabotropic glutamate receptors (mGluR1/5) play important roles in drug reinforcement and drug seeking and, therefore, have been pursued as promising targets for therapeutic development. Here, we examined the role of mGluR1/5 receptors in abstinence drug seeking using animal models of cocaine self-administration. Male Sprague-Dawley rats were trained to self-administer cocaine (FR1; 1.0 mg/kg/200 µl/inf) during either 2-hr (ShA) or 6-hr sessions (LgA) for 14 days. Subsequently, animals were left undisturbed in the home cage for 3, 10, or 60 days. Following abstinence

period, rats were tested under context-primed extinction condition for cocaine seeking after systemic administration of either saline or an mGluR1/5 receptor antagonist (MTEP or JNJ16259685). Following a short abstinence period (3 or 10 days), the blockade of mGluR5 receptor reduced drug seeking only in ShA subjects without affecting the LgA animals, while mGluR1 receptor blockade was effective in reducing drug seeking in both groups. However, after a long abstinence period (60 days), the systemic blockade of either of receptors significantly reduced drug seeking in ShA and LgA rats. Using separate groups of rats, it was demonstrated that intracerebral infusion of MTEP (3µg/side) after 10 days of abstinence into either NAcore or NAshell led to a decrease in drug seeking in ShA rats. However, our data suggest that inhibition of mGluR5 receptors by MTEP in either NA subregions was not effective in reducing drug seeking in LgA rats. These results suggest that exposure to cocaine produce a transient intake dependent plasticity in mGluR5, but not in mGluR1, signaling in the brain. Moreover, our data point to Nucleus accumbens as the anatomical substrate contributing to the selective modulation of mGluR5 signaling in LgA rats. Importantly, mGluR5 receptor is suggested as an important molecular target for regulation of cocaine craving and the propensity to relapse during prolonged abstinence periods. In addition, understanding the mechanism of cocaine mediated effects on group I metabotropic glutamate receptors may reveal new molecular targets for the treatment of cocaine addiction.

Disclosures: M. Ghasemzadeh: None. **L. Metko:** None. **T. Maxim:** None. **N. Abraham:** None. **C. Johnston:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 7R37DA054370-03

Title: Inhibition of Cocaine Seeking By Rescue of Serotonin Receptor Function in the Rostromedial Tegmental Nucleus

Authors: *M. HOHMEISTER[1], T. C. JHOU[2];
[1]Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD; [2]Neurosci., Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD

Abstract: Inhibition of Cocaine Seeking By Rescue of Serotonin Receptor Function in the Rostromedial Tegmental Nucleus[*]M. Hohmeister[1], T. Jhou[1], J. Parilla-Carrero[21]University of Maryland Baltimore School of Medicine, Baltimore, Maryland; [2]Southern Illinois University School of Medicine, Springfield, Illinois

In addition to its rewarding effects, cocaine also possesses aversive properties that strongly influence drug seeking. We have recently demonstrated that aversive responses to cocaine are mediated by excitatory serotonin 2c receptor (5HT2cR) signaling in the rostromedial tegmental nucleus (RMTg), a major inhibitory afferent to midbrain dopamine neurons. Furthermore, we have shown that weakened aversive responses to cocaine is mediated by the downregulation of 5HT2cR signaling, and predictive of increased cocaine seeking. Our lab has also identified an inbred strain of rat, the Lewis rat, that has uniformly low aversive reactions to cocaine as opposed to outbred strains where aversive responses to cocaine vary considerably between individuals. These Lewis rats also exhibit reduced 5HT2cR signaling in the RMTg accompanied by increased cocaine seeking after initial cocaine exposure. In the present study, we further investigated whether this reduction in Lewis rats is mediated by dephosphorylation of the 5HT2c receptor via phosphatase and tensin homolog deleted on chromosome 10 (PTEN). Using a runway operant task, we found that intracranial administration of the peptide Tat-3L4F, which blocks PTEN's interaction with 5HT2cRs, into the RMTg significantly increased latency to obtain cocaine in Lewis rats (n=5). In contrast, no change in latency to obtain cocaine was observed in a separate group of animals receiving a scrambled control peptide (n=2). These results strongly support the role of PTEN in increased cocaine seeking driven by the loss of sensitivity to the drugs aversive properties, and points towards its therapeutic potential for treating addiction vulnerability.

Disclosures: M. Hohmeister: None. **T.C. Jhou:** None.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R25NS080687
Neuro ID Fund

Title: Reward Anticipation Reduces H-Current in Midbrain Dopamine Neurons

Authors: *G. T. A. M. TIRADO;
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Abstract: Substance abuse is a recurring and intricate brain disorder marked by drug pursuit despite harmful consequences. The mesocorticolimbic system (MCL), governing pleasure, reward, and motivation, is profoundly impacted by substance abuse. Dopaminergic (DA) neurons, abundant in the Ventral Tegmental Area (VTA) of the MCL, represent a primary target for addictive substances. Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels

conduct a depolarizing current that significantly influences neural processes like resting membrane potential firing frequency and intrinsic excitability of VTA DA neurons. Previous results from our laboratory demonstrated that after non-contingent cocaine sensitization, VTA DA neurons I_h amplitude and membrane capacitance (C_m) are significantly reduced. However, it is unknown how a contingent drug self-administration paradigm alters these intrinsic properties and if pairing cues during drug administration are essential in altering VTA DA cells' intrinsic properties. This study investigates the impact of paring cues during passive cocaine and sucrose administration on I_h in VTA DA neurons, exploring the hypothesis that the associative learning of reward predictive cues modulates I_h. The objective was to elucidate whether cocaine self-administration or reward anticipation modulates VTA DA neurons I_h. Animal groups were divided into cocaine and saline IntA. Two groups of yoked controls were paired with the cocaine IntA subjects for comparison between drug administration and cue presentation during drug delivery. Yoked controls received non-contingent cocaine administration either with a light cue (Yoked + cue) or without it (Yoked - cue). Another cohort of animals underwent sucrose self-administration under conditions analogous to the IntA protocol and yoked controls. Following behavioral training, animals were sacrificed, and brain slices with VTA were isolated from their brains. Through in-vitro electrophysiology in rat brain slices, we analyzed I_h current, synaptic integration, Cm, and evoked action potentials. Our findings demonstrate a significant reduction in I_h amplitude in animals in the cocaine IntA and Yoked + cue control groups. I_h amplitude reduction was also evident in the sucrose IntA group and in the yoked +cue sucrose controls. These results suggest that the associative learning of cues modulates the I_h of VTA DA neurons. In conclusion, I_h modulation after cocaine and sucrose anticipation could be a general mechanism of reward processing.

Disclosures: G.T.A.M. Tirado: A. Employment/Salary (full or part-time):; Neuro ID. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Grant Number - R25NS080687.

Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR299.22/O4

Topic: G.09. Drugs of Abuse and Addiction

Support: R01DA053070

Title: Astrocyte Ca^{2+} in the dorsal striatum suppresses neuronal activity to oppose cue-induced reinstatement of cocaine seeking.

Authors: *N. TAVAKOLI[1], S. MALONE[2], T. ANDERSON[1], R. E. NEELEY[1], A. ASADIPOOYA[1], M. T. BARDO[2], P. I. ORTINSKI[1];
[1]Dept. of Neurosci., [2]Dept. of Psychology, Univ. of Kentucky, Lexington, KY

Abstract: Understanding cocaine use disorder remains a significant challenge within neuroscience community. Recent literature indicates that astrocytes may actively regulate cocaine seeking behavior. Astrocyte Ca^{2+} is speculated as a prominent mechanism underlying such regulation. However, the specific impact of astrocyte Ca^{2+} on neuronal activity in brain areas associated with cocaine seeking and reinstatement have not been previously investigated. To fill this gap, we overexpressed the cytosolic Ca^{2+} extruder pump, HPMCA2, in the dorsal striatum astrocytes of rats trained to self-administer saline or cocaine along with the neuronal Ca^{2+} reporter, GCaMP6f, in the same brain area. While no significant differences were noted during saline self-administration, HPMCA2 animals exhibited increased cocaine self-administration compared to animals injected with the control tdTomato virus. No behavioral differences were observed between either group during extinction. While no significant differences were observed between saline HPMCA2 and saline tdTomato animals during cue induced reinstatement, suppression of astrocytic Ca^{2+} led to increased cue-induced reinstatement in cocaine HPMCA2, relative to cocaine tdTomato animals. Subsequently, brain slices were collected from each animal for *ex vivo* calcium imaging. In slice imaging experiments, suppression of astrocyte Ca^{2+} increased the amplitude, an indirect measure of cell excitability, in the cocaine animals, but not the saline animals. Furthermore, suppression of astrocyte Ca^{2+} decreased the duration of neuronal Ca^{2+} transients in cocaine self-administering animals. Together these results were not associated with differences in neuronal response to exogenous cocaine and were not linked to differences in the overall extracellular Ca^{2+} levels. In a separate cohort of animals, we investigated the effects of reduced astrocyte Ca^{2+} on neuronal activity in awake behaving animals. Fiber photometry experiments, conducted throughout extinction and reinstatement sessions, were used to link neuronal activity to discrete behavioral events. Preliminary results indicate that the associations between lever press behavior and neuronal activation in the dorsal striatum may be regulated by astrocyte Ca^{2+}. On-going analyses investigate whether astrocyte Ca^{2+} regulation of neuronal excitability during discrete extinction and reinstatement of cocaine-seeking parallel the overall increase in neuronal excitability observed in the slice preparation.

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Poster

PSTR299: Cocaine: Seeking, Reinstatement, and Motivation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR299.23/O5

Topic: G.09. Drugs of Abuse and Addiction

Support: DA000633

Title: Dopamine D_3-receptor preferring D_3/D_2 partial agonists decrease psychostimulant use disorders-like behaviors in rats

Authors: *O. SOLER-CEDENO[1], H. ALTON[2], G.-H. BI[1], E. LINZ[3], C. D. VOGT[1], E. S. GOGARNOIU[1], Z. XI[4], A. H. NEWMAN[5];
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Abstract: While substance use disorders continue to pose a significant global public health challenge, there are currently no approved medications for treating psychostimulant use disorders (PSUD). Extensive research over the past decades suggests that targeting the dopamine D_3 receptor (D_3R) holds promise in treating PSUD. However, most of the research has focused on developing highly selective D_3R antagonists or partial agonists, which have shown limited efficacy in suppressing psychostimulant self-administration under conditions of low cost and high reward, often requiring high doses to attenuate drug-seeking behaviors in preclinical rodent models. Here, we propose that D3R-preferring partial agonists with concurrent D_2 receptor (D_2R) partial agonism may offer enhanced pharmacological efficacy against PSUD. In this study, we evaluated two novel D_3R-preferring D_3R/D_2R partial agonists, ESG-1-60 and ESG-1-61, derived from cariprazine, a clinically used D_3R-preferring D_3R/D_2R partial agonist for the treatment of bipolar disorder and schizophrenia, in animal models of addiction. We first investigated their effects on intravenous cocaine self-administration and cocaine-primed reinstatement of drug-seeking behavior and then evaluated their potential adverse effects in open-field locomotion and conditioned place preference/aversion (CPP/CPA) tests. Our results demonstrate that cariprazine, ESG-1-60, and ESG-1-61 reduced cocaine self-administration in both male and female rats, shifted cocaine self-administration dose-response curves downwards, attenuated motivation for cocaine-taking under progressive-ratio reinforcement, and inhibited cocaine-induced reinstatement of drug-seeking behavior. Chronic administration of ESG-1-60 also suppressed cocaine self-administration over five consecutive days of treatment. Importantly, these compounds did not induce locomotor inhibition or impairment. In CPP/CPA assays, cariprazine and ESG-1-61 induced significant CPA, while ESG-1-60 did not, supporting further evaluation. In conclusion, our study provides robust preclinical evidence supporting the potential efficacy of D_3R-preferring D_3R/D_2R partial agonists in treating PSUD without significant unwanted side effects commonly associated with D_2R blockade. (Supported by NIDA IRP)

Disclosures: O. Soler-Cedeno: None. **H. Alton:** None. **G. Bi:** None. **E. Linz:** None. **C.D. Vogt:** None. **E.S. Gogarnoiu:** None. **Z. Xi:** None. **A.H. Newman:** None.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR300.01/O6

Topic: G.09. Drugs of Abuse and Addiction

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Ministero dell'Università e della Ricerca PRIN 2022 PNRR Progetti di Rilevante Interesse Nazionale P202274WPN (DC, RC, FF)

Title: Translating human drug use patterns into rat models: exploring spontaneous interindividual differences via refined drug self-administration procedures

Authors: *G. D'OTTAVIO[1], S. PEZZA[2,1], I. REVERTE[2,1], C. MARCHETTI[2,1], S. ZENONI[1], J. MODONI[1], M. RUANO[3,1], M. S. MILELLA[5], R. LATTANZI[2], R. CICCOCIOPPO[6], F. FUMAGALLI[7], M. VENNIRO[8], A. BADIANI[4], F. BOIX[9], D. CAPRIOLI[2,1];
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Abstract: Background: The failure of preclinical research to translate into significant treatment advances may be due to animal models' failure to capture crucial aspects of human substance use disorder. Heroin and cocaine users exhibit substantially different patterns of drug-use, including distinct dosage, route, and frequency of administration. Yet, preclinical addiction research often uses drug self-administration and choice procedures based on discrete, as opposed to continuous dimension strategies, featured by pre-selected experimenter-imposed unit-doses spaced by timeouts. Implementation of these constraints result in standardized and similar behaviors (including patterns of drug-taking) across different drugs. Here, we contrasted discrete to continuous dimension strategies (i.e., self-selected doses without timeout) that allow to do so. Methods: We analyzed the drug-taking patterns and estimated (though a PK model) drug concentrations in the brain in distinct self-administration training conditions, characterized by discrete or continuous dimension strategies. We also assessed the motivation to take and seek drugs under these varying conditions and within the framework of drug-versus-social choice scenarios. Results: Overall, the implementation of continuous, as opposed to discrete dimensions strategies significantly heightened the motivation to take and seek heroin and cocaine. Drug-taking patterns and related pharmacokinetics profiles differed substantially between the two

dosing strategies and depending on the substance used. Finally, the continuous dosing method revealed, for the first time, volitional social withdrawal post-heroin administration—a phenomenon not observed with cocaine—in a subset of rats. Notably, interindividual differences in social withdrawal were predicted by the severity of heroin-taking patterns. Conclusions: The findings support the use of continuous dosing strategies in self-administration and choice experiments. Such approaches more faithfully replicate the nuanced behaviors associated with human drug use and are likely to reflect the underlying neural changes more accurately.

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Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

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Topic: G.09. Drugs of Abuse and Addiction

Support: Purdue Women's Global Health Institute
Grace M. Showalter Research Trust

Title: Escalation of intravenous fentanyl self-administration and assessment of withdrawal behavior in male and female mice

Authors: *Y. CHEN[1], T. XIAO[2], A. J. KIMBROUGH[2];
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Abstract: Background: The rise in overdose deaths from synthetic opioids, especially fentanyl, necessitates the development of preclinical models to study fentanyl use disorder (FUD). While there has been progress with rodent models, additional translationally relevant models are needed to examine excessive fentanyl intake and withdrawal symptoms. **Methods:** The study performed intravenous self-administration (IVSA) of fentanyl in male and female C57BL/6J mice for 14 days. Mechanical pain sensitivity during withdrawal was assessed using the von Frey test. Anxiety-like behavior was evaluated via the open field test one-week into abstinence and incubation of craving for fentanyl was assessed four weeks into abstinence. **Results:** Both male and female mice demonstrated a significant escalation in fentanyl intake over the 14 days of self-administration, with significant front-loading observed in the final days of self-administration. Increased mechanical pain sensitivity was present from 36- to 48-hour into withdrawal and increased anxiety-like behavior was found 1 week into abstinence. Four weeks into abstinence,

mice showed significantly higher active lever pressing than the final self-administration session prior to abstinence. **Conclusions:** The study establishes a translationally relevant mouse model of IVSA of fentanyl, effectively encapsulating critical aspects of FUD, including escalation of drug intake, front-loading behavior, withdrawal symptoms, and prolonged craving for drug into abstinence. This model offers a robust basis for further exploration into behavioral and neurobiological mechanisms involved in fentanyl dependence and potential therapeutic strategies.

Disclosures: Y. Chen: None. **T. Xiao:** None. **A.J. Kimbrough:** None.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

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Program #/Poster #: PSTR300.03/O8

Topic: G.09. Drugs of Abuse and Addiction

Title: Escalated oxycodone self-administration is associated with activation of specific gene networks in the rat dorsal striatum.

Authors: *A. Y. WABREHA[1], A. DAIWILE[3], M. T. MCCOY[2], J. L. CADET[2];
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Abstract: Escalated oxycodone self-administration is associated with activation of specific gene networks in the rat dorsal striatum.
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AbstractThe number of individuals diagnosed with opioid use disorder (OUD) has risen steeply because of the increased prescribing of opioid drugs like oxycodone for chronic pain relief. OUD is characterized by loss of control of drug taking, continued drug use in the presence of adverse consequences, and repeated relapses to drug taking. Repeated exposure to oxycodone self-administration can lead to addiction in certain rats while others remain unaffected. Understanding the molecular mechanisms between these two groups holds promise for developing strategies to combat addiction. To identify signaling pathways associated with oxycodone addition, this study used male Sprague-Dawley rats to self-administer oxycodone for 20 days according to short-(ShA, 3 h) and long-access (LgA, 9 h) paradigms. Animals were euthanized after 2 hours of self-administration cessation and their dorsal striata were used for RNA sequencing analysis. LgA rats escalated their oxycodone intake and developed into 2 phenotypes, named long-access high (LgA-H, addicted) and long-access lower (LgA-L, non-addicted) rats, based on the level of escalation and drug taken during the self-administration experiment. RNA sequencing revealed many differentially expressed genes in the oxycodone-

addicted rats in comparison to other groups. DAVID analysis indicated that some of these genes were involved in potassium transport, ATP binding, and regulation of synaptic processes. Ingenuity pathway analysis (IPA) revealed previous involvement of some genes in OUD and cognitive processes. RNA sequencing and RT-PCR analysis of dorsal striatum samples unveiled a significant upregulation of potassium channel genes in LgA-H rats, suggesting a potential molecular mechanism underlying addiction susceptibility.
Acknowledgments and Disclosures This work is supported by the department of health and human services/National Institutes of Health/National Institute on Drug Abuse/Intramural Research Program, Baltimore, MD, USA.

Disclosures: A.Y. Wabreha: None. **A. Daiwile:** None. **M.T. McCoy:** None. **J.L. Cadet:** None.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

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Program #/Poster #: PSTR300.04/O9

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA-NIH [1ZIADA000434-23]
FI2GM142476

Title: 'Dose-extending placebo effect' in a rat model of buprenorphine maintenance treatment

Authors: *K. PITTS[1], L. COLLOCA[2], Y. SHAHAM[3], J. J. CHOW[4];
[1]Natl. Inst. of Drug Abuse, BALTIMORE, MD; [2]PTSS, Univ. of Maryland Sch. of Nursing, Baltimore, MD; [3]Behavioral Neurosci. Br., IRP/NIDA/NIH, Baltimore, MD; [4]NIDA IRP, Baltimore, MD

Abstract: Background: Clinical studies reported that effective medication doses can be lowered by combining lower doses with cues previously paired with higher doses. This 'dose-extending placebo effect' has been shown for pain and other medical conditions, but not drug addiction. Here, we tested if the dose extending placebo effect can be observed in a rat model of opioid (buprenorphine) maintenance treatment. **Methods:** We first trained rats (n=8) to self-administer remifentanil (5 µg/kg/infusion, fixed ratio (FR) 5 schedule, 6 days). Next, we implanted buprenorphine minipumps (3 mg/kg/day) and continued training for self-administration but under an FR10 schedule in the presence of a discriminative cue (tone + houselight) for 20 sessions (2 sessions/day) for 10 days. Finally, we removed the minipumps and retrained the rats for remifentanil (FR5 schedule) without the buprenorphine-paired discriminative cue. During this time, we tested the effect of the cue, low- and high-buprenorphine doses (0.15 and 0.3 mg/kg, i.v.), and the low dose plus the cue (the dose-extending placebo manipulation) on remifentanil self-administration. **Results:** Rats learned to self-administer remifentanil and self-

administration was suppressed during buprenorphine maintenance. The discriminative cue and the low dose had no effect on remifentanil self-administration. The high dose decreased self-administration in 7 out of 8 rats and combining the discriminative cue with the low dose strongly decreased self-administration in all rats. **Conclusion:** We provide evidence suggesting that the 'dose-extending placebo' effect can be studied in a rat model of opioid maintenance. This treatment modality may translate into dose reduction during opioid maintenance treatment in humans.

Disclosures: K. Pitts: None. **L. Colloca:** None. **Y. Shaham:** None. **J.J. Chow:** None.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

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Program #/Poster #: PSTR300.05/O10

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA-IRP

Title: Modeling opioid maintenance treatment in Oprm1-Cre knock-in rats: effect of chronic buprenorphine on context-induced reinstatement of heroin seeking

Authors: *J. M. BOSSERT, K. E. CALDWELL, H. BONBREST, K. NEGISHI, R. MADANGOPAL, Y. SHAHAM;
NIH, NIDA, IRP, Baltimore, MD

Abstract: Background: We recently modeled opioid maintenance treatment in rats and found that chronic buprenorphine decreased several relapse-related behaviors, including context-induced reinstatement of heroin seeking. We also recently introduced a novel CRISPR-based *Oprm1-Cre* knock-in rat that allows us to visualize and manipulate μ-opioid receptor (MOR)-expressing cells in the brain. Here, we used the *Oprm1-Cre* rats to identify brain MOR-expressing cells involved in the inhibitory effect of buprenorphine on context-induced reinstatement of heroin seeking.Methods: We trained male and female *Oprm1-Cre* rats to self-administer heroin in Context A for 14 days (6-h/day). Two to three days later, we implanted osmotic minipumps containing buprenorphine (0 or 9 mg/kg/day). Five days after minipump surgery, we began extinction training in Context B for 8 days (6-h/day), followed by context-induced reinstatement in Contexts B and A (1-h/day). After the second test in Context A, we anesthetized the rats and extracted their brains for *in situ* hybridization. Results: There were no sex differences in heroin self-administration, extinction responding in Context B, or in context-induced reinstatement of heroin seeking. In both sexes, chronic buprenorphine decreased context-induced reinstatement of heroin seeking. We are currently performing RNAscope for c-fos (a neuronal activity marker) and iCre (marker of MOR-expressing cells) to determine which

MOR-dense brain areas are activated during context-induced reinstatement of heroin seeking and subsequently inhibited by chronic buprenorphine.Conclusions: Our study using the new *Oprm1-Cre* rats confirms previous results on context-induced reinstatement of heroin seeking. We will present our findings at the meeting.

Disclosures: J.M. Bossert: None. **K.E. Caldwell:** None. **H. Bonbrest:** None. **K. Negishi:** None. **R. Madangopal:** None. **Y. Shaham:** None.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

Location: MCP Hall A

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Topic: G.09. Drugs of Abuse and Addiction

Support: DA33641
DA045765

Title: Sensation seeking phenotype predicts motivation for fentanyl in male rats

Authors: *S. A. DE GUZMAN, T. J. SACKO, S. O'CONNOR, S. E. SWINFORD-JACKSON, M. H. JAMES, C. PIERCE;
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Abstract: Understanding behavioral biomarkers that are predictive of substance use disorder (SUD) is important for identifying individuals with a higher risk of developing a SUD. Clinical studies have established a link between heightened sensation seeking and an increased risk of SUD. In rats, sensation seeking can be measured by examining locomotor reactivity to a novel environment. Prior research by our group indicates that high-responder (HR) rats exhibit higher preferred baseline cocaine intake compared to low-responder (LR) rats on a behavioral economics procedure. Following cocaine self-administration on an intermittent access paradigm, HR rats displayed higher baseline cocaine intake, as well as lower demand elasticity (higher cocaine motivation) on a behavioral economics (BE) procedure after intermittent drug self-administration. The present study tested if these outcomes extend to the opioid fentanyl. Male HR and LR rats acquired fentanyl self-administration on a fixed ratio 1 schedule of reinforcement. Rats were then evaluated daily on a BE procedure for fentanyl until baseline demand was established. Rats then self-administered fentanyl under an intermittent access schedule for 14 days and were re-evaluated on the BE procedure. Our preliminary results indicated that HR rats displayed lower demand elasticity (higher motivation) for fentanyl compared to LR rats only after intermittent access to fentanyl, consistent with our previous findings with cocaine. These data suggest that the sensation seeking trait may serve as a

behavioral biomarker for predisposition to opioid use disorder. Future experiments should extend these studies to female rats.

Disclosures: S.A. de Guzman: None. **T.J. Sacko:** None. **S. O'Connor:** None. **S.E. Swinford-Jackson:** None. **M.H. James:** None. **C. Pierce:** None.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Contract No. 75N95019D00026

Title: Evaluation of the abuse liability of gabapentin and duloxetine in male and female rats using two approaches: intravenous self-administration and conditioned place preference

Authors: *Q. CHANG[1], E. DUGAN[2];
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Abstract: Evaluation of the abuse liability of gabapentin and duloxetinein male and female rats using two approaches:intravenous self-administration and conditioned place preference

Q. Chang[1], E.A. Dugan[1], W. Min[1], H. Buechler[1], P. V. Severino[1], D. A. Nicholson[1], S.A. Woller[2], W. Lacsina[1], S. Iyengar[2], T. Hanania[11]PsychoGenics, Paramus NJ; [2]NINDS/NIH 6001. Executive Boulevard, Rockville, MD 20852

In collaboration with the NIH HEAL Initiative Preclinical Screening Platform for Pain (PSPP), we evaluated the abuse liability properties of gabapentin and duloxetine in the intravenous self-administration (SA) and conditioned place preference (CPP) tests in male and female SD rats. SA took place in operant chambers where rats pressed a lever that delivered the test compound through a jugular vein catheter. An independent group design with n=10-12 was used. Rats were allowed to self-administer saline or vehicle (6% DSMO for duloxetine study), oxycodone (0.06 mg/kg/infusion, as positive control for both studies), gabapentin (0.3, 1 and 3 mg/kg/infusion) or duloxetine (0.3, 1 and 3 mg/kg/infusion) on an FR3 schedule. Acquisition training lasted 20 days. The results indicated that oxycodone 0.06 mg/kg/infusion possessed strong abuse liability, gabapentin did not show signs of abuse potential, and the groups with higher doses of duloxetine had significantly fewer infusion compared to the vehicle group. This suggests duloxetine may have aversive effects in the SA model. In the CPP study, an independent group design (N=14-16) was used. In the gabapentin study, there were five groups (saline, oxycodone 3 mg/kg, and gabapentin 3, 10 and 30 mg/kg); in the duloxetine study, there were also five groups (saline, oxycodone 3 mg/kg, and duloxetine 10, 30 and 100 mg/kg.) Compounds were administered

orally (5 ml/kg and 0 minute pretreatment, except saline and oxycodone, which were i.p. dosed with 1 ml/kg and 0 min pretreatment). Perceptive cues were applied to create a distinctive texture and visual features for the two compartments. A 10-day protocol was used and the study was videotaped. Rats were treated with saline on days 2, 4, 6 and 8, and with either oxycodone or gabapentin / duloxetine on days 3, 5, 7, 9. Animals were confined in the “drug compartment” or “saline compartment” immediately after administration for 20 minutes. Compared to saline, oxycodone induced significant CPP. None of the gabapentin or duloxetine groups showed bias between the two compartments. These studies confirmed the validity of both assays in screening the potential abuse liability as part of the NIH HEAL Initiative’s PSPP program towards discovering novel non-addictive analgesics.

Disclosures: Q. Chang: None. **E. Dugan:** None.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

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Topic: G.09. Drugs of Abuse and Addiction

Support: R21DA055047
R01DA053752

Title: Development of Wireless Equipment for Autonomous Rodent Infusion Tasks

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[2]Anesthesiol., [1]UCLA, Los Angeles, CA; [3]Anesthesiol., Washington Univ. In St. Louis, Saint Louis, MO; [4]Anesthesiol., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Despite major advances in both the preclinical and clinical addiction fields, the number of US citizens afflicted by substance use disorders (SUDs) and the lethal overdose outcomes has continuously increased over the past two decades. To tackle this alarming health issue, intravenous self-administration procedures have been used as the gold standard for translational rodent SUD models. However, despite being one of the most reliable procedures, with clear face validity, this procedure is still limited by its tethered nature, constraining its use to restricted spaces in which rodents are exposed to unenriched environments with limited or no access food, water, or social interaction. Availability of volitional social interaction and non-social rewards such as wheel running, and operant-delivered palatable foods can decrease consumption, escalation, and reinstatement of drug self-administration.To tackle the limitations imposed by current intravenous self-administration approaches, we are developing a wireless-controlled wearable drug reservoir connected to intravenous indwelling catheters and using Bluetooth mesh technology to enable homecage-based intravenous self-administration

procedures.Using a combination of 3D printed encapsulation and commercially available reprogrammed micro-pumps together with a non-invasive pulse oximeter, we demonstrate that wireless infusion of fentanyl (50ug.kg-1) is sufficient to produce respiratory depression in freely moving rats. Further, we demonstrate that our wearable devices do not impair horizontal locomotion and performances in operant sucrose self-administration in adult male and female rats. Additionally, we show that these devices can be effectively used in combination with other tethered approaches. Here, we used fiber photometry to reliably record neuronal activity in the ventral tegmental area following administration of fentanyl via our wireless device.Together, those newly developed wearable devices seem to be adapted to 1) perform home cage drug self-administration and 2) be combined with other tethered approaches to investigate neuronal circuits and ensemble responsible for consumption, escalation, and reinstatement of drug use.Overall, we envision that these new open-source approaches will broaden the translational value of pre-clinical SUD models by enabling new experimental designs to improve current strategies aiming at developing substance use disorders treatments.

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Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR300.09/O14

Topic: G.09. Drugs of Abuse and Addiction

Support: R01DA025634

Title: Sensor sensibility: Divergent measurements of dopaminergic neural activity to acute morphine administration via fiber photometry

Authors: *R. M. DONKA[1], M. K. LOH[2], M. F. ROITMAN[2], J. D. ROITMAN[2];
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Abstract: Opioid abuse remains a significant burden to public health despite reduction efforts in part due to their highly addictive nature. Morphine modulates the mesolimbic dopamine (DA) system, a critical pathway for reward processing, however the temporal dynamics of DA activity are not well defined. Here, we measured dopamine neural activity in response to acute morphine treatment using *in vivo* fiber photometry at both dopamine cell bodies in the VTA and terminals in the NAc. To measure VTA DA cell body dynamics, we injected Long Evans rats (TH:Cre^{+}) with a Cre dependent GCaMP6f (n = 16). To measure DA release dynamics, we injected Long Evans rats with GRABDA2H (n = 16) or dLight1.3b (n = 16) targeting the NAc lateral shell. Fiber optic cannulae were implanted 0.1mm dorsal to the injection site. Experimentation began four weeks post-surgery. Subjects were randomly assigned to saline control or morphine

administration groups. Two recording sessions were conducted per week on subsequent days: a saline control session followed a morphine administration test day session. Each session consisted of a 15-minute baseline and 60-minute post injection recording period. Doses were administered in escalating order (2.5, 5.0, 7.5, and 10 mg/kg, i.p.). Saline control animals received a volumetrically matched injection of saline on both baseline and test days. For each session, data were normalized to the pre-injection baseline and transient events were detected using a criterion of a 3SD increase above the local minimum value. GCaMP6f recordings of DA cell body activity demonstrated a dose-dependent increase in transient frequency relative to control sessions, with higher doses resulting in greater increases. dLight1.3b recordings demonstrated a similar dose-dependent increase in transient frequency. By contrast, GRABDA2H recordings demonstrated a robust and substantial decrease in both transient frequency and amplitude, conflicting with results obtained with both GcAMP6f and dLight1.3b. These experiments demonstrate, with high temporal precision, changes exhibited by VTA DA neurons in response to escalating doses of morphine, which is corroborated by release dynamics capture with dLight1.3b in the NAc lateral shell. GRABDA2H results are vastly divergent, potentially indicating saturation of the sensor. Together, these results support the use of multiple sensors and approaches to validate experimental findings. Future studies will evaluate the effects of chronic morphine administration and subsequent withdrawal on DA dynamics, which may help define neural processes underlying the rewarding effects of opioid use and the negative affective aspects of withdrawal.

Disclosures: R.M. Donka: None. **M.K. Loh:** None. **M.F. Roitman:** None. **J.D. Roitman:** None.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR300.10/O15

Topic: G.09. Drugs of Abuse and Addiction

Title: When passion persists: Deep brain recording from the nucleus accumbens shell reveals differential reactivity to opioid cues in a patient with extended recovery from opioid use disorder.

Authors: *A. CHILDRESS[1], L. QIU[2], Y. NHO[2], R. L. SEILHEIMER[1], P. S. REGIER[1], A. TUFANOGLU[1], N. HAGER[1], B. WILENT[2], K. SCANGOS[1], C. H. HALPERN[2,3]; [1]Psychiatry, Univ. PENN Perelman Sch. Med., Philadelphia, PA; [2]Neurosurg., Univ. PENN Perelman Sch. Med., Philadelphia, PA; [3]Surgery, Corporal Michael J. Crescenz Veterans Affairs Medical Center, Philadelphia, PA

Abstract: AIM: Individuals in active addiction often recount the power of drug “reminder cues” (e.g., sights, smells, sounds, and memories) to trigger drug desire (“craving”), drug-seeking and relapse. The deep brain circuits (e.g., nucleus accumbens [NAc], ventral pallidum, amygdala) activated by drug reward cues have been the focus of many functional imaging studies, but little is known about the longevity of cue-triggered brain vulnerability for individuals in sustained addiction recovery. We recently had a unique opportunity to record brain responses in the NAc to opioid cues in an individual with extended (4 year) recovery. We predicted that we would detect a low-frequency “reward anticipation” signal to the opioid cues, despite prolonged recovery -- and 160 mg daily methadone. METHODS: A 26 year-old male with a long history of severe opioid use disorder (OUD) had achieved 4 years of remission with deep-brain-stimulation (DBS) of the NAc. His pulse generator had been removed due to wound erosion, with a return of opioid craving. Prior to re-implantation of the pulse generator, we interrogated his existing electrodes for the NAc response to opioid cues, with several cue task sessions featuring alternating 6 s videos of drug (matched to the patient’s injection paraphernalia) and non-drug cues. We also tracked both the brain and subjective (“craving”) response to the cues during intermittent DBS stimulation of the NAc. RESULTS: As predicted, we demonstrated a robust (cluster-corrected, $p< 0.05$) low-frequency (1-8 Hz) response in the NAc to opioid (vs. non-drug) cues. The patient was dismayed that explicit visual cues proximal to self-administration (e.g., "booting", bringing blood up into the injection syringe) were highly evocative, despite several years without opioid misuse, and despite daily methadone. The brain response was correlated with drug craving, persisted across multiple sessions/days, and was subsequently blunted by DBS (5-6 mA) in the NAc (Please see concurrent 2024 posters, Drs. Qiu and Nho). CONCLUSION: To our knowledge, these recordings represent the first demonstration that a differential brain response (low-frequency, 1-8Hz) to opioid cues in the NAc may persist *even after several years of recovery*. The low-frequency brain response to opioid cues in our OUD patient is strikingly similar to the NAc brain response during palatable food cues in our patients with loss-of-control eating, suggesting a “cue-vulnerable endophenotype” that may be shared across multiple disorders. Cue-reactivity probes may be useful for revealing this vulnerability, and for optimizing the interventions (e.g., neuromodulation tools and their parameters) to address it.

Disclosures: A. Childress: None. **L. Qiu:** None. **Y. Nho:** None. **R.L. Seilheimer:** None. **P.S. Regier:** None. **A. Tufanoglu:** None. **N. Hager:** None. **B. Wilent:** None. **K. Scangos:** None. **C.H. Halpern:** None.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR300.11/O16

Topic: G.09. Drugs of Abuse and Addiction

Title: Assessing the influence of hyperbaric oxygen treatment on the rewarding effects of morphine and the alleviation of morphine withdrawal in male and female mice

Authors: *M. T. G. SOUTHARD[1], L. G. BAILEY[1], R. M. QUOCK[2], T. E. BROWN[1]; [1]Integrative Physiol. and Neurosci., [2]Psychology, Washington State Univ., Pullman, WA

Abstract: Opioid use disorder (OUD) presents a multifaceted challenge marked by its high relapse rates, profound health and social repercussions, extensive impact on individuals and communities, and the intricate interplay of physical and psychological factors driving the disorder. To combat the opioid epidemic effectively, it is imperative to find better treatment options. Recent independent studies have demonstrated the therapeutic potential of hyperbaric oxygen treatment (HBOT)—high pressure exposure to 100% oxygen—in alleviating opioid withdrawal symptoms in both humans and male mice. We have previously shown that HBOT significantly attenuates the physical symptoms of morphine withdrawal in male mice; however, no studies have examined this effect in females. Additionally, it remains uncertain whether HBOT can influence the rewarding and aversive behaviors associated with morphine exposure. Our experiments were designed to address this gap in knowledge by first, expanding upon the initial findings regarding therapeutic effects of HBOT on physical withdrawal symptoms by replicating this experiment and including female mice and second, exploring the potential therapeutic effects of HBOT on the rewarding properties of morphine and the aversion toward naloxone-induced morphine withdrawal. In addressing the potential sex differences in the efficacy of HBOT on physical withdrawal symptoms, we anticipated that treating morphine withdrawal with HBO may yield sex-dependent effects due to the different sensitivities toward opioids, opioid withdrawal, and the development of OUD between males and females. Our preliminary data supports the potential for sex effects of HBOT on the physical symptoms of morphine withdrawal. Future experiments will be conducted to explore the observed trends. For our second aim, we hypothesized that HBO exposure would diminish morphine induced conditioned place preference (CPP) and naloxone induced conditioned place aversion (CPA) in morphine dependent male C57BL/6 mice. HBOT resulted in a trend towards reduced withdrawal-associated aversion compared to control animals (Sham: -276.6 ± 42.0 sec, n=12; HBO: -208.8 ± 52.0 sec, n=14; $p=0.07$; $t= 2.0$). However, there was no statistically significant effect of HBOT on morphine-induced CPP ($p>0.05$). These outcomes suggest that while HBOT may not alter the rewarding properties of morphine, it holds promise in mitigating aversion toward environments associated with withdrawal. Further investigating the effects of HBOT on contextual drug-seeking and morphine withdrawal will provide insight into the mechanisms of HBOT and how it can best be utilized to treat OUD.

Disclosures: M.T.G. Southard: None. **L.G. Bailey:** None. **R.M. Quock:** None. **T.E. Brown:** None.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR300.12/O17

Topic: G.09. Drugs of Abuse and Addiction

Support: UG3DA050325
F30DA057043
Dr. Ed Saylor and his wife Janis Saylor

Title: Glucagon-like peptide-1 receptor agonist, liraglutide, attenuates fentanyl seeking behavior and associated c-Fos and tyrosine hydroxylase activation patterns in rat brains

Authors: *B. EVANS, K. T. NEWMASTER, F. A. KRONMAN, Y. KIM, P. S. GRIGSON; Neural and Behavioral Sci., Penn State Col. of Med., Hershey, PA

Abstract: Although there are pharmacological treatments for opioid use disorder (OUD), relapse rates remain high. New treatments, then, are essential. To that end, treatment with glucagon-like peptide-1 receptor agonists (GLP-1RAs), known satiety agents, reduce responding for ethanol, nicotine, cocaine, and opioids. Here, we used fentanyl self-administration and light sheet microscopy to measure fentanyl seeking, the associated brain activation patterns (i.e., c-Fos and tyrosine hydroxylase, TH), and reversal of both by treatment with the GLP-1RA, liraglutide, in rats. Male Sprague-Dawley rats were given 6 h to self-administer fentanyl (1.85 ug/infusion) across 14 trials. On day 15, all rats were injected with saline or liraglutide (0.3 mg/kg sc) and 6 h later placed in the drug self-administration chamber for a 90 min extinction test where cues were presented, but no drug was delivered. Afterwards, the rats were deeply anesthetized, and brains prepared for light sheet microscopy. High drug takers self-administered more fentanyl/6h across trials 1 - 14 than did the low drug takers. During the 90 min extinction test, all rats pretreated with saline exhibited high seeking for fentanyl and an increase in expression of c-Fos and TH in areas associated with reward and aversion; this seeking behavior, and brain activation, was blocked by treatment with the GLP-1RA. Together, these data show that fentanyl taking and seeking in the most vulnerable drive neuronal activity in substrates involved in drug need and in reward/seeking and that treatment with the satiety agent, liraglutide, reverses both drug-seeking behavior and the associated patterns of brain activation.

Disclosures: B. Evans: None. **K.T. Newmaster:** None. **F.A. Kronman:** None. **Y. Kim:** None. **P.S. Grigson:** None.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR300.13/O18

Topic: G.09. Drugs of Abuse and Addiction

Title: Oxytocin attenuates stress-induced oral oxycodone-seeking behavior under a progressive ratio schedule in male and female rats

Authors: *N. E. CORNEJO, T. Q. YATES, M. KANEKO, R. J. RAGER-AGUIAR, S. HO, K.-C. LEONG;
Neurosci., Trinity Univ., San Antonio, TX

Abstract: Opioid use disorder (OUD) has become increasingly prevalent in the United States with increased availability and access to prescription opioids. OUD is characterized by chronic opioid abuse, craving, and periods of relapse which have been shown to be instigated and exacerbated by stress. Oxycodone (OXY) is the most commonly prescribed opioid and the third-leading cause of opioid overdose deaths. Stress often prompts and increases OXY-seeking behavior. Oxytocin (OXT), a neuropeptide with known anxiolytic effects, has been shown to attenuate drug-seeking behavior within rat models and is a promising therapeutic for treatment of OUD. We trained male (n = 8) and female (n = 7) Sprague-Dawley rats in an operant conditioning paradigm to observe the possible attenuating effect of OXT on stress-induced OXY-seeking behavior. Briefly, a sucrose-fading paradigm was used to train animals to press an active lever for a short period of access to OXY solution (0.1 mg/ml). After establishing stable responding at FR5, animals were administered the pharmacological stressor yohimbine (2 mg/kg, i.p.) prior to a progressive ratio test where increased active lever presses were required for OXY access. When yohimbine and OXT (1 mg/kg, i.p.) were concurrently administered, OXT attenuated the yohimbine-induced increase in progressive ratio responding across both males and females as shown through a one-way ANOVA. Break point was significantly lowered from the yohimbine-only test relative to the concurrent yohimbine and OXT test. These results demonstrate OXT's amelioration of stress-induced OXY-seeking behavior across sexes and invite further investigation of OXT as a treatment for OXY addiction and broader OUD.

Disclosures: N.E. Cornejo: None. **T.Q. Yates:** None. **M. Kaneko:** None. **R.J. Rager-Aguiar:** None. **S. Ho:** None. **K. Leong:** None.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR300.14/O19

Topic: G.09. Drugs of Abuse and Addiction

Title: The effect of peripheral and central routes of oxytocin administration on oxycodone conditioned place preference in male and female rats

Authors: *K. N. DIXON, P. BENSING, J. GRIFFIN, K.-C. LEONG;
Neurosci., Trinity Univ., San Antonio, TX

Abstract: Opioid addiction presents significant health and socio-economic challenges, with existing limited viable pharmacological interventions. Based on its known ability to modulate reward processing, current research suggests the neuropeptide oxytocin (OT) as a promising contender in the treatment of opioid addiction. The present series of experiments examined the effects of centrally and peripherally administered OT on oxycodone (OXY) preference behavior utilizing a conditioned place preference (CPP) in adult male and female Sprague-Dawley rats. Briefly, animals are placed in a two-chamber apparatus where one chamber is paired with OXY (3 mg/kg; i.p.) and the other with saline. Vehicle-treated males and females displayed moderate OXY CPP. Interestingly, peripheral administration of OT (1 and 3 mg/kg, i.p.) prior to testing had no effect on OXY CPP. However, central administration of OT at test resulted in attenuation of OXY CPP. Together, these results suggest that centrally-administered, but not peripherally-administered, OT may modulate OXY preference behavior. Overall, these results support a growing body of literature indicating that OT may be a viable modulator of addiction related behaviors and that route of administration should be taken into consideration in evaluating the viability of OT as a therapeutic target.

Disclosures: K.N. Dixon: None. **P. Bensing:** None. **J. Griffin:** None. **K. Leong:** A. Employment/Salary (full or part-time):; Trinity University.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR300.15/O20

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA IRP

Title: Developing A Demand Elasticity Task For Opioid Self Administration in Squirrel Monkeys

Authors: *M. ZARCONE[1], A. JOHANSEN[2], J. C. HECKER[3], J. J. CHOW[4], H. P. JEDEMA[5], C. W. BRADBERRY[5];
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Abstract: Recent literature suggests that effort based economic demand curve analyses are an effective way to assess differences in motivation to consume drugs. Relevant variables obtained are demand elasticity (α) and Q_0: the point at which animals would consume drug under unrestricted conditions. Using squirrel monkeys trained on the economic choice task shown on the accompanying poster, wherein 60 sec intertrial intervals (ITI) were in place, a shorter ITI was desired for conducting the effort-based demand procedures. Therefore, we compared responses on a 20 sec ITI task with those of the 60 sec. Evaluating the animals' performance on the new reduced ITI task would inform the feasibility of developing a demand elasticity task. On a touchscreen, subjects choose stimuli corresponding to differing amounts of either a remifentanil (0.08 µg/kg per symbol) or milk reward (75µl/kg per symbol). At baseline, there was a 60 second ITI between each trial. The following week, the ITI was reduced to 20 seconds. Response latencies significantly increased from the 60 to 20 second ITI task which indicates that animals self-regulate drug intake, safeguarding against overdose. The shortened ITI task did not alter animals' preference for either food or drug reward. These data suggest that animals would safely self-administer drug when repeatedly given the option to with limited time out in a demand elasticity task. We then developed a within session demand elasticity task for the purpose of investigating the effects of different pharmacological pretreatments on consumption of drug in response to changes in price. In this task, animals are presented with a single drug symbol at random locations on the screen. The task is comprised of 11 10 minute blocks, where the dose of remifentanil decreases with each block. As such, the price per unit amount of drug increases as the blocks advance. Animals' responses for remifentanil are decreasing across blocks in an orderly way, with an average Q_0 of 5.39 ± 1.13 and average α of 0.008 ± 0.002 across subjects (*n*=6). We intend to administer treatments for opioid use disorder that are clinically available, such as buprenorphine, as well as novel compounds to investigate their effects on motivation to acquire drug.

Disclosures: M. Zarcone: None. **A. Johansen:** None. **J.C. Hecker:** None. **J.J. Chow:** None. **H.P. Jedema:** None. **C.W. Bradberry:** None.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR300.16/O21

Topic: G.09. Drugs of Abuse and Addiction

Support: Supported by NIDA IRP

Title: The Effects of Orexin Antagonism on Remifentanil Choice and Demand in Squirrel Monkeys

Authors: *A. N. JOHANSEN[1], M. J. ZARCONE[2], J. C. HECKER[3], H. P. JEDEMA[4], C. W. BRADBERRY[4];
[1]NIDA Intramural Res. Program (NIH), Baltimore, MD; [2]NIDA Intramural Res. Program, NIH, Baltimore, Baltimore, MD; [3]Natl. Inst. on Drug Abuse, NIH, NIDA IRP, Baltimore, MD; [4]NIDA Intramural Res. Program, NIH, Baltimore, MD

Abstract: Recently, the literature has focused on the orexin system as a possible therapeutic target for substance use disorders. Pre-clinical rodent studies suggest that high doses of both single and dual orexin receptor antagonists may attenuate opioid self-administration, decrease opioid-seeking behavior during reinstatement, and increase demand elasticity. The present study's primary aims are (i) to elucidate the effects of orexin antagonists on economic choice between remifentanil and milk and (ii) to examine the impact of orexin antagonism on motivation for remifentanil in squirrel monkeys. Namely, we hypothesized that dual orexin antagonist suvorexant and orexin-1 antagonist SR-9659 would dose-dependently attenuate drug choice. To this end, squirrel monkeys (n = 7) received daily intravenous pre-treatments of orexin antagonists before behavioral sessions. For the choice task, indifference values (the point at which subjects displayed an equal probability of selecting drug or milk) served as our primary outcome of interest. Effective treatments shift responding away from drug toward the non-drug alternative. Because suvorexant treats insomnia, we also examined sleep using actigraphy monitors. Results on the choice task showed no effect of either antagonist on indifference values. Suvorexant increased sleep, while the SR compound had no effect. Moving forward, we will also evaluate this class of compounds using an effort based economic demand approach to evaluate their effect on remifentanil demand elasticity. For this task, we designed a remifentanil threshold self-administration procedure. In short, sessions were divided into 11 10-minute blocks, where the unit dose of drug systematically decreases between blocks within each session. Varying "price" by decreasing the unit dose of drug available for the same amount of effort allows us to construct demand curves. The accompanying poster presents the behavioral results following adaptations to the self-administration procedure needed for the new task, and initial demand curve analyses.

Disclosures: A.N. Johansen: None. **M.J. Zarcone:** None. **J.C. Hecker:** None. **H.P. Jedema:** None. **C.W. Bradberry:** None.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR300.17/O22

Topic: G.09. Drugs of Abuse and Addiction

Support: VABHS Core Funds

Title: Progesterone decreases opioid seeking during abstinence in a rat model of opioid use disorder

Authors: *J. A. BABB[1], E. B.-L. MANESS[2];
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Abstract: Recent evidence suggests that intensity of withdrawal symptoms is a predictor of relapse to opioid use in women but not in men. Additionally, relapse risk is generally attenuated during the luteal phase of the menstrual cycle when progesterone is relatively high, compared to the follicular phase when progesterone is low, suggesting that this hormone may have clinical utility for decreasing relapse risk among people with opioid use disorder (OUD). In this study, we tested the hypothesis that acute treatment with progesterone could decrease opioid seeking behavior during acute abstinence in female rats using a rat model of OUD. Young adult female rats were initially trained to intravenously self-administer oxycodone in daily 1-hour sessions for 4 days. Rats were then shifted to extended access sessions (6 hr/day) daily for 10 days. Estrous cycle stages were monitored via daily vaginal lavage. The morning after the 10th extended access self-administration session, rats were injected s.c. with either vehicle (n = 7) or 2 mg/kg progesterone (n = 10). Thirty minutes later, rats were tested for cue-induced oxycodone seeking for 30 minutes. All rats acquired self-administration behavior. Rats in both treatment groups significantly escalated their oxycodone intake ($F_{1.764, 26.47} = 38.89$; $p < 0.0001$) and responding for oxycodone ($F_{1.73, 25.95} = 18.09$; $p < 0.0001$) over the 10 days of extended access self-administration. There was no impact of estrous cycle stage on oxycodone responding (i.e., lever presses) or intake during self-administration. However, rats that were treated with progesterone had significantly fewer active lever presses during the cue-induced oxycodone seeking test than rats that were treated with vehicle ($t_{15} = 2.27$; $p = 0.038$). These data suggest that progesterone can reduce drug seeking (i.e., craving) for oxycodone during acute abstinence. These data could have important implications for relapse prevention and/or treatment of OUD in women.

Disclosures: J.A. Babb: None. **E.B. Maness:** None.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR300.18/O23

Topic: G.09. Drugs of Abuse and Addiction

Title: Transcranial Magnetic Stimulation of Dorsal Striatum Circuits in Relapse to Oxycodone Seeking

Authors: *S. LIN[1], Z. MA[1], Y. DUAN[1], H. NGUYEN[1], H. LU[2], Y. YANG[3];
[1]NIDA IRP, Baltimore, MD; [2]Natl. Inst. on Drug Abuse, Natl. Inst. on Drug Abuse, Baltimore, MD; [3]Natl. Inst. of Drug Abuse, Baltimore, MD

Abstract: Addiction is a chronic brain disease often characterized by uncontrolled drug taking and seeking behavior despite harmful consequences. Recent studies using functional magnetic resonance imaging (fMRI) in the rat brain showed that changes in functional connectivity between the orbitofrontal cortex and dorsal striatum is associated with oxycodone seeking after abstinence. In a follow-up study, we found that inactivation of the dorsal medial striatum (DMS) increases its functional connectivity with multiple frontal cortex regions. Recently, our lab developed a rodent model of transcranial magnetic stimulation (TMS) neuromodulation paradigm with advanced pulse patterns such as intermittent theta burst stimulation (iTBS) and continuous theta burst stimulation (cTBS). Previously, we combined the iTBS paradigm with our rodent drug relapse model to non-invasively modulate the DMS-based circuit and examine the effects on incubated relapse to drug seeking behavior. When comparing rats that received iTBS with those receiving sham TMS, we observed excitatory effects resulting in more lever presses within the TMS modulation group. In this study, to further understand the neural mechanisms underlying impulsive choice decision-making in drug taking, we modulated the DMS-based circuit with cTBS, a type of TMS modulation pulse pattern with opposite effects of iTBS modulation, to see if this new stimulation paradigm can reduce relapse to oxycodone seeking significantly. Overall, we observed that there was a general decreasing trend in lever presses when comparing the cTBS group with a sham group. In the future, we will perform additional experiments to confirm the effects of iTBS and cTBS paradigms on the drug seeking behavior.

Disclosures: S. Lin: None. **Z. Ma:** None. **Y. Duan:** None. **H. Nguyen:** None. **H. Lu:** None. **Y. Yang:** None.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR300.19/O24

Topic: G.09. Drugs of Abuse and Addiction

Support: National Institute of General Medical Sciences of the NIH under award number: 1SC3GM130430-03 to RR

Title: Environmental enrichment reduces cue-induced reinstatement of heroin seeking in the extended-access heroin self-administration model

Authors: *A. VASHISHT[1,2], S. VINOD[2], T. ALI[2], N. SANDOVAL[2], Z. NACHSHON[2], S. GUBIN[2], R. RANALDI[2], H. GOLDSTEIN[2];

[1]Grad. Center, City Univ. of New York, New York, NY; [2]Queens College, City Univ. of New York, Flushing, NY

Abstract: Contemporary long-term treatments for heroin use disorder demonstrate only limited efficacy against relapse. Our behavioral studies have previously demonstrated that environmental enrichment (EE), administered as a treatment after stable heroin seeking behavior is established, reduces cue-induced reinstatement of heroin seeking in rats. This study was aimed at extending these "anti-relapse" effects of EE to rats that received *extended access* to heroin intravenous self-administration (IVSA). The experimental design had 4 operant conditioning phases: 3-hr acquisition of heroin IVSA (phase 1), 6-hr *extended access* heroin IVSA (phase 2), extinction (phase 3), followed by a cue-induced reinstatement test. In Phase 1, male and female rats were trained to self-administer heroin over daily 3-hr sessions for 15 days. In Phase 2, rats were transitioned to daily 6-hr IVSA sessions for 15 days. Then, rats were assigned to EE or non-EE housing where they remained for the rest of the experiment. Three days following assignment, rats started Phase 3 consisting of 15 daily 2-hr extinction sessions. During all phases, active and inactive lever presses were recorded to establish and test for drug seeking. Following the last extinction day, all rats were tested for cue-induced reinstatement of heroin-seeking where active presses produced the drug-cue but no drug infusion. We found that EE significantly reduced cue-induced reinstatement. These data indicate the generality of the anti-relapse effects of EE even to the extended access IVSA model, a model thought to closely represent human addiction. This underscores the promising potential of implementing EE as a long-term sustainable treatment strategy for heroin use disorder.

Disclosures: A. Vashisht: None. **S. Vinod:** None. **T. Ali:** None. **N. Sandoval:** None. **Z. Nachshon:** None. **S. Gubin:** None. **R. Ranaldi:** None. **H. Goldstein:** None.

Poster

PSTR300: Opioids: New Models, Techniques, and Therapeutics

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR300.20/O25

Topic: G.09. Drugs of Abuse and Addiction

Title: Environmental Enrichment Attenuates Fentanyl Use and Stress Induced Drug Seeking Behavior

Authors: *J. DEARMAN[1,2], J. A. HIGGINBOTHAM[3], J. MORON-CONCEPCION[4]; [1]Anesthesiol., Washington Univ. in St. Louis, St. Louis, MO; [2]Anesthesiol., Washington Univ. Sch. of Med., St. Louis, MO; [3]Washington Univ. Sch. of Med., st. louis, MO; [4]Washington Univ., Saint Louis, MO

Abstract: Opioid misuse is a universal growing crisis. Between 1999 and 2021, nearly 645,000 people in the United States had a fatal opioid overdose. Furthermore, during the COVID-19 pandemic in 2020, with increasing stress and isolation, there was a 30 % increase in drug overdose deaths from 2019. This crisis has been primarily driven by fentanyl, a potent and synthetic opioid: 50 times stronger than heroin and 100 times stronger than morphine. According to human and animal model studies, a key driver in increased opioid seeking behavior and relapse is stress. Furthermore, animal studies have shown that implementation of environmental enrichment (EE) can decrease reinstatement for both drug and natural rewards. However, it has not been examined whether stress-induced reinstatement of opioid seeking behavior, opioid intake, or stress hormone signaling can be mitigated by implementation of long-term EE. Therefore, we hypothesized that long term exposure to novel EE is sufficient to reduce fentanyl use and prevent stress-induced reinstatement of drug seeking behavior, and these effects will be paralleled by blood serum corticosterone concentrations as a quantitative measure of stress. Using an animal model of stress-induced relapse, male and female Long-Evans rat (n=15) received EE (n=6) or non-enriched (NE; n=9) housing conditions and underwent 15 two-hour sessions of fentanyl self-administration (5 and 2 µg/kg/infusion) followed by 7 extinction sessions (no fentanyl), and a final test of stress-induced reinstatement pharmacologically induced by Yohimbine (2.5, mg/kg, i.p.). Samples of blood serum corticosterone were obtained weekly following training sessions to assess blood serum corticosterone levels at critical time points throughout training. Consistent with our hypothesis, EE decreased fentanyl intake throughout fentanyl self-administration and decreased drug seeking behavior during reinstatement relative to NE. Interestingly, serum corticosterone levels were similar between EE and NE groups, but overall, females had elevated corticosterone levels compared to males. These findings suggest that EE can suppress maladaptive fentanyl use and stress induced relapse and reveal sex differences in stress reactivity associated with opioid use. Together, this work highlights the need to better understand individual vulnerabilities to stress during opioid use and points to the therapeutic potential of environmental factors influencing the effects of stress on opioid use.

Disclosures: J. Dearman: None. **J.A. Higginbotham:** None. **J. Moron-Concepcion:** None.

Poster

PSTR301: Neural Mechanisms of Decision Making in Human and Non-Human Primates

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR301.01/O26

Topic: H.03. Decision Making

Support: NIH/NEI R01019041
NIH T32GM007281
DOD VBFF
NIH F30EY033648

Title: Neuronal encoding of rapid categorical decisions and behavioral biases across primate oculomotor areas LIP, FEF, and SC.

Authors: *O. ZHU[1], V. SHIRHATTI[2], K. W. LATIMER[3], S. DAVID[3], S. CHANG[4], A. MEDOFF[1], D. J. FREEDMAN[5];
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Abstract: Our ability to rapidly categorize stimuli is an essential cognitive process that imparts meaning to sensory input and guides behavior. While previous studies have explored the oculomotor system's role in categorical decisions, few have examined this process when motor plans are rapidly updated by incoming sensory information. In this study, we designed an urgent oculomotor motion categorization task based on the compelled-saccade paradigm. This paradigm limits the viewing time of motion stimuli by a random duration on each trial, exploiting the natural variability in reaction times to assess behavior across a range of viewing times: from uninformed decisions with short viewing times to accurate decisions with longer viewing times. We recorded populations of neurons using up to six linear microelectrode arrays (Plexon V-Probes) from the lateral intraparietal area (LIP), frontal eye field (FEF), and superior colliculus (SC) in two monkeys. Each session yielded about 50 well-isolated single units per area. Using linear support vector machines, we found that population responses in each area reliably encoded task-related variables such as stimulus category and saccade direction prior to saccade onset. In both monkeys, FEF encoding of motion category and saccade direction occurred significantly earlier than in SC and LIP ($p < 0.001$, two-sample K-S test), while LIP encoding of motion direction was significantly earlier than in FEF and SC ($p < 0.001$, two-sample K-S test). These results demonstrate that FEF is the leading area mediating oculomotor categorical decisions. Both monkeys exhibited significant category and saccade biases on trials with short stimulus viewing times, leading them to preferentially select specific categories and saccade directions when stimulus information was limited. In ongoing analyses, we quantify how the population activity of FEF, LIP, and SC relates to these behavioral biases. First, we trained binary linear classifiers (both SVM and logistic regression) to predict category or saccade direction using neural activity around saccade onset. When tested on activity around stimulus onset (where classifiers typically show chance accuracy), these classifiers exhibited similar biases in predicting one category or saccade direction that are consistent with the monkeys' behavior. This suggests that the monkeys' rapid behavior relies on priming neural activity to be close to the final neural states of categorical decisions and motor outputs, enabling faster decisions when stimuli align with their existing plans but causing slower decisions when these plans must be overwritten.

Disclosures: O. Zhu: None. **V. Shirhatti:** None. **K.W. Latimer:** None. **S. David:** None. **S. Chang:** None. **A. Medoff:** None. **D.J. Freedman:** None.

Poster

PSTR301: Neural Mechanisms of Decision Making in Human and Non-Human Primates

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

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Title: Rapid emergence of learned abstract encoding in primate FEF, LIP, and hippocampus during saccade based foraging

Authors: *V. SHIRHATTI[1], S. DAVID[2], G. NELSON[2], S. CHANG[4], K. SERGEEVA[1], S. M. LANDI[5], E. A. BUFFALO[6], D. J. FREEDMAN[3];
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Abstract: Humans and other animals are adept at rapid learning and generalization across sequentially learned experiences. To study the neural basis of rapid task learning in non-human primates we developed a foraging-based associative learning paradigm under which monkeys rapidly learned novel stimulus-reward associations in single sessions. In the saccade foraging task (SFT) monkeys freely explored an array of 16 or 24 visual stimuli for 15s on each trial and harvested associated rewards by fixating a chosen stimulus for a specified duration (1.2s). Stimuli were associated with different levels of rewards, with the stimulus features (e.g. color, category) predictive of reward size. The monkeys could optimize their reward amounts by learning to choose targets with features associated with higher rewards. They successfully learned stimulus-reward associations for stimulus sets with hierarchies between 3 or 4 distinct color-reward associations, or 3 space-reward maps or even 2 natural categories (e.g. faces, vehicles) within single sessions, demonstrating remarkably rapid within-session learning. During each problem set, we recorded neuronal populations simultaneously in 2 to 5 brain areas using up to 7 multi-electrode arrays (32 channels each) to examine population encoding during task performance in brain regions involved in oculomotor control - frontal eye field (FEF), lateral intraparietal area (LIP), and superior colliculus (SC) - and areas engaged in reward-based learning and memory, including orbitofrontal cortex (OFC) and hippocampus. FEF activity exhibited abstract encoding of reward value which generalized across successively learned problems with distinct visual stimuli. By contrast, encoding in hippocampus was more context specific, exhibiting rapid discrimination between learned stimuli within a problem set during learning, but not generalized across sequentially learned problems. Population responses in LIP showed a mixture of generalized and problem specific representations. These results suggest

complementary roles for these areas during learning of problem sets, wherein the cortical areas FEF and LIP develop and maintain abstract schema representations which generalize across contexts, while the hippocampus forms context specific cognitive maps that may facilitate rapid learning of novel problems within a context. Ongoing analyses of simultaneous recordings from subsets of these and other recorded brain regions are aimed at determining their specific roles during the learning process, and understanding how they contribute to the animals' foraging behavior during this naturalistic decision-making task.

Disclosures: V. Shirhatti: None. **S. David:** None. **G. Nelson:** None. **S. Chang:** None. **K. Sergeeva:** None. **S.M. Landi:** None. **E.A. Buffalo:** None. **D.J. Freedman:** None.

Poster

PSTR301: Neural Mechanisms of Decision Making in Human and Non-Human Primates

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Topic: H.03. Decision Making

Support: NIH U-19 Grant ID 5U19NS107609-03

Title: Cross-problem generalization in primate hippocampus and prefrontal cortex underlies schema-based 'learning to learn'

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Abstract: Learning from one's environment is fundamentally rooted in prior experience: constructing and adapting mental 'schemas' to interpret new situations and respond appropriately. As a schema is formed and applied to new scenarios, the rate of learning increases exponentially, demonstrating the concept of 'learning to learn'. Here we examine neuronal encoding within and interactions between primate hippocampus (HPC) and prefrontal cortex (PFC) during rapid associative learning across multiple problems. We trained one female, and are preparing one male rhesus macaque monkey, to learn 24 sequential problems of a visual association task. In each problem, animals learn to associate two arbitrary stimulus images with two patterned targets and indicate their choice with a saccade. After reaching the learning criterion of 180/200 correct consecutive trials, a novel problem pair of images is introduced, and must be learned and mapped to the same target patterns. Consistent with learning to learn, the first monkey demonstrates an exponential decrease ($R2 = 0.778$) in trials-to-criterion across 24 learned problems. We used semi-chronic electrode arrays to record from PFC (areas 9/46, 21-26 multiunit channels / session) and HPC (primarily CA3, 14-25 / session) during learning. All but the earliest sessions include both familiar and novel problems. Since target location is

randomized, we can dissociate saccade planning from image-target association, and identify separate neural subspaces specific to these computations, as well as decode task variables independently. Demixed Principal Component Analyses reveal response and association subspaces shared across problems in both PFC and HPC. These subspaces guide low-dimensional trajectories of neural activity, which diverge increasingly according to their respective task variables as learning progresses. Within session Support Vector Machine decoding of saccade direction and image association similarly supports cross-problem generalization of neural information, increasing with problem progression. Our combined preliminary results of increased low-dimensional trajectory separation and cross-problem decoder accuracy across learning suggest a cross-regional computational basis for schema-based learning to learn. This simultaneous multi-region evidence of gradual changes in neural task representations and coding generalization during learning demonstrates an underlying neural basis of primates' application of internalized schemas to new situations.

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Poster

PSTR301: Neural Mechanisms of Decision Making in Human and Non-Human Primates

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

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Topic: H.03. Decision Making

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Title: Cognitive variables are encoded in primate oculomotor networks and in eye movements

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Abstract: Intelligent behavior is facilitated by abstraction - the capacity to generalize learned information flexibly across stimuli and contexts. Our lab has studied abstract decisions through neurophysiological recordings in macaques performing a delayed match-to-category (DMC) task. In DMC, animals group dot-motion stimuli into categories according to a learned category boundary, and indicate via a manual report whether sequences of these stimuli belong to the same category or not. During the delay period (>= 1s) between sample and test stimuli, animals need to maintain information about the sample stimulus in an abstract format that generalizes across motion directions within a category. Recently, we found that the superior colliculus (SC) and lateral intraparietal area (LIP), core regions involved in oculomotor processing, rapidly and strongly encode abstract categories during DMC, and that their inactivation impairs DMC performance. These findings implicate the primate oculomotor network in mediating abstract

cognitive tasks, even those without demands to covertly shift attention or to report decisions with eye movements. To study the oculomotor system's role in mediating abstract cognitive tasks, and how it relates to the system's known role during eye movements, we re-analyzed neural activity and behavior during DMC performance across 18 datasets from 11 macaques (1058 sessions total, range: 18-115 per dataset, ~300 correct trials/session). In all datasets, we found evidence that these areas' cognitive and oculomotor roles are not fully orthogonal: small eye movements differed according to the sample category (0.38 +/- 0.08 dva, mean +/- SEM across datasets), particularly during DMC's memory delay. This trend emerged well after the onset of category encoding in LIP/SC, and despite the requirement to maintain central fixation for the entirety of every trial. Analysis of neural activity in oculomotor areas (LIP, SC, and the frontal eye fields, FEF) revealed a strong tendency toward category selectivity during the delay (mean category tuning index: LIP: 0.19, SC: 0.24, FEF: 0.35). These neural/behavioral signatures were attenuated during a task with the same stimuli and timings, but which did not require grouping stimuli into categories. This suggests that oculomotor areas may specifically be engaged by DMC's demand for abstraction, rather than just by maintenance of information in short term memory. Ongoing analyses of behavior during inactivation of LIP/SC aim to determine the extent to which this role is independent of these regions' known role in eye movement control.

Disclosures: M. Rosen: None. **D.J. Freedman:** None.

Poster

PSTR301: Neural Mechanisms of Decision Making in Human and Non-Human Primates

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Topic: H.03. Decision Making

Support: Project PRIN 2022 (CUP B53D23014270006)

Title: Dynamics of neural oscillations in the dorsal Premotor Cortex during a Transitive Inference task

Authors: *I. B. MARC[1,2], V. GIUFFRIDA[1,2], F. DI BELLO[1], P. PANI[1], S. FERRAINA[1], E. BRUNAMONTI[1];
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Abstract: The transitive inference task assesses inferential reasoning in humans and animals by leveraging prior knowledge to handle novel scenarios. It involves manipulating items based on their rank order. Participants first learn the hierarchical relationships between adjacent pairs of items (e.g., A>B>C>D>E>F), then infer relationships between non-adjacent items during testing (e.g., B>D). This setup enables parameterizing the inferential difficulty level, known as the

Symbolic Distance (SD) effect. Participants create a spatially organized mental schema of the ranked items, organizing closely ranked items nearby. However, noise from item proximity complicates determining their relative rank. Previous research has found neuronal modulation in the PMd's single-unit activity after presenting item pairs, suggesting its involvement in encoding decision-making variables. Yet, untangling its dual role in cognitive and motor processing, particularly in tasks linking motor actions to decision outcomes, remains challenging. Here we investigate how PMd's oscillatory activity, particularly in Theta (4-7.5Hz), Alpha (8-13Hz), Beta (16-26Hz), and High Gamma (70-120Hz) bands -a proxy for neural spiking- relate to the complexity of manipulation introduced by the SD. Two monkeys were trained to select higher-ranked items, regardless of screen position, and then tested on inferring relationships between new item pairs. Performance during these comparisons showed decision difficulty modulation by the SD effect (recorded sessions: 10 Mc, 11 Mp). As item distances increased, monkeys' Accuracy and Reaction Times improved significantly ($p<.001$ one-way rANOVAs). Time-frequency analysis of lower frequency bands shows SD modulation during later item pair presentations (300 ms>). Significant variations in power activity across SDs were observed ($p<.001$, one-way ANOVAs; Bonferroni corrected with $\alpha=.01$). Correlation analysis of power average activity during significant periods showed significant negative correlations for both monkeys (all $ps<.001$). However, as movement onset approached, the encoded information in the lower frequency bands exhibited signs of degradation, while modulation of synchronized high Gamma activity across SDs ($p<.001$, one-way ANOVAs), positively correlated with Accuracy (all $ps<.001$). We further quantified this interplay through phase-amplitude coupling analysis of $\theta\gamma$ and $\alpha\gamma$, finding that coupling strength varies with SD, stronger at larger distances. These results support PMd's top-down role, where low-high frequency interactions support decision-making and motor response preparation.

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Poster

PSTR301: Neural Mechanisms of Decision Making in Human and Non-Human Primates

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR301.06/O31

Topic: H.03. Decision Making

Title: Reward-based cues influence movement preparention: insights from Drift diffusion model and Premotor spiking activity

Authors: *V. GIUFFRIDA[1,2], I. B. MARC[1,2], G. BARDELLA[1], E. BRUNAMONTI[1], P. PANI[1], S. FERRAINA[1];

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Abstract: In everyday life, we are continuously required to make decisions. Many of these decisions require a proper balance between sensory evidence and internal preferences. In this balancing, the reward, or the expectation of it, plays a key role in decision-making. The dorsal premotor cortex (PMd) is one of the areas most involved in decision-making and recent research has highlighted its role in integrating the representation of reward expectations into movement preparation. To explore how this process is reflected in the neuronal activity of PMd, we trained two monkeys to perform a variant of the stop signal task (SST) designed to modulate the expectation of reward by providing cue-based evidence at the beginning of each trial. In a typical SST, there are two types of trials: Go trials, in which the monkey is asked to perform an action when the Go signal appears and Stop trials, in which the monkey must inhibit the action when an unexpected Stop signal follows the Go signal. In our version of the SST, each CUE was associated with different contexts: 'Go plus', 'Stop Plus', and 'Neutral'. In the Go plus, a greater reward was anticipated for acting (Go trials) than inhibiting (Stop trials); vice versa in the Stop plus. 'Neutral' offered an equal amount of rewards for both trial types. We adopted the drift-diffusion model to infer from behavioral data the computational strategies employed by the monkeys in Go trials. This model depicts movement preparation as an accumulation process that initiates from a starting point (z), representing CUE-informed beliefs and evolves with a drift rate (v) towards a bound. We found that the z was closer to the bound and the accumulation process had a steeper v, in Go plus compared to the Stop plus condition, demonstrating reward-context-dependent dynamics that explained the differences in response time distributions. We then investigated whether the spiking single neuron activity in PMd reflected the modeling findings. Our results indicate that a significant percentage of neurons were modulated by reward context, consistent with the drift-diffusion model hypothesis, with higher drift values observed in the “Go plus” context, indicative of a faster accumulation process. These findings show how neuronal activity in PMd may reflect adaptive decision-making strategies to adjust movement preparation to specific reward contexts.

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Poster

PSTR301: Neural Mechanisms of Decision Making in Human and Non-Human Primates

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Support: Brain R01 - NS104923
MURI: W911NF-16-1-0368

Title: Multiregional mechanisms of exogenous attentional selection in the posterior parietal cortex

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Abstract: Exogenous mechanisms of attentional selection override internally generated stimulus filtering mechanisms due to the automatic capture of attention by a salient stimulus, such as a bright light or loud sound. Area 7A and lateral intraparietal area (LIP) are two regions within the posterior parietal cortex (PPC) that are directly anatomically interconnected and have been shown to play a crucial role in processing exogenous attention. However, a direct understanding of how area 7A and LIP individually and together contribute to the processing of exogenous attention remains poorly understood.
Here, we investigated the neural population dynamics during exogenous attentional selection in PPC. We simultaneously recorded spiking and LFP signals from populations of neurons (~200 per experimental session) using a 10 mm long multi-contact probe- Neuropixel probes. The presence of unit activity at depths along the neuropixel probe allowed us to simultaneously record neural activity from area 7A and LIP. We conducted experiments on non-human primates performing a two-target, free choice, eye-movement luminance-selection task (LRS). Additionally, we analyzed the neural responses in a single-target oculomotor task (center-out task) to determine the traditional response field.
Consistent with prior work, on center-out trials, PPC neurons showed elevated firing when the target inside the response field (In-RF) was selected compared to the target outside the response field (Out-RF). Interestingly, area 7A neurons displayed an earlier response to the target onset compared to LIP neurons (mean ST: 30 vs 58 ms). On LRS task trials, area 7A neurons showed similar responses on trials involving In-RF and Out-RF selection. In comparison, LIP neurons showed an elevated response to In-RF selection. Consistent with these results, analysis of the neural population dynamics demonstrated that the dominant mode of area 7A represents visual activity whereas the dominant mode of LIP represents selection activity during the LRS task. Based on these results, we propose that area 7A is involved in the initial processing of sensory information and the detection of salient stimuli. In contrast, LIP is more specifically involved in the allocation of attention to the selected target for subsequent saccade. We suggest that sensory information from area 7A is communicated to LIP, allowing for further integration and modulation of sensory information to guide exogenous attentional selection.

Disclosures: A. Dubey: None. **K. Wingel:** None. **B. Pesaran:** None.

Poster

PSTR301: Neural Mechanisms of Decision Making in Human and Non-Human Primates

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Topic: H.03. Decision Making

Title: State coding in the primate hippocampus during abstract maze task

Authors: *H. LIANG[1], D. LEE[2];
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Abstract: Sequential decision-making can be guided by multiple types of learning, including model-free reinforcement learning (RL) in which estimates of future outcomes or values are updated by trial and error and model-based RL in which values can be computed by mental simulation using the knowledge of the environment. Although the neural mechanisms for model-free learning have been extensively studied, how the brain can update the value functions more flexibly by learning and simulating the changes in the environment remains unknown. The goal of this study is to understand the function of the primate hippocampus and prefrontal cortex in using the animal's knowledge for sequential planning. To this end, we developed an abstract maze navigation task in which the animal can navigate inside a graph with its nodes arranged in the shape of the letter H. Each node in this graph was uniquely associated with a fractal image, and the animal's current position in the graph was indicated by the fractal image shown at the center of a computer screen. The animal transitioned from one node to another by choosing one of the fractal images appearing randomly in 3 possible peripheral locations on the computer screen, preventing the animal from using a strategy based on a spatial sequence. The animal received a small reward for each choice, but a large reward upon arriving at a goal node. The start and goal nodes were chosen randomly, and the fractal image for the goal node was displayed at the bottom of the screen throughout the trial. We found that the accuracy of the animal's choice was higher than the chance level even immediately after the goal change. Results from modeling also suggested that the animals used a hybrid strategy of model-free and model-based reinforcement learning algorithms. Neural recordings were made from the hippocampus of a monkey performing this abstract navigation task. Hippocampal neurons were selectively activated by saccadic movements toward specific states in the abstract maze as well as specific locations on the screen. Simultaneous recordings from the hippocampus and prefrontal cortex and further analyses will be performed to understand how the coordination between these two regions supports the outcome prediction and selection of sequential decisions.

Disclosures: H. Liang: None. **D. Lee:** None.

Poster

PSTR301: Neural Mechanisms of Decision Making in Human and Non-Human Primates

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Topic: H.03. Decision Making

Title: Schema in the pre-motor cortex of monkeys accelerates learning

Authors: *K. TIAN[1,2], Z. ZHAO[3], Y. CHEN[1], S. CAO[1,4], J. GU[5], S. YU[1,2];
[1]Inst. of Automation, Chinese Acad. of Sci., Beijing, China; [2]Sch. of Future Technology, Univ. of Chinese Acad. of Sci., Beijing, China; [3]ophthalmology, The First Hosp. of Jilin Univ., Changchun, China; [4]Sch. of Artificial Intelligence, Univ. of Chinese Acad. of Sci., Beijing, China; [5]Strategic Support Force Med. Ctr., Beijing, China

Abstract: Schema refers to a relatively stable mental representation that we use to accelerate learning in new but similar contexts. Although several studies have demonstrated the existence of schema-like neural population dynamics in rodents, the electrophysiological evidence of it in non-human primates remains very limited. Here, in the dorsal premotor cortex (PMd) of monkeys, we show the possible existence of schema by demonstrating 1) shared low-dimensional features of neural population dynamics across tasks accompanied by accelerated learning, and 2) the lack of such shared dynamic patterns associated with slowed learning. Specifically, we recorded neural population activities from the PMd in 3 macaques trained to perform a series of visual-motor mapping tasks. During the similar learning phase, the monkeys learned to perform the same visual-motor mapping with novel visual stimuli, while during the reversal learning phase, the monkeys needed to learn the reversed mapping rule. We found that during similar learning tasks, monkeys demonstrated accelerated learning for new stimuli pairs compared to the learning of the first one. Importantly, in this condition, we identified shared features in population dynamics, i.e., manifolds, across tasks. Subspace decomposition uncovered that the reused manifolds resided in the low-dimensional subspace of motion decision. In contrast to the similar learning phase, monkeys encountered difficulties during the reversal learning phase. We calculated the similarity of decision subspace manifolds between the similar and reversal learning phases and found that the reused manifolds in the former were not present in the latter. To explore the relationship between the degree of manifold reuse and the speed of learning, we regressed the number of trials needed to learn a task with the similarity of manifolds in the decision subspace and found a significant linear relationship between the two. Taken together, these findings suggest that the activity of the pre-motor cortex in monkeys exhibits a schema-like architecture for integrating prior knowledge to facilitate subsequent learning. Furthermore, by revealing the linear relationship between schema use and learning speed, our results shed light on how neural networks balance between reliance on previously acquired knowledge and the learning of new one.

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Poster

PSTR301: Neural Mechanisms of Decision Making in Human and Non-Human Primates

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Topic: H.03. Decision Making

Support: STI2030-Major Projects (2021ZD0203800)

Title: Neural mechanisms of memory-based evidence integration in perceptual decision making

Authors: *Y. ZHANG, Y. ZHOU;
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Abstract: In real-world decision-making scenarios, successful decisions often require the integration of evidence from multiple sensory inputs gathered over time. However, most perceptual decision-making studies in laboratory settings only involved single stimulus, leaving the neural mechanisms of memory-based evidence integration poorly understood. In this study, we trained two macaques to perform a color integration task. The monkeys were tasked with observing two separate colored dot patches sequentially and then making a saccade choice based on the dominant color resulting from the combination of the two temporally separated stimuli. Concurrently, we recorded neural activity in the frontal eye field (FEF), lateral intraparietal area (LIP), and superior colliculus (SC) using multi-channel linear probes. Our findings revealed that neurons in all the three brain areas encoded the abstract proportion of colors, serving as a presumed form of decision variable. Notably, the FEF appeared to play a more significant role in integrating color evidence compared to LIP and SC. FEF neurons exhibited the strongest encoding of color evidence from both sensory stimuli during both the presentation and memory intervals, with highly correlated encoding of the two stimuli at the single-neuron level. In contrast, LIP seemed more involved in encoding and maintaining color evidence for each sensory stimulus, as evidenced by robust encoding during delay periods and less correlated encoding of the two stimuli. Additionally, SC demonstrated earliest encoding of color evidence for both stimuli during presentation periods, suggesting an early role in sensory integration during decision-making processes, a function traditionally less appreciated. Overall, our results highlight distinct roles of frontal-parietal and mid-brain areas in complex decision-making and offer insights into the neuronal basis of memory-based evidence integration.

Disclosures: Y. Zhang: None. **Y. Zhou:** None.

Poster

PSTR301: Neural Mechanisms of Decision Making in Human and Non-Human Primates

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Topic: H.03. Decision Making

Support: NIH Grant R01 EY022411

Title: Perceptual decision-related single-unit activity in the monkey substantia nigra pars reticulata

Authors: *J. A. BHATTI[1,2,3], K. ROGERS[1,2], J. I. GOLD[1,2], L. DING[1,2];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Dept. of Neurosci., Philadelphia, PA; [3]Neurosci. Grad. Group, Philadelphia, PA

Abstract: How the basal ganglia (BG) contribute to visual perceptual decision-making is not yet well understood. Theoretical studies have posited various computational roles for different BG nuclei in converting noisy evidence to discrete choices. These ideas have received support from experimental studies of the major input nuclei of the BG, the striatum and subthalamic nucleus, including the identification of neural activity patterns that encode evidence accumulation and, when perturbed, affect decision-making behaviors. In contrast, these ideas have received less support from experimental studies of a major output nucleus of the BG, the internal segment of the globus pallidus, which have identified neural representations of already-formed choices but only rarely evidence-related quantities. To reconcile these results, we hypothesize that the BG's causal contributions to decision formation are substantiated via the other major BG output nucleus, the substantia nigra pars reticulata (SNr). As the first step to test this hypothesis, we recorded single-unit SNr activity in each of two monkeys performing a visual motion discrimination saccade task. The monkeys made saccades at a self-chosen time to indicate their decision about the direction of a random-dot motion stimulus. For each trial, the motion strength and direction were chosen randomly from 5 values and two directions, respectively. The monkeys were given liquid rewards for correct choices. In our preliminary sample, we identified 56 SNr neurons with changes from baseline activity after motion stimulus onset and/or surrounding saccade onset. Using multiple linear regression analyses, we found that 18 units (32%) showed choice-selective activity from motion onset to saccade onset (i.e., during evidence accumulation), the majority of which (14 units) showed greater activity for trials with contralateral choices. We also found 20 units (36%) with motion-strength modulation, 13 of which showed increasing response suppression for stronger motion. Within a 200-ms window prior to saccade onset (i.e., around the time of decision commitment), 26 units (46%) showed choice-selective activity. These preliminary results support the idea that the BG pathway that exits via the SNr does not simply represent already-made choices but instead contributes to decision processes involving evidence accumulation and decision commitment. Further work is needed to specify the exact, causal nature of these contributions to decision formation.

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Poster

PSTR301: Neural Mechanisms of Decision Making in Human and Non-Human Primates

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Topic: H.03. Decision Making

Support: NINDS 5K99NS133475

Title: Dimensionality and dynamics of decision-making in simultaneous recordings from the hippocampus, prefrontal, and premotor cortex of virtually navigating macaques

Authors: *R. A. GULLI[1], R. HASHIM[1], W. J. JOHNSTON[1], S. FUSI[1], C. D. SALZMAN[1,2]; [1]Columbia Univ., New York City, NY; [2]NYSPI, New York, NY

Abstract: Humans have an exquisite ability to integrate vast amounts of information from the environment and prior experiences to make adaptive choices in novel situations. This cognitive function is believed to rely on a distributed network of brain areas implicated in learning, decision-making, and action execution, but remain poorly understood. We have developed an experimental platform for probing these mechanisms in non-human primates. Our approach employs a novel virtual reality experimental task to study decision-making and generalization in novel situations (Gulli et al., 2022, SfN). During task performance, we are simultaneously recording neural activity in large neural ensembles using Neuropixels probes in the hippocampus, prefrontal cortex (areas 8a, 9/46d, 9/46v), and pre-motor cortex.
In experiments, trained monkeys perform a two-alternative forced choice decision-making task while exploring a large open-field virtual environment. As monkeys navigate, they are occasionally presented with two differently colored objects. Through trial-and-error, monkeys learn that certain objects are only rewarded in certain parts of the environment. For example, black objects may be rewarded in the West half of the environment, with white objects rewarded in the East. While learning the spatial rule, choices are made only at a subset of locations in the environment. After learning, monkeys must make choices in completely novel locations within the environment, with new visual perspectives. Choices at novel locations are presented only once, and they cannot be based on memorized scenes or singular landmarks. Instead, the ability to make correct choices must rely on generalization using the previously-learned rule and knowledge of one's location.
During experiments, we have employed a newly developed NHP Neuropixels Rail Drive Insertion System that facilitates simultaneous recording of 1000+ units across hippocampus, prefrontal cortex, and premotor cortex. We hypothesize that brain areas supporting generalization behavior should represent the learned spatial rule within a lower-dimensional subspace before the computation of a decision. By contrast, brain areas implicated in the decision computation should exhibit, at least transiently during deliberation, higher dimensionality. The results of this work will yield insight into precisely how the balance of high- and low-dimensional neural representations and their dynamics enable our ability to make adaptive decisions and generalize in novel situations.

Disclosures: R.A. Gulli: None. **R. Hashim:** None. **W.J. Johnston:** None. **S. Fusi:** None. **C.D. Salzman:** None.

Poster

PSTR301: Neural Mechanisms of Decision Making in Human and Non-Human Primates

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Title: Task-irrelevant stimuli evoke reliable pupil responses, but do not affect the concomitant formation of a perceptual decision

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Abstract: The arousal systems of the brainstem, specifically the locus coeruleus-noradrenaline system, respond “phasically” during decisions. These central arousal transients are accompanied by dilations of the pupil. Attempts to understand the impact of phasic arousal on cognition would benefit from the ability to manipulate arousal in a temporally precise manner. Here, we evaluated a candidate approach for such a manipulation: task-irrelevant sounds presented during the execution of a challenging task. Such sounds drive responses of brainstem nuclei involved in the control of pupil size, but it is unknown whether the sound-evoked responses mimic the central arousal transients recruited by cognitive computations. We aimed to test to which level of temporal precision and reliability task-irrelevant sounds can be used to systematically manipulate pupil responses, and how these responses relate to phasic arousal boosts that occur naturally during decisions.
We tested a total of 97 participants in three challenging perceptual decision-making tasks. In each experiment, we compared pupil responses evoked by the task with the pupil responses evoked by task-irrelevant white noise sounds of varying onset latencies or durations. Participants were asked to judge whether a low-contrast visual grating stimulus was superimposed onto dynamic visual noise (Exp. 1 and 2) or whether the average orientation of a stream of eight gratings belonged to a “diagonal” or “cardinal” category (Exp. 3).
The pupil dilated in response to both task engagement and the task-irrelevant sounds. The latter consistently drove robust and precisely timed pupil responses that superimposed onto the task-evoked pupil responses and were therefore separable through a linear subtraction. We replicated a negative correlation between the amplitude of task-evoked pupil responses and choice bias observed in previous studies. Yet, in neither experiment did the task-irrelevant sounds affect bias, nor any other aspect of choice behavior.
Our findings suggest that task engagement and task-irrelevant sounds may differentially recruit

neural systems that are all involved in the control of pupil size but have distinct influences on cognitive computation.

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Poster

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German Federal Ministry of Education and Research (BMBF, project numbers 01EW2007B and 01GQ1907)
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Title: Imaging the changes in cortex-wide human circuit dynamics under pharmacological manipulation of GABA-A and NMDA receptors

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Abstract: Microcircuit properties such as the ratio between synaptic excitation and inhibition are critical for the computations underlying cognitive behavior and seem to be disturbed in cognitive disorders. Theory indicates that several parameters of neural mass dynamics (assessable with non-invasive electrophysiological recordings) are sensitive to changes in such microcircuit properties. Further, microcircuit properties exhibit heterogenous patterns across cortical areas, reflecting the cortical hierarchy. We performed a placebo-controlled pharmacological MEG study aiming to quantify the spatial patterns of the *changes* in spontaneous cortical dynamics under pharmacological manipulation of GABA-A or NMDA receptors in healthy human participants. Each session entailed the oral administration of memantine (NMDA receptor antagonist), lorazepam (GABA-A receptor agonist), or placebo, each for two repeated sessions in randomized order. Participants (N=20, cross-over design) completed (among other tasks) a block of 10 min eyes-open resting-state MEG recordings. Following MEG source reconstruction and modeling of the source power spectra, we estimated six distinct parameters quantifying different features of spontaneous cortical dynamics, for 180 areas covering the cortical sheet. These included the slope of the aperiodic component and the power and peak frequency of neural oscillations. We computed the change between each drug and placebo condition, separately for each condition repeat. Under placebo, the maps of all six parameters were heterogeneous across

the 180 cortical areas, and most exhibited a large-scale gradient from the back to the front of the cortex. This gradient reflected the anatomical hierarchy of cortical areas estimated from an anatomical MRI proxy (ratios T1w to T2w maps). Lorazepam and memantine administration both changed the dynamics parameters in distinct spatial patterns that were again heterogenous and highly reproducible across the two repeats of each manipulation. For the slope of the aperiodic component, these spatial patterns reflected not the anatomical hierarchy, but the cortical distribution of GABA-A receptor densities, with opposite directions for lorazepam and memantine effects.
We conclude that controlled manipulations of specific synaptic receptors alter cortical dynamics along reliable and distinctive large-scale spatial patterns, which are partially explained by synaptic properties. These spatial patterns provide a reference for interpreting the patterns of corresponding changes due to the same manipulation during cognitive tasks or due to cognitive disorders in our ongoing work.

Disclosures: A. Arazi: None. **A. Toso:** None. **T.H. Donner:** None.

Poster

PSTR301: Neural Mechanisms of Decision Making in Human and Non-Human Primates

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Title: Distributed coding of decision and associated confidence across competing accumulators in the human cortical motor system

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Abstract: Neural circuit models of decision-making postulate that distinct populations of choice-selective neurons accumulate time-varying input signals supporting one versus another alternative. In this framework, the decision variable (DV) is encoded in a distributed (for two choice alternatives: 2-dimensional) fashion. It is unknown whether this distributed coding of DV

predicts key aspects of decision-making, which commonly used 1-dimensional reductions (e.g., difference between accumulators for two-choice tasks) fail to predict. Here, we tested the idea that distributed coding governs the confidence and reaction time (RT) associated with a choice. Human participants (N=20) performed a visual choice task during magnetoencephalography (MEG) recordings. In each visual hemifield, a stream of 10 "samples" (circular gratings, 100 ms each) of fluctuating contrasts was presented. Participants then reported the "stronger" side (left vs. right) and their confidence in that choice (high vs. low) by button press. We estimated the impact of evidence fluctuations on choice and confidence reports and fit accumulator models to both behavioral features. Moreover, we trained decoders to predict choices from patterns of source-level MEG signals in the dorsal premotor and primary motor cortex (PMd/M1) of each hemisphere.
Choice and confidence reports were biased by evidence fluctuations across all 10 samples, but more so for early samples. For choice this impact was symmetric for "chosen" and "unchosen" streams but dominated by the chosen stream for confidence. Confidence reports and RTs exhibited signatures of statistical decision confidence. Behavior was well explained by a model of two competing (feed-forward inhibition), leaky accumulators for each stream, racing to hit a collapsing bound. The output of PMd/M1 choice decoders built up during decision formation with an evidence-dependent slope. Critically, RTs and confidence reports (i) depended on the balance between "left" and "right" supporting decoder outputs, and (ii) were more closely related to the state of the decoder supporting the chosen side.
Our results establish a distributed representation of decision states in the brain, whereby the balance between competing neural accumulators shapes overt behavior (RT) and the sense of confidence.

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Poster

PSTR301: Neural Mechanisms of Decision Making in Human and Non-Human Primates

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Title: Non-linear estimates of nutritional properties lead to systematic errors in healthy food choice

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Abstract: Maintaining a daily caloric deficit while dieting underlies most successful weight loss programs. As the majority of foods in our environment don't come with nutrition labels, dietary food choices are primarily guided by subjective nutritional estimates. These estimates are often informed by perception and experience, but how accurate are they? We identified in a previous study that, while the estimated fat and sugar values of 36 foods were highly correlated with their actual values (Carrington et al., 2023), the relationship between estimated and actual values was logarithmic, rather than linear, **thus becoming increasingly inaccurate with increasing values.** This relationship mirrors the Weber-Fechner principle in perceptual psychophysics as well as other findings about numerical magnitude estimation (Algom, 2021; Dehaene, 2003). In the present study, we asked a set of 303 online participants to estimate a larger set of nutritional values of a set of 68 different foods, which we prepared and photographed under controlled lighting and presentation conditions. As in our previous study, the relationships between estimated and actual values of carbohydrates, fat, and caloric content of these foods were better explained by a logarithmic than a linear model (all $X^2 > 16$, $p < 0.001$). Following this, we presented another set of 114 participants 100 pairs of these food images, in which each pair of foods differed by approximately 200 calories. Participants selected the highest calorie food from each pair. As predicted, **as the average calorie content of the image pairs increased, participants' accuracy at choosing the correct image significantly decreased** ($F = 35.1$, $p < 0.001$). These results suggests that reliance on subjective nutritional estimates when dieting could lead individuals to make systematic errors in food choice, on the order of a few hundred calories per day, particularly when choosing between more calorie dense foods. These errors could then lead to gradual and significant increases in weight over time and accompanied health effects.

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Poster

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ARC Grant FT230100656

Title: Combining electrophysiology and event-related fMRI to understand how humans make perceptual decisions

Authors: J. WONGTRAKUN[1], S.-H. ZHOU[1], R. G. O'CONNELL[2], T. T.-J. CHONG[1], M. A. BELLGROVE[1], ***J. P. COXON**[1];
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Abstract: Perceptual decisions require that sufficient sensory evidence is accumulated to form a judgement. In the macaque brain, accumulation-to-bound dynamics have been observed in single-cell recordings of several brain regions, including the lateral intraparietal area. Isolating regions of the human brain that display evidence accumulation properties using functional magnetic resonance imaging (fMRI) has proven a challenge. Here, we sought to address this via the combination of electroencephalography (EEG) and event-related fMRI. We had 40 right-handed adult humans (25 ± 6 years) perform a random dot motion kinematogram task during EEG, and subsequently, during fMRI. Participants monitored random dot motion and responded with a keypress when making perceptual decisions about subtle instances of coherent motion within the kinematogram. For each participant, we determined the slope of the centroparietal positivity (CPP), an EEG signal that demonstrates characteristic evidence accumulation dynamics (O'Connell & Kelly, 2021). We regressed the CPP against the event-related fMRI response to coherent motion onset. We find that the blood-oxygen-level-dependent signal scales inversely with CPP slope in the supplementary motor area (SMA) proper, the bilateral inferior parietal lobule, and the left intraparietal sulcus ($p < .05$, FWE-corrected at cluster-level). Our findings clarify the brain regions responsible for the centro-parietal positivity and provide compelling evidence for a role of SMA and the intraparietal sulcus in the process of evidence accumulation during human perceptual decision-making.

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Poster

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Topic: H.03. Decision Making

Support: F30MH134507

Title: The neural mechanisms of categorical decision-making

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Abstract: When we are confronted with unclear or vague information about our world, we rely heavily on memory to fill in missing data and ultimately guide behaviors. What mechanisms in the brain are responsible for recognizing, identifying, and categorizing objects? To better understand the temporal dynamics of how categorization occurs in the human brain, we recruited 27 participants who were being treated for epilepsy with intracranial electrode implants. Participants completed a task where they were shown images and were asked to categorize them into one of four categories. Images were either unambiguous and clear (i.e., nonblurred) or ambiguous and vague (i.e., blurred). Participants categorized the nonblurred images significantly faster and more accurately than their blurred counterparts. We examined the neural correlates of where categorization occurs in the human brain, how it occurs under perceptual ambiguity, and how previous memories may mediate these neural computations. We found evidence that the anterior temporal lobe (ATL) contains categorical-level information that correlated with reaction time. Furthermore, the medial temporal lobe (MTL), which is crucial for memory recall, increases is functional connectivity with areas in the ventral visual stream to a greater degree when presented with more ambiguous images. Together, these two analyses suggest that semantic memory is reinstated and informs decision-making signals in the ATL. Next steps include investigating the nature of decision-making signals in the ATL and prefrontal cortex using a drift-diffusion modeling to determine which area computes a decision variable.

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Poster

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ONR grant N00014-22-1-2699
NIH Fellowship 5K00NS105204-05

Title: Support vector machine classification in multi-attribute decision making in human intracranial recordings

Authors: *T. OUYANG[1], A. L. SAMPSON[2], E. E. EMERIC[1], W. J. LIPSKI[6], S. MOREIRA GONZALEZ[8,9], A. DAMIANI[7], J. A. GONZÁLEZ-MARTÍNEZ[6], S. V. SARMA[3], V. STUPHORN[4], E. NIEBUR[5];
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Abstract: Real-world decisions typically require considering multiple options, each of which may be defined by multiple attributes. To study these kinds of multi-attribute decisions, we have implemented a tablet-based task in which participants choose between two options. On two thirds of the trials (the 'win domain'), both options offered cash rewards of different amounts and with different probabilities. On one third (the 'loss domain') both options could result in losing money, again with specified amounts of loss and probabilities of losing. Attribute types (amounts and probabilities) are represented by separate symbols. By default, these symbols are "covered" by masks indicating the attribute type "hidden" under them, and only when participants tap on these masks are the corresponding attribute values displayed momentarily. Participants can freely inspect both attributes of both options as many times as desired, but only one attribute of one option may be inspected at a time. When the participant has come to a decision, they select the chosen option by tapping a separate button labelled 'select'. This sequence of events gives us the ability to precisely observe when each piece of decision-relevant information is being collected.
To study the neural correlates of this decision process, we collected stereoelectroencephalogram (sEEG) recordings from patients undergoing treatment for medically intractable focal epilepsy while they performed the multi-attribute decision task (85 ± 72 win domain trials and 40 ± 36 loss domain trials, mean and standard deviation across 13 patients). We then use Support Vector Machines (SVM) to classify the behavioral state based on the sEEG data within various cerebral regions during decision-making. The multi-attribute decision making task presents a number of behavioral states of interest that can be studied as binary classes. We use a 5-fold cross-validation procedure to train and test the performance of SVMs with averaged balanced accuracy as measure of classification performance. As features, we use spectral power for multiple narrow and broad frequency bands at specific peri-event time windows. These spectral features are computed from the sEEG recordings aligned with specific task-related events, e.g., inspections of options or the outcome presentation. As a first validation of the approach, we show that sEEG activity in both the motor cortex and frontal cortex show activity differences when indicating choices with hand movements. Based on these results, we apply the same approach to classifying win domain and loss domain option inspections, decisions, and outcomes, as well as positive and negative outcomes in both domains.

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Poster

PSTR301: Neural Mechanisms of Decision Making in Human and Non-Human Primates

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Topic: H.03. Decision Making

Title: Distinct roles for perceived realism and memory recall in complex decision making

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Abstract: One key neural component in complex decision making is the retrieval of information. Cognitive neuroscientists emphasize two types of information retrieval: episodic memory (e.g. detailed remembrance of events) and semantic memory (e.g. remembrance of facts and general knowledge). Additionally, narrative models discuss how retrieved information is used in complex decisions. Specifically, narrative models indicate that deciding between alternative explanations of events requires retrieved information about real world events to determine which explanation is more realistic. We refer to this as perceived realism. In most studies, the contributions of episodic memory, semantic memory, and perceived realism are examined in isolation. However, in real world decisions, it is likely that all three views of retrieved information contribute to a person's choice. Here, we examined the relationship between each type of retrieved information's effect on complex choice. In the current study, jury eligible subjects (N = 83) were asked to read mock cases consisting of crime accusations and varying levels of evidence. For each case, subjects were asked to rate the strength of the case against the accused, the strength of their episodic memories and semantic memories for similar events, and their perceived realism of the case. Results of mixed effects modeling revealed that both traditional memory processes and perceived realism significantly influence case strength ratings. Additionally, variance partitioning indicated that the two largest contributors to case strength include a combination of all three types of retrieved information and an independent contribution of perceived realism. Using structural equation modeling to interpret variance partitioning indicated that episodic and semantic memory load onto a common factor that significantly predicts case strength and is mediated by perceived realism. Our findings support an independent role of perceived realism in complex decision making that is separate from episodic memory and general knowledge. A separate role for perceived realism is consistent with assessing the validity of retrieved information.

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Poster

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MnDRIVE

Title: A General Framework for Single-Trial Population Decoding using LFPs in Humans during Visual and Cognitive Tasks

Authors: *S. KOENIG[1], X. YAN[2], A. S. WIDGE[3], A. B. HERMAN[4], D. P. DARROW[5]; [1]Psychiatry and Neurosurg., Univ. of Minnesota, Minneapolis, MN; [2]Dept. of Psychiatry and Behavioral Sci., Univ. of Minnesota, Twin Cities, Roseville, MN; [3]Psychiatry, Univ. of Minnesota, Minneapolis, MN; [4]Univ. of Minnesota, Minneapolis, MN; [5]Neurosurg., Univ. of Minnesota, Minneapolis, MN

Abstract: Neural encoding models determine how pseudo-populations of signals across participants in specific brain areas encode information about task variables or participant choices. Conversely, neural decoding asks how well we can predict task variables or participant choices from either pseudo-populations of neural signals recorded across participants or within a population of simultaneously recorded signals obtained from a single participant. Despite their similarities, neural decoding is typically much harder to implement than neural encoding. Furthermore, most successful population decoding has been implemented in sensorimotor areas. However, in humans very limited population decoding has been applied in more cognitive areas like the prefrontal cortex where mixed selectivity is very common.Here we propose a general framework for decoding neural signals from human intracranial local field potentials (LFPs) using monte-carlo-cross validation with linear decoders (e.g. logistic decoder) with elastic net regularization on multiple features derived from canonical frequency bands extracted from simultaneously recorded neural signals. We test the proposed methods on the decoding of stimuli in the visual cortex during a visual perception task and decoding stimuli in the prefrontal and temporal cortices during the Multi-Source Interference Task (MSIT). The proposed method yields a median peak decoding accuracy of 95.0% in the visual cortex (n = 12) during the visual task but only 74.1% in the prefrontal and temporal cortices (n =6) during the MSIT. The proposed method outperforms dPCA (Kobak et. al., 2016) at the population-level in the visual cortex (90.25%) and prefrontal and temporal cortices (65.4%). However, dPCA can perfectly decode pseudo-population data in the visual cortex. We also built encoding models for multiple brain areas at the pseudo-population level to determine how each brain area encodes information on average using cluster-based permutation tests with GLMEs (König et. al., 2024). Results from encoding models were not always good at predicting stable decoder features, but individual features at the channel-level with at least weak encoding (np2 > 0.01) were more often used in the population decoders. In conclusion, neural decoding can be much more difficult in cognitive areas like the prefrontal cortex than in sensorimotor areas like the visual cortex. Ultimately, building single-trial, inferential decoders like these are important for determining good

neuromodulation targets for various psychiatric and cognitive disorders in humans. This decoding framework could easily be extended to real-time applications.

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Poster

PSTR301: Neural Mechanisms of Decision Making in Human and Non-Human Primates

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FRQNT
natural sciences, mathematics sciences and engineering (FRQNT)

Title: Neural Dynamics of Decision-Making and Performance Monitoring in Human PMd Under Sensory Conflict and Choice Uncertainty

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Abstract: Humans and animals are regularly tasked with making decisions about how to interact with an environment rich with conflicting sensory cues that support multiple potential actions. Central to this critical brain function is the dorsal premotor cortex (PMd), which orchestrates voluntary arm movements by integrating sensory, contextual, and action-related information. Kalaska et al. have explored PMd's role in decision-making tasks in which humans and non-human primates (NHP) determined the dominant color of a multicolored checkerboard Decision Cue (DC) to select one of two color-coded targets for a reaching movement. Variations in the proportion of differently colored small squares set the level of sensory conflict and choice uncertainty of the DCs. In the Targets-First (TF) task, targets were presented before the DC. In the Checkerboard-First (CF) task, the DC was displayed before the targets, allowing subjects to make an independent perceptual judgment about the DC's dominant color before deciding how to report it after the targets appeared. Neural recordings in NHPs showed that PMd activity reflected the selected direction of reach (correct or incorrect) after the DC appeared in the TF task and after the targets appeared in the CF task. PMd activity was also modulated by the strength of the evidence in the DC. However, PMd activity expressed little activity related to the critical color-discrimination aspects of the decision process. In this study, we made whole-brain

magnetoencephalography (MEG) recordings while human subjects performed variants of the tasks, called TFD and CFD because they included an imposed delay after the presentation of each instructional cue (targets, DC). As before, subjects determined the dominant color of a DC with different numbers of blue and orange squares to select one of two color-coded targets on opposite sides (R, L) of the DC. However, they reported their decision by pressing the R or L mouse button with their R or L index finger, respectively. One second after the button press, a differential auditory beep tone provided feedback (knowledge of results, KR) indicating whether the chosen target was correct (high beep) or not (low beep). One novel finding was that beta-band power in PMd increased transiently after correct KR tones, whose amplitude increased as DC evidence strength and choice certainty decreased. KR-evoked responses in PMd also differed significantly after incorrect choices and from those evoked by random high and low beeps delivered outside of the task, supporting their role in performance monitoring rather than simple auditory reactions. This indicates a reward prediction error-like signal expressed in PMd.

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Title: Shifting Expectations: Individual differences in criterion shift association of Electroencephalography, in a recognition memory security patrol paradigm.

Authors: *C. G. BOARDMAN[1], E. LAYHER[2], J. M. VETTEL[3], M. B. MILLER[4];
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Abstract: At times it might be prudent to alternate between liberal and conservative criterion. For instance, security personnel ought to adopt a liberal criterion when interrogating people at a security checkpoint. Whereas in situations where the possibility of using physical force against suspects is more likely, a conservative criterion may reduce the risk of unjust harm. Prior investigations have demonstrated significant inter-individual variability in the tendency to transition between liberal and conservative criteria. This study employs electroencephalography (EEG) to explore the temporal and oscillatory patterns associated with individual tendency to shift between maintaining a conservative versus liberal criterion during a recognition security patrol task.

Participants underwent rigorous screening, leading to the selection of individuals demonstrating a notable tendency to shift (N=59, 46 female, aged 17-29) for the EEG phase. Response-locked and stimulus-locked event-related spectral perturbations was averaged across conservative and liberal old and new answers and studied and unstudied images. After visual inspection of these data, we also decided to average correct rare (liberal correct rejections and conservative hits) and default (liberal hits and conservative correct rejections) conditions. An initial Pierson analysis of stimulus-locked ERSP data indicated modest correlations between band power and individual tendency to shift in beta (r=-0.04 to -0.47) and delta (r=0.39 to 0.30) ranges.
To elucidate these data, we employed a simple-multi kernel learning, which distinguished between poor (N=29) and strong (N=29) shifters. Temporal patterns in the high beta (22-24 Hz) range for conservative old answers distinguish between strong and poor shifters in the 1000 ms before participant response, with 91% accuracy and a significance of 0.0003 using 10,000 permutations of the training model. Similarly, patterns in the 25-27 Hz range from 0-1000ms post participant response distinguish between poor and strong shifters with 84% accuracy and a significance of 0.0017 using 10,000 permutations of the training model. Initial results (using only 1,000 model permutations) indicate that in both rare responses and conservative old answers temporal patterns in lower frequency ranges in the 2000 to 1000 ms before response reliably distinguish between poor and strong shifters (4-6 Hz, with 80% accuracy p=0.002 and 7-9 Hz with 78% accuracy p=0.016, for rare responses and conservative old answers respectively). We suggest that differences between strong and poor shifters are mediated by interactions between low and high-frequency oscillations.

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Poster

PSTR302: Decision-Making: Neural Mechanisms: Risk and Ambiguity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR302.01/Web Only

Topic: H.03. Decision Making

Support: JSPS KAKENHI Grant Number JP24K10718

Title: Sequential risky choices: An insights from risk-sensitive foraging theory

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Abstract: Maximization of utility under static risk preference is typically used to explain human risk choices when optimal decisions depend only on the properties of the current options. In contrast, recent studies have investigated human economic risk choices in tasks adapted from foraging theory, in which optimal decisions need consideration of the outcomes of past choices or expected future opportunities. In such foraging tasks, human economic risk choice is explained by the principle of fitness maximization, which naturally leads to dynamic risk preference. Here, we investigated whether fitness maximization contributes to non-foraging economic decisions. In two online experiments, we investigated participants' risk preference dynamics as they made a series of risky economic decisions under changing environmental richness. We found that participants' risk preference was modulated by the current and past environments in accordance with fitness maximization. The participants were more risk averse during and after a rich environment as compared to a poor environment. Our results suggest that human economic decisions are better explained by the principle of fitness rather than utility maximization even in non-foraging situations.

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Poster

PSTR302: Decision-Making: Neural Mechanisms: Risk and Ambiguity

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Program #/Poster #: PSTR302.02/P12

Topic: H.03. Decision Making

Support: NIMH Grant R01MH128187

Title: Interictal epileptiform discharges and their influence on decision-making in epilepsy

Authors: *N. SHAHDOUST[1,2], R. COWAN[3], A. PRICE[4], B. KUNDU[5], T. DAVIS[3], J. D. ROLSTON[6], S. RAHIMPOUR[3], E. H. SMITH[3];
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Abstract: IEDs (interictal epileptiform discharges) are high-amplitude bursts of neural activity occurring between seizures, known to interfere with cognitive processes and memory. In this research we explored the impact of IEDs on cognitive control and decision-making during the Balloon Analog Risk Task (BART) in individuals with epilepsy. BART is used to assess decision-making under risk by simulating the inflation of a balloon, which increases potential rewards, but also the risk of popping and losing all gains.In our research 43 patients (21 females) undergoing invasive neuromonitoring for drug-resistant epilepsy, completed an average of

233.24 (± 23.81) BART trials. During these trials, real-time neural activity was recorded via 3,259 electrodes implanted in regions critical for cognitive control, such as the prefrontal and cingulate cortex. We identified IEDs occurrence per trial and timing in the neural recordings using a combination of band-pass filtering with a 4th-order Butterworth filter with a bandwidth of 5-25 Hz, applying a Hilbert transform on the output signal, and utilizing peak detection method to find the peak of the resulting signal. Our study examined how these IEDs impacted response time (RT) and inflation time (IT) during three 500 ms time windows relative to stimulus onset (pre: 500 ms before balloon onset to balloon onset; peri: 250 ms before to 250 ms after balloon onset; post: 500 ms following balloon onset).The findings showed that the presence of IEDs significantly lengthened both RTs and ITs, particularly when they occurred in brain regions linked to cognitive functions, such as the parietal, prefrontal, basal ganglia, and thalamic areas. This suggests that IEDs disrupt normal decision-making processes by affecting the neural computation in these cognitive regions. Additionally, while most patients showed increased RTs, some displayed shorter ITs, indicating a potentially heightened risk-averse behavior in certain brain conditions. The study emphasizes the profound influence of IEDs on behavioral responses in risk-based decision-making tasks and suggests a complex interplay between IEDs and cognitive control mechanisms. This research sets a foundation for future exploration into the neural dynamics underlying decision-making in epilepsy. Our next goal is to explore the impact of IEDs on specific features of brain activities such as spectral representations and neural firing rates associated with BART performance.

Disclosures: N. Shahdoust: None. **R. Cowan:** None. **A. Price:** None. **B. Kundu:** None. **T. Davis:** None. **J.D. Rolston:** None. **S. Rahimpour:** None. **E.H. Smith:** None.

Poster

PSTR302: Decision-Making: Neural Mechanisms: Risk and Ambiguity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR302.03/Q1

Topic: H.03. Decision Making

Support: HKRGC-CRF C4012-22G
HKRGC-GRF 14115821
Theme-Based Research Scheme T13-605/18-W), CUHK 7104252

Title: Cognitive flexibility in decision-making under uncertain conditions

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Abstract: Cognitive flexibility refers to the capacity to adapt and adjust one's decision-making process in response to changing or ambiguous conditions. An effective strategy in decision-making under uncertain situations is to rely on previously learned information, an ability that is known to be impaired in some diseases like Parkinson's disease. This study explores the involvement of the prefrontal cortex (PFC) in this ability. C57BL/6 mice were employed to establish an auditory decision-making behavioral paradigm. Through training, all mice were able to associate sounds of different frequencies (3kHz and 16kHz) with different buttons to get rewards, achieving a success rate over 80%. When sounds of never-learned frequencies were introduced (4.5 kHz, 7 kHz and 10.6 kHz), animals tended to choose the side closer in frequency to the previously learned sounds, generating a sigmoidal curve with a midpoint of 10.6 kHz at which the animals made random decisions. Manipulating the ratio of low-frequency and high-frequency sounds enabled the animals to make choose the side that was associated with the higher proportion of the sound. Post-mortem immunohistochemistry results showed that changing the ratio of sounds during the ambiguous conditions led to a specific increase in c-fos expression in the orbitofrontal cortex of PFC. This finding suggests that the orbitofrontal cortex may play a role in the cognitive flexibility observed in the mice, facilitating their ability to utilize previously perceived information to make decisions under uncertain situations.

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Poster

PSTR302: Decision-Making: Neural Mechanisms: Risk and Ambiguity

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Program #/Poster #: PSTR302.04/Q2

Topic: H.03. Decision Making

Support: R01MH128187

Title: Reward probability encoding in human neurons

Authors: *A. PRICE[1], R. L. COWAN[1], T. S. DAVIS[1], S. RAHIMPOUR[1], B. SHOFTY[1], M. M. BOTVINICK[2], T. H. MULLER[3], S. W. KENNERLEY[4], J. D. ROLSTON[5], E. H. SMITH[1]; [1]Neurosurg., Univ. of Utah, Salt Lake City, UT; [2]Univ. Col. London, London, United Kingdom; [3]Univ. of Oxford, Oxford, United Kingdom; [4]Exptl. Psychology, Oxford Univ., Oxford, United Kingdom; [5]Neurosurg., Brigham and Women's Hosp., Boston, MA

Abstract: To survive and succeed in a complex world, individuals must make decisions that lead to uncertain or delayed rewards (Kahneman & Tversky, 1979). Risk can be defined as the amount of uncertainty associated with a future reward. Previous neuroimaging work in humans has identified signatures of reward and risk encoding in the brain. Reward is encoded linearly and Risk is encoded quadratically across reward probabilities (Preuschoff et al., 2006, 2008).

Here, we sought to understand how decision uncertainty was represented in populations of neurons in human decision-making circuits. Single neuron activity (334 well-isolated units) was recorded using Behnke-Fried microwires extending from the distal tip of the clinical macroelectrodes. Units were recorded from the Anterior Cingulate Cortex (ACC), ventromedial Prefrontal Cortex (PFC), Orbitofrontal Cortex (OFC) and Mesial Temporal Lobe (MTL). Subjects performed the Balloon Analog Risk Task (BART) where they initiate and stop a computerized balloon's inflation. Each trial results in either the inflation being stopped and points being awarded proportional to the size of the balloon, or the balloon pops and the subject doesn't receive nor lose any points. BART also has passive and active trials to modulate the amount of risk involved. Generalized linear models were fit to firing rates and it was found that a significant proportion of units encoded reward probability monotonically and quadratically. Across units, we found significant encoding of reward in all of the brain areas except OFC (binomial test, $p < 0.05$). The majority of neurons that significantly predicted the reward probability categories in response to the cue, exhibited a reversal of their reward probability encoding in response to outcome (60% of units in ACC, 50% in MFC, 74% in MTL, and 50% in OFC). Each of the brain areas had significant quadratic encoding of reward probability at cue and outcome, indicating a significant encoding of risk.These data confirm and extend previous fMRI studies of encoding of reward and risk signals in the human brain. We find cortical units that perform computations that have also been found in subcortical structures.
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Preuschoff, K., Bossaerts, P., & Quartz, S. R. (2006). Neural Differentiation of Expected Reward and Risk in Human Subcortical Structures. *Neuron*, *51*(3), 381–390. https://doi.org/10.1016/j.neuron.2006.06.024
Preuschoff, K., Quartz, S. R., & Bossaerts, P. (2008). *Human Insula Activation Reflects Risk Prediction Errors As Well As Risk.*

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Poster

PSTR302: Decision-Making: Neural Mechanisms: Risk and Ambiguity

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Program #/Poster #: PSTR302.05/Q3

Topic: H.03. Decision Making

Support: Wellcome Trust Grant HMR05400 HM00.01
Rhodes Trust

Title: Decision making under uncertainty: impact of small vessel white matter disease

Authors: *F. C. WICKHAM[1], B. ATTAALLAH[1], S. G. MANOHAR[1,2], M. HUSAIN[1,2]; [1]Nuffield Dept. of Clin. Neurosci., [2]Exptl. Psychology, Univ. of Oxford, Oxford, United Kingdom

Abstract: Decisions are often made to maximize reward. Information gathering prior to making a definitive choice is critical to optimize such a process. Here, we examine how, in the context of uncertainty, people decide when they have sufficient information to make a final decision, comparing individuals with small vessel white matter disease (SVD) to healthy age-matched controls (HCs). We used variants of a paradigm consisting of both active sampling and passive choice tasks to rigorously evaluate decision making (DM) under uncertainty (Petitet et al. 2021 Nat HumanBehaviour). Two types of passive choice task were deployed: one where uncertainty and reward were the key variables that participants had to evaluate, and one where in addition the amount of physical effort required to obtain a reward was varied. 27 SVD patients and 36 HCs participated, allowing for assessment of the influence of vascular pathology on uncertainty perception, risk aversion, effects of sampling cost and reward, effort avoidance and adaptive decisional strategies. Measures of cognition, apathy, motivation, impulsivity, anxiety, and depression were also obtained, together with structural and diffusion-weighted MRI, and resting state fMRI. Overall, the SVD group who was significantly more apathetic and depressed scored significantly lower on the Addenbrooke's Cognitive Examination (ACE) than our control group. The results of the experimental tasks showed that while SVD patients' ability to perceive and respond appropriately to uncertainty in DM remained intact, their response to reward modulation was impaired. However, the introduction of physical effort in DM restored this and also heightened it in controls. In the active sampling task, patients gathered significantly fewer (information) samples and tolerated significantly greater uncertainty than HCs prior to committing to a decision. They were also significantly less sensitive to sampling cost. Analysis of the neuroimaging data revealed key white matter regions which are part of a brain network related to performance parameters underlying DM under uncertainty, with and without effort being a factor.

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Poster

PSTR302: Decision-Making: Neural Mechanisms: Risk and Ambiguity

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Program #/Poster #: PSTR302.06/Q4

Topic: H.03. Decision Making

Support: R01 DA049147

Title: Effect of potential debt and mixed gambles on risk-attitude

Authors: *K. LEE, V. STUPHORN;
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Abstract: Many everyday decisions involve some degree of uncertainty about its outcome. Such decision-making under risk is influenced by the individual's risk-attitude, which is highly variable and depends on both the internal state and perceived external context (e.g., current wealth, whether the choice regards potential losses or potential gains) at the moment of choice. Despite its significance in everyday life, the underlying neuronal mechanisms by which risk-attitude is shaped remains unclear. Previous work in our lab addressed this issue by relating core concepts from prospect theory, the most influential model to date for describing choices under risk, with neuronal activity in the anterior insula. Here, we extend our previous work by introducing changes to the design of our initial token-based gambling task that will allow novel tests of neuronal activity and behavior under risk. In this task, monkeys must choose between a smaller sure outcome and a larger probabilistic outcome. Monkeys are given tokens ("wealth") that can accumulate over multiple trials, which correspond to the amount of reward they will receive at the end of "cash-out" trials that occur intermittently. In particular, we newly introduce "anti-tokens" that signify sub-zero-token wealth levels thereby allowing the individual be in debt, and mixed gambles that have both potential gain and loss components (e.g., 75% chance of +2, 25% chance of -3). Under our new design, we will be able to probe the neural basis of the reference point, a central concept of prospect theory, and test the possibility of multiple reference points and their effect on risk-attitude. Moreover, we will investigate the differential effect of mixed gambles and pure gambles (i.e., gambles that can only result in either gain or loss domain; e.g., 75% chance of +2, 25% chance of +0) on behavior and neural activity. Together, our work will provide a richer mechanistic account of how risk-attitude is formed, changed, and implemented in the brain. We will present preliminary behavioral results from rhesus monkeys and humans performing this updated token-gambling task. This will allow us to directly compare risk-taking behavior across species, as well as provide grounds for investigating the neural basis of risk attitude.

Disclosures: K. Lee: None. **V. Stuphorn:** None.

Poster

PSTR302: Decision-Making: Neural Mechanisms: Risk and Ambiguity

Location: MCP Hall A

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Program #/Poster #: PSTR302.07/Q5

Topic: H.03. Decision Making

Support: NIH Grant R01

Title: Cumulative Lifetime Stress Exposure is Differentially Related to Ambiguity Attitudes in a Clinical Population with Opioid Use Disorder

Authors: *J. J. STUCKEY[1], F. LOFARO[1], J. KONG[1], S. HAFEZI[2], C. M. RAIO[3], A. B. KONOVA[1];
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Abstract: Human decision-making often occurs in contexts laden with uncertainty. This uncertainty can take at least two forms: decisions in which the probability of an outcome is known (risky) and those in which the probability of an outcome is unknown (ambiguous). Neuroeconomic models capture how these sources of uncertainty impact choice and have revealed human decision makers vary significantly in their risk and ambiguity attitudes. Notably, these attitudes only weakly correlate and have largely dissociable roles in health behavior, including substance use. Recent work has begun to identify factors that help shape how these attitudes might form and are maintained, showing that ambiguity (but not risk) attitudes can be explained, in part, by individual differences in major psychosocial stressor exposure across the lifespan. Here, we sought to examine the generalizability of these findings to a clinical population previously shown to exhibit distinct attitudes toward uncertainty and heightened exposure to psychosocial stress - individuals with opioid use disorder (OUD). Treatment enrolled OUD patients (N = 109, mean age = 49.3) and age- and sex-matched controls (N = 49, mean age = 45.4) completed a comprehensive lifetime stress assessment (the Stress and Adversity Inventory for Adults or STRAIN) and a well-validated economic decision-making task that evaluates both known-risk and ambiguity attitudes. Confirmatory analysis showed that patients exhibited a wide range of risk and ambiguity attitudes and reported a significantly higher number of stressful life events (20-30 more on average) and higher perceived severity of these events (2-3 times more) compared to controls and the original published sample. Interestingly, in patients, greater number and severity of lifetime stressors were selectively correlated with higher ambiguity aversion, but not known-risk aversion. The strength of this correlation did not differ significantly from that observed in controls. Moreover, across all participants, this relationship between stressor number and ambiguity aversion held separately for stressors reported during prenatal development, early life, and adulthood. These findings underscore the potential differential impact of stress across the lifespan on ambiguity attitude within clinical populations. Further, they show that decision-making tendencies in adulthood may be influenced by stressors that occur as early as gestation. This research could offer insights into how environmental and psychosocial stressors shape decision-making in addiction and broader populations.

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Poster

PSTR302: Decision-Making: Neural Mechanisms: Risk and Ambiguity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR302.08/

Topic: H.03. Decision Making

Title: Modelling metabolic influences on human risky choice

Authors: *S. GEYSEN[1], A. BRANDS[1], H. SCHULTZ[2], M. TITTGEMEYER[3], J. KOENIG[4], J. PETERS[1];
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Abstract: Processing risk and probabilities is central to various decision scenarios. These functions may be influenced by potential interactions of homeostatic hormones with the dopaminergic system and its central structures. However, previous studies reported both increased and decreased risk-taking after homeostatic modulations. Here, we investigated the effects of two homeostatic systems on risky choice behaviour and its underlying cognitive processes. In two separate experiments, homeostatic hormone levels were modulated by a short-term fasting intervention (N_{FAS} = 37) or one night of total sleep deprivation (N_{TSD} = 40), and compared to control conditions (within-subjects). In both experiments, healthy male volunteers performed a probability discounting task with simultaneous fMRI recording. All participants further provided blood samples in each condition to assess ghrelin and insulin. Model-free analyses revealed moderate evidence against manipulation effects on risky choice and response times in both studies. After fasting, there was strong evidence for increased ghrelin levels and decreased insulin levels. Frequentist statistics showed a moderate increase in ghrelin following TSD, which was inconclusive using Bayesian statistics. To investigate the homeostatic effects on the underlying cognitive processes we applied a hierarchical Bayesian modelling approach, comparing the common hyperbolic probability discounting (HPD) model and prospect theory (PT) model. However, due to recent success of choice perseveration, we extended these models by also accounting for perseveration. Here we consider first-order (repetition of the choice on the previous trial) and higher-order preservation which considers information spanning over a longer period. Overall, we compared 8 different models covering different combinations of the base models and proposed extensions. The PT model without perseveration performed best in both studies. Closer examination of the model parameters revealed strong evidence for increased sensitivity to probabilities and decision noise after TSD. Attractiveness to risk did not change meaningfully after TSD. No reliable changes in model parameters were observed after fasting. There was moderate evidence against associations between hormonal levels and parameter values in both studies. FMRI analyses are pending and will examine homeostatic effects on activity in the prefrontal cortex and insula during risky choice. Together, our results are inconsistent with the idea that hunger generally affects risk-taking, but confirm effects of sleep deprivation on specific computational components of the choice process.

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Poster

PSTR302: Decision-Making: Neural Mechanisms: Risk and Ambiguity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR302.09/Q6

Topic: H.03. Decision Making

Support: NSF-1948752

Title: Reward Prediction Updates: What Happens When the Probability of Reward Changes

Authors: *Z.-Y. YAN[1], S. F. BUCHER[2,3], B. SHEN[4], P. DAYAN[2], P. W. GLIMCHER[4]; [1]New York Univ., New York City, NY; [2]Max Planck Inst. for Biol. Cybernetics, Tübingen, Germany; [3]MIT Sloan School of Management, Boston, MA; [4]Neurosci. Inst., NYU Grossman Sch. of Med., New York, NY

Abstract: It is widely established that a reward prediction error signal can be observed in the human brain. When subjects believe there is a 50% chance of earning a reward and then receive it, areas like the ventral striatum and the ventromedial prefrontal cortex show BOLD activations correlated with a positive prediction error. This finding is based on the belief of a 50% reward chance, a reward expectation. This study aims to determine where and how these reward expectations are updated. Specifically, it asks whether BOLD signals in the ventral striatum and ventromedial PFC, known to encode reward prediction errors (Schultz, Dayan, and Montague, 1997; Caplin et al., 2010; Rutledge et al., 2010; Niv et al., 2012), also reflect updates to a subject's reward expectations, even without actual rewards. In an fMRI experiment, participants faced binary lotteries represented as partially occluded pie charts. While in the scanner we sequentially revealed more and more of the pie chart, dynamically resolving ambiguity about the probability of winning $20 or $100 as a trial progressed. The pie chart's composition was revealed only gradually to participants, so that they could sequentially update their belief about the probability of winning the prize. At a random time during this gradual resolution, as early as after the first view of the occluded pie chart or as late as after the full pie chart had been revealed, we elicited participants' valuation of the current pie chart-lottery using an incentive-compatible procedure. Behavioral data show that, in line with theoretical predictions, participants' valuations closely track the lottery's winning probability when comparing two lotteries with identical prizes (which amounts to pure belief elicitation). Our procedure results in a gradually updated reward expectation that is under clean experimental control. In the presence of ambiguity, participants' revealed beliefs are slightly more pessimistic, indicating ambiguity aversion. When the lotteries' prizes are not identical, participants' elicited probability equivalents reveal their degree of risk aversion.

The neuroimaging portion tests whether updates to reward expectations produced BOLD activations in reward prediction areas. This study seeks to see if reward prediction-error encoding areas represent updates to reward expectations, mirroring punishment expectation errors seen in similar locations (Seymour et al., 2003). This approach tests neuroeconomic theories making joint predictions about beliefs, behavior, and neural data, offering new empirical constraints on models of belief evolution.

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Poster

PSTR302: Decision-Making: Neural Mechanisms: Risk and Ambiguity

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR302.10/R1

Topic: H.03. Decision Making

Title: A prefrontal to Amygdala pathway for contextual selective awakening

Authors: *H. ZHU[1], R. WIMMER[2], N. H. LAM[2], J. SCOTT[2], Y. WANG[2], M. HALASSA[2]; [1]Tufts Univ. Sch. of Med., Boston, MA; [2]Sch. of Med., Boston, MA

Abstract: Sleep is an evolutionarily conserved behavior with multiple functions such as energy homeostasis, toxic waste removal and systems-level memory consolidation. Despite progress in understanding these associated functions, how sleep itself is coupled to rapid changes in environmental contingencies is poorly understood. Research on human subjects suggests that both associative and contextual contributions to this decision-making process. Subjects are more likely to awaken when their name is called(associative), highlighting the associative component. Sleeping through fireworks on new year's eve, but otherwise rapidly awakening to their sound highlights the contextual component. Neuroimaging studies have implicated the prefrontal cortex (PFC) and amygdala in goal-directed sleep interruption. However, due to limitations in accessing the human brain, the precise mechanisms remain unexplored. Here, we aim to identify neural mechanisms that selectively couple sleep interruption to behaviorally relevant environmental signals, a process we term goal-directed sleep interruption. We first developed a goal-directed sleep interruption paradigm in mice by leveraging an active avoidance paradigm to overcome the limitations. Our data indicate that mice selectively interrupt their sleep in an associative and contextual manner. Specifically, following appropriate training, mice awaken in response to a conditioned stimulus but only in the environment in which that conditioning occurred. Using advanced circuit dissection tools, we found that both mouse amygdala and PFC are involved in this process. The amygdala showed enhanced encoding of task cues after conditioning. Intervention of the different subdivisions of amygdala selectively disrupted CS+, but not CS- induced sleep interruption in the conditioned environment. Furthermore, we investigated the role

of the PFC in regulating the contextual component of sleep interruption, by having the mice sleep in both neutral and condition environment. PFC inactivation reinstated CS+-induced interruption only in neutral contexts. This suggests that the PFC may exert top-down control over learned associations, suppressing amygdala-mediated sleep interruption in neutral environments. Overall, by understanding how environmental cues influence sleep behavior at the neural level, we may uncover insights that could lead to novel therapeutic interventions for sleep-related disorders.

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Poster

PSTR302: Decision-Making: Neural Mechanisms: Risk and Ambiguity

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Topic: H.03. Decision Making

Support: NSTC 111-2628-H-A49 -004 -MY4
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Title: Detecting regime shifts in financial markets

Authors: *S.-W. WU;
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Abstract: Detecting regime shifts is ubiquitous, from assessing whether a pandemic has passed to the onset of a bear market. Previous studies showed that people systematically under- or overreact to changes: overreactions were primarily found when environments are stable (small transition probability) but signals are noisy (low signal diagnosticity), while underreactions were seen in unstable environments with precise signals. However, it seems possible that the valence of a regime—whether a certain regime is favorable or desirable—can also impact regime-shift judgments. For example, in a bull market, people may be less willing to accept a shift to the bear market, therefore causing them to underreact to change. In this study, we investigated how regime valence might impact regime-shift detection in a task where subjects had to decide whether to invest in a virtual financial market. At the beginning of each trial, subjects received information about market-shift direction (from good to bad markets, from bad-to-good markets), transition probability and signal diagnosticity. Subjects then went through 10 periods where at each period she or he received a signal (market value up or down). They were told that market can shift at any period but can only shift once. The task was to judge whether the market had shifted. At the conclusion of 10 periods, subjects were asked whether to invest on the market she or he believed to be in. We replicated previous findings on over- and underreactions to change.

Further, participants' sensitivity (n=33) to system parameters (transition probability and signal diagnosticity) were significantly correlated between the two market-shift directions. For investment decisions, both transition probability and signal diagnosticity significantly impacted investment decisions in both market-shift directions and there was a significant transition probability-signal diagnosticity interaction—signal diagnosticity had greater impact on investment decisions when participants were in more stable environments (low transition probability). However, participants' sensitivity to system parameters only partially impacted investment decisions, with only sensitivity to transition probability affecting investment decisions when subjects were in a good market with a potential to switch to a bad market. Together, these results suggest that people in general respond similarly to different market-shift directions. It is only when they face the potential of entering a bad-market regime that sensitivity to transition probability begins to impact financial choice.

Disclosures: S. Wu: None.

Poster

PSTR302: Decision-Making: Neural Mechanisms: Risk and Ambiguity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR302.12/R3

Topic: H.03. Decision Making

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r01mh120118
NSF Grant 2139936
NSF Grant 2003830
NSF Grant 1810758

Title: Coglink networks reveal computational mechanisms of uncertainty management and its perturbation in schizophrenia

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Abstract: Animals navigate uncertainty to optimize rewards, but neural mechanisms and their deviations in Schizophrenia (SZ) remain unclear. We introduce CogLink Networks, a novel class of neural models integrating biophysical mechanisms with normative models to address this gap. We model networks of prefrontal cortex (PFC), mediodorsal thalamus (MD) and basal ganglia (BG) and include mechanisms such as thalamic modulation of cortical activity (Rihkye et al. 2018) and plasticity (Canto-Busto et al. 2022) through cortical interneurons. To model uncertainty linked to associations and contexts, we consider a probability reversal bandit task.

Our model is more accurate than the standard algorithm in dynamic bandits (+4.9±0.8%, p=4e-12) and shows contextual tuning in MD consistent with our past work. Additionally, our model switches in fewer trials (-6.8±1.3, p=2e-13) and learns to switch faster (ρ=-0.29, p=4e-5). PFC-MD synapses also learn the contextual model of the environment. To understand the efficacy, we take the CogLink approach and approximate the model to a normative one. Specifically, our model approximates CUSUM, an optimal algorithm to detect environmental change, providing a basis for flexible switching. It also learns value and model estimates without forgetting them across contexts. From the cortical interneuron activity, we discover that MD gates plasticity based on contextual uncertainty through interneurons to achieve continual learning. To show CogLink's ability to connect circuit-level perturbation and behavioral changes, we consider an MD lesion experiment and a striatal D2 receptor abundance SZ model to replicate experimental findings. Rihkye et al. show that MD inactivation disrupts flexible switching and indeed the silenced model takes more trials to switch (+64.1±2.4, p=6e-60). Waltz (2017) reports that SZ patients show longer switching time and elevated win-switch rates and our SZ model also has higher switching time (+16±2.7, p=4e-3) and win-switch rates (+6.3±0.9%, p=5e-18). Strikingly, PFC-MD synapses cannot learn an accurate model of the environment, potentially reflecting delusional thinking. Following the CogLink approach, we approximate the impaired model into a normative one and derive that the threshold of the underlying normative model to output environmental change becomes much smaller, indicating a strong prior in environmental volatility. Recently, Zhou et al. show MD stimulation rescues cognitive deficits in SZ model mice and indeed MD stimulation reduces switching time (-18.8±3.3, p=3e-16) and win-switch rates (-3.5±1.1%, p=0.04) and recover model's capacity to learn the correct model of the environment.

Disclosures: B. Wang: None. **N. Lynch:** None. **M. Halassa:** None.

Poster

PSTR303: Executive Functions: Prefrontal Mechanisms in Humans

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Program #/Poster #: PSTR303.01/R4

Topic: H.04. Executive Functions

Support: NIH Grant U01NS117839
NSF Grant BCS-2219800

Title: Non-orthogonal population geometry underlies coding of action representations by single neurons in human medial frontal cortex during cognitive control

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Abstract: The capacity to monitor one's behavior and enact control when necessary is crucial for intelligent, goal-directed behavior. While it is generally agreed that the medial frontal cortex (MFC) plays a central role in performance monitoring, it is unclear whether it does so by (1) maintaining representations of primed and correct responses or (2) selectively amplifying and suppressing information based on task demands. In the present study, we investigated these hypotheses by re-analyzing recordings of single neurons from 34 human patients who performed a Stroop task while undergoing epilepsy monitoring (Fu et al., 2022). We found that the pre-supplementary motor area (preSMA, N=607 neurons), a subregion of MFC, encodes the specific identity of both the correct and primed responses, favoring hypothesis (1). Interestingly, however, population-level decoders trained in one context exhibited below-chance performance when applied to the other, suggesting that preSMA uses a non-orthogonal representational geometry that may serve to disentangle task-relevant and irrelevant information reminiscent of hypothesis (2). This geometry was not present in the dorsal anterior cingulate cortex (dACC, n=584 neurons). Computational modeling suggests that this representational geometry is incompatible with models that rely on competition between cued responses but is exhibited by feedforward neural networks trained to perform error monitoring in a Stroop context. These networks also encode conflict in a manner similar to human preSMA despite not being explicitly trained to do so, suggesting that the representational geometry we observed in preSMA may be specifically relevant to error monitoring. Consistent with this hypothesis, individual neurons that signal errors following incorrect responses often exhibit firing rate patterns consistent with this representational geometry before response onset.

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Poster

PSTR303: Executive Functions: Prefrontal Mechanisms in Humans

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Program #/Poster #: PSTR303.02/R5

Topic: H.04. Executive Functions

Support: R01MH110831
BCS-1554105
U01NS117839

Title: Relationship between single-neuron correlates of action monitoring and reward processing in the human medial frontal cortex

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Abstract: We investigate the relationship between errors in goal-directed behaviors that are resulted from cognitive control failures (referred to as “action errors”) and reward-related outcome signals. The medial frontal cortex (MFC) is one of the key substrates for signaling action outcomes, reward prediction errors (RPEs) as well as action errors. A major open theoretical question is whether RPEs and action errors are detected and signaled by the same or different neural substrate. We propose that action errors, which are signaled by the error-related negativity (ERN), reflects a local process within the MFC that compares a corollary discharge of the executed action with a predicted action outcome generated by action forward models. The RPE, on the other hand, is a result of input from midbrain dopamine neurons to the MFC. If this hypothesis is true, these two types of error signals should involve separate groups of MFC neurons. In this study, we tested this hypothesis by designing a novel task that elicits uncorrelated action errors and reward prediction errors.We designed a one-arm bandit task where subject needs to make a choice, but it requires physical skills to make such choices. To maximize rewards, subjects must be able to learn a skill to execute choice and to learn about the reward value for each option. We recorded single neurons in the MFC in 8 patients implanted with hybrid depth electrodes for evaluation for drug resistant epilepsy. Subjects made action errors in ~20% of trials in attempting to reach the chosen option. We construct reinforcement learning model to parametrize subjects’ trial-to-trial learning of reward values. We construct an observer-controller model to evaluate subjects’ moment-to-moment monitoring and control of their skill performance within a trial. We analyzed the encoding of action errors, reward outcome and reward prediction errors (RPE) at the neuronal level. Across the N=159 recorded MFC neurons, ~25% of neurons signaled action errors and ~20% of neurons signaled reward prediction error. Remarkably, there was little overlap between these two groups of neurons. On the population level, these two groups of error neurons support robust decoding of action errors and RPEs. These results support our hypotheses that the action errors and RPE are separate signals in the MFC, useful for updating different state representations: the former for updating the state of cognitive control, whereas the latter for updating the state of the external world.

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Poster

PSTR303: Executive Functions: Prefrontal Mechanisms in Humans

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Program #/Poster #: PSTR303.03/R6

Topic: H.04. Executive Functions

Support: U01NS132788
U01NS117839

Title: Causally influencing momentary happiness and RPE representations by microstimulation of the human Substantia Nigra compacta

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Abstract: Computational modelling has demonstrated that the experience of happiness in a probabilistic reward task is determined by a combination of expected reward and reward prediction errors (RPE), with RPEs explaining most variance (Rutledge et al., 2014). To test this model causally, we hypothesized that microstimulation of neurons in the Substantia Nigra compacta (SNc would manipulate RPEs, and therefore subjective happiness). We conducted behavioral experiments while stimulating and recording from 93 single neurons in SN in 25 Parkinson´s patients during DBS surgery. We used a probabilistic reward task in which subjects chose between a certain option and a gamble with equal probabilities of two outcomes. In addition, every 3 trials, subjects were asked to answer, "How happy are you right now?". We used microstimulation (0.03mA, 130Hz) for half of the trials in blocks of 3. We fit a computational model to examine the relationship between happiness ratings and chosen certain rewards (CRs), the expected values (EVs) of chosen gambles, and Reward Prediction Errors (RPEs). First, RPEs explained more variance in happiness than EV, confirming earlier findings. Second, we evaluated whether subjects modified how they decided whether to gamble or not as a function of the expected value of the offered gamble relative to the certain reward (CR-EV) and whether stimulation was on or off with a 2-way ANOVA. The main effect of stimulation was significant, with the mean value of CR-EV significantly lower (and thus the gamble worth more) when they gambled when stimulation was on. The total earnings obtained by patients were higher across all stimulated compared to non-stimulated trials. This effect was due to patients obtaining higher rewards, on average, when they gambled and not by a difference in rewards obtained when they chose the certain reward. Strikingly, stimulating SN also led to higher happiness ratings. This effect is potentially explained by higher EV and RPE values. Furthermore, we found a significant interaction between Gamble Type offered (Gain/Mix and Loss) and Stimulation (ON/OFF) for likelihood of gambling, indicating patients gambled more for stimulated Mix and gain trials but not loss trials. 19/93 of SN neurons were responsive to momentary happiness during the happiness rating period. These neurons also tracked happiness throughout the rest of the experiment. Microstimulation modified the firing rate of these neurons in a brain state dependent manner, indicating that their excitability changed as a function RPE

and happiness. We can conclude that stimulation modified the decision threshold so that subjects were more likely to accept gambles with higher EVs.

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Poster

PSTR303: Executive Functions: Prefrontal Mechanisms in Humans

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR303.04/S1

Topic: H.04. Executive Functions

Support: Friends of BrainHealth

Title: Mindful emotion regulation: greater present moment awareness precedes better executive control

Authors: *G. BATCHALLI MARUTHY[1], L. HIMES[2], A. CAMPOS[1], B. P. RYPMA[3]; [1]The Univ. of Texas at Dallas, Richardson, TX; [2]Behavioral and Brain Sci., [3]Behavioral & Brain Sci., Univ. of Texas At Dallas, Dallas, TX

Abstract: Trait mindfulness (TM) is dispositional awareness to the present moment with nonjudgmental acceptance. While salutary benefits of mindfulness have been demonstrated, neural mechanisms underlying them remain unknown. Some studies report recruitment of top-down regulatory areas (eg, prefrontal cortex) and others report recruitment of bottom-up emotion processing areas (eg, amygdala, insula). This between-study variability suggests that different strategies may be invoked by task variables such as stimulus type, extent of individuals' TM levels, and time course dynamics of mindful emotion regulation. Behavioral data suggest a time course dynamic in which higher mindful individuals adapt and update strategies across the duration of an affective task. Specifically, if awareness to emotional stimuli enhances affective influence initially, we expect increased activity in emotion processing areas. When such emotions are then regulated through nonjudgmental acceptance, we expect increased activity in top-down regulatory areas. We tested whether individual differences in TM predicted changes in brain areas employed across the duration of an emotional Stroop task. During scanning, participants saw emotional words and pressed one of four buttons that corresponded to the font color of the word. Words were chosen from the English Lexicon Project and manipulated on valence and arousal resulting in 4 experimental conditions (eg, Positive valence High arousal). Neutral words served as a control condition. Words were equivalent in length and dominance ratings. TM was measured using Five Facet Mindfulness Questionnaire. Functional echo planar images were acquired on a 3T Prisma SIEMENS scanner. Anatomical MPRAGE T1-weighted images were also obtained. fMRI data were preprocessed with a standard pipeline. Linear mixed

effects analyses were conducted on the beta coefficients obtained from the first level general linear models for the experimental-control contrasts. Amygdala and insula showed significant decreases in signal over the course of the task, conditional on TM levels. Prefrontal cortex showed significant increases in signal over the course of the task, conditional on TM levels. These results indicate that higher mindful individuals process, update and regulate emotions generated by external environments differently over time than lower mindful individuals. It may be that such time-course changes, in emotion processing and regulation areas, account for between-study variability in the TM literature and comprise the mindful emotion regulation mechanism that underlies the salutary cognitive benefits attributed to mindfulness.

Disclosures: G. Batchalli Maruthy: None. **L. Himes:** None. **A. Campos:** None. **B.P. Rypma:** None.

Poster

PSTR303: Executive Functions: Prefrontal Mechanisms in Humans

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Program #/Poster #: PSTR303.05/S2

Topic: H.04. Executive Functions

Support: Haverford College Faculty Research Grant

Title: Adversity and error-monitoring: effects of emotional context

Authors: D. SHUDRENKO, E. NG, K. MANN, E. TURDUKULOV, *R. COMPTON; Haverford Col., Haverford, PA

Abstract: Based on prior evidence that early-life stress impacts frontal lobe development, this EEG/ERP study tested whether self-reports of childhood adversity predict alterations in neural markers of error processing, a crucial element of executive functioning. We further investigated whether any such associations would differ under emotional versus non-emotional task conditions, reasoning that conditions with emotional distractors are especially relevant to information processing in everyday life. $N = 99$ undergraduates completed two selective attention tasks, a classic color-word Stroop task and a modified task using emotional words, while EEG was recorded. Participants also completed self-report measures of adverse and positive childhood experiences, executive functioning challenges, depression, and current stress. Reports of adversity were robustly correlated with self-reported challenges in executive functioning ($r = 0.41$, $p < .001$), even when controlling for depression and stress (partial rs > 0.20, ps < .05). However, adversity was not correlated with performance (reaction time or accuracy) in the color-word Stroop (ps > .25) or the emotion-word Stroop (ps > .11). EEG/ERP markers of error processing included the error-related negativity (ERN), which reflects initial error detection; the error positivity (Pe), which reflects sustained error processing; and error-

related alpha suppression, which reflects arousal-related oscillatory changes following errors. When controlling for individual differences in task accuracy, greater adversity predicted an enhanced ERN and blunted Pe (partial *r*s < -0.20, *p*s < .05), but only during the emotion-word blocks of the task. Alpha oscillations were also predicted by adversity, in a pattern that implies less error-responsiveness during the emotion block compared to the color block among participants with higher adversity ($r = 0.22$, $p < .05$). Overall, results indicate that in this high-functioning sample, adverse life experiences were correlated with alterations in several neural markers of error-monitoring. In an emotional context, initial error detection was enhanced and sustained error processing was blunted among those who reported greater adversity, even in the absence of overt performance changes. The findings imply that the emotionality of the task context is an important factor when examining the potential impact of adversity on executive functions.

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Poster

PSTR303: Executive Functions: Prefrontal Mechanisms in Humans

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Program #/Poster #: PSTR303.06/S3

Topic: H.04. Executive Functions

Support: R00MH126161

Title: Investigating the neural basis of social anxiety disorder using a novel social judgement approach-avoidance task

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Abstract: Social anxiety disorder (SAD) is the experience of intense fear in social situations and diagnoses are rising precipitously since the pandemic. The cognitive processes impacted by symptoms of SAD and their corresponding neural correlates are poorly understood. Existing literature points to two relevant constructs: inaccurate anger perception for ambiguous faces and increased avoidance during perceived anger. To investigate the relationship of these constructs to symptoms of SAD, we developed a novel task that required participants to make a social judgement (anger perception) and then perform an approach or avoid behavior. In our study, participants that were diagnosed with SAD using the Mini International Neuropsychiatric Interview for the DSM-V completed the social judgement approach-avoidance task (SJ-AAT) while high-density electroencephalography (EEG) was recorded. Severity of SAD symptoms

were quantified along multiple dimensions using the Liebowitz Social Anxiety Scale. We found that our task captured meaningful differences in social judgement by manipulating the degree of angry-neutral morph. Furthermore, participants with SAD were slower and less accurate when required to approach, relative to avoid, an angry face. Our analyses dissociate individual differences in social judgement and approach-avoidance that are related to prefrontal control over the motor and perceptual system. The neural mechanisms identified in this study will be targeted in a future study using non-invasive brain stimulation to establish causal evidence for these correlational findings.

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Poster

PSTR303: Executive Functions: Prefrontal Mechanisms in Humans

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Topic: H.04. Executive Functions

Support: This work was supported by the Polish National Science Center under Grant No: 2019/33/B/NZ7/01980

Title: Impact of high-intensity interval exercise on executive function, prefrontal cortex activation, and peripheral neuroprotective molecule concentration among the elderly

Authors: *S. KUJACH[1,2], M. SKUREWICZ[2], Z. JOST[3], A. SAWICKA[1], M. CHROBOCZEK[2], R. LASKOWSKI[2], R. A. OLEK[4];
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Abstract: Background: The aging process naturally involves not only a decline in physical fitness, but also in cognitive functions. Both factors present considerable challenges to the independence and quality of life of the elderly. Our previous research has shown that high-intensity interval exercise (HIIE) can improve the physical fitness and cognitive function of young people, but the results of studies among seniors are still ambiguous. Multiple animal and human studies have revealed that exercise enhances human cognition via exercise-enhanced neurotrophins and catecholamine synthesis, which is known to mediate neural plasticity and energy metabolism in the brain. However, the neural mechanisms responsible for the post-exercise improvement of cognitive functions in elderly are still being sought. **Aim:** The purpose of the present study was to assess the acute effect of HIIE on executive function focusing on

underlying neural and neurobiological factors among the elderlies. **Methods:** The study involved 19 females and 5 males (69.4 ± 3.4 years old). The counterbalanced, crossover, randomized trial consisted of two sessions: control (CTL) and high-intensity interval exercise (HIIE). The HIIE protocol comprised eight 60s cycling bouts at ~90% HR_{max} intensity separated by 60s passive rest. The participants performed the Stroop test and Trial Making Test (TMT-A and TMT-B) before and after exercise or control condition. Cortical activation has been measured by applying functional Near-Infrared Spectroscopy (fNIRS). Moreover, before and after the HIIE to assess the concentration of brain-derived neurotrophic factor (BDNF), Klotho, Cathepsin B, Irisin, as well as Tryptophan (Trp) metabolite, venous blood samples were collected. **Results:** HIIE contributed to a significantly shorter execution time in the TMT-B test. Moreover, an increased prefrontal activation (dorsolateral prefrontal cortex and middle frontal gyrus) has been observed following an acute bout of HIIE. Additionally, we found a significant increase in peripheral Klotho and Cathepsin B concentration. Interestingly, the obtained results showed a decrease in the ratio of neurotoxic to neuroprotective tryptophan metabolite levels from pre- to post-HIIE. **Conclusions:** The results suggest that the proposed HIIE protocol can effectively improve executive function in the elderly, which can be attributed to increased activation in cortical areas relevant to cognitive functioning and synthesis or release of neuro-supportive molecules.

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Poster

PSTR303: Executive Functions: Prefrontal Mechanisms in Humans

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Program #/Poster #: PSTR303.08/S5

Topic: H.04. Executive Functions

Support: KMDF-RS-2022–00140478

Title: Interactive Multi-Touch Games Enhanced Prefrontal Inter-Brain Synchrony and Cognitive Flexibility Related Network Connectivity in the Elderly

Authors: *E. KIM[1,2], J. KIM[3], S.-H. LEE[2], Y.-H. KIM[2,4];
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Abstract: This study explored the impact of interactive multi-touch cognitive games on prefrontal activation, cognitive flexibility related network connectivity, and inter-brain synchrony in the elderly. Thirty-two elderly participants (13 males; mean age 74.5 ± 4.3)

participated in a single-blind, randomized, controlled trial, divided into an interactive multi-touch game-based cognitive intervention (ICI) group and a traditional cognitive intervention (TCI) group. Over four weeks, both groups engaged in twelve 40-minute cognitive training sessions. The ICI group partook in gamified cognitive tasks on a multi-touch screen designed for collaborative play across key cognitive domains using HAPPYTABLE® (Spring Soft Co. Ltd, Seoul, Republic of Korea), whereas the TCI group engaged in traditional paper-and-pencil tasks. We assessed changes in cognitive flexibility-related prefrontal activity and network changes using a 20 channels of functional near-infrared spectroscopy (fNIRS) system (NIRScout®, NIRx Medical Technologies, Germany) during color-word Stroop task. Inter-brain synchrony was evaluated through hyperscanning techniques during the intervention, utilizing the same 20-channel topographic map across participants. After 10 intervention sessions, the ICI group showed significant improvement in interference scores and interference ratios of Stroop test compared to TCI group. Significant differences were noted in oxyHb concentration during Stroop interference at the prefrontal cortex between the ICI and TCI groups. In the ICI group, oxyHb concentration increased in these areas at post-intervention, whereas they decreased in the TCI group. Connectivity within the prefrontal cortex significantly enhanced in the ICI group after 10 intervention sessions. While some connections in the TCI group showed significant increases, others were reported to decrease. During the intervention, intra-brain synchrony within the prefrontal cortex was stronger in the ICI group compared to the TCI group. Remarkably, inter-brain synchrony, reflecting connectivity between participants, was significantly stronger in the ICI group than in the TCI group, especially between the dorsolateral prefrontal cortex and orbitofrontal cortex of two subjects. Interactive multi-touch game-based interventions effectively enhance cognitive flexibility, prefrontal activity, network connectivity, and inter-brain synchrony among the elderly. These findings may advocate for incorporating collaborative digital games into cognitive training programs as a viable strategy for modulating age-related cognitive decline.

Disclosures: E. Kim: None. **J. Kim:** None. **S. Lee:** None. **Y. Kim:** None.

Poster

PSTR303: Executive Functions: Prefrontal Mechanisms in Humans

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Program #/Poster #: PSTR303.09/S6

Topic: H.04. Executive Functions

Title: Cerebellar vermis atrophy associates with executive dysfunction in multiple system atrophy

Authors: *Y. TANIGUCHI[1], Y. UCHIDA[1,2], N. MATSUKAWA[1];
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Abstract: Background: Executive dysfunctions are observed in Multiple System Atrophy (MSA). Neuropathological studies have revealed that there was a significant neuronal loss in the frontal cortex of MSA patients with executive dysfunctions. Further, an anatomical connective network between the prefrontal cortex and cerebellar vermis has been reported. This study aimed to investigate the association between the executive functions and anatomical volumes of the whole brain in MSA patients using voxel- and atlas-based morphometric analyses. Methods: We retrospectively analyzed magnetic resonance imaging (MRI) to acquire magnetization-prepared rapid gradient echo images from 27 patients and 19 healthy controls. MRI data were analyzed using voxel-based morphometry (VBM) and atlas-based analysis, in which the cerebellar atlas created by Johns Hopkins University was used. Executive dysfunctions were assessed using Frontal Assessment Battery (FAB). We analyzed the correlation between the FAB scores and atlas-based volumes. Results: In the VBM analysis, patients with MSA exhibited gray matter volume reductions of the basal ganglia and cerebellar lobes, compared to healthy controls. Atlas-based analysis revealed white matter volume reductions of the cerebellar vermis X and frontal lobe including the orbitofrontal area in MSA patients. The FAB scores were correlated with the volumes of the cerebellar vermis VI and VII. Conclusions: Our study revealed that executive dysfunctions in MSA were associated with volume reductions of the cerebellar vermis.

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Poster

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Program #/Poster #: PSTR303.10/T1

Topic: H.04. Executive Functions

Support: NCN 2022/47/B/HS6/02748

Title: Prospective executive control in humans: combined analysis of EEG and single-neuron recordings

Authors: *M. MAGNUSKI[1], A. A. KOLODZIEJ[2], A. STANISZEWSKA[2], K. PALUCH[3], W. M. SREDNIAWA[3], W. FORTUNA[4], D. IVANOVSKI[5], R. BURNS[5], P. TABAKOW[4], H. BABU[5], A. BRZEZICKA[2], J. KAMINSKI[3];
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Abstract: In our daily lives, we frequently encounter situations where we need to flexibly switch between encoding new information and maintaining what's already stored in our working memory. Knowing how many pieces of information need to be encoded (such as a short PIN code or an entire telephone number) enables us to allocate memory resources effectively. This awareness also helps us anticipate when to engage executive control, transitioning from encoding to maintenance mode.
To investigate this cognitive function we combined analysis of EEG and single-neuron recording in humans. We first focused on a classic EEG Sternberg procedure, where healthy subjects are unaware of the number of memory items they will encounter in each trial. We found that uncertainty about the onset of maintenance is reflected in the amplitude of maintenance-evoked P300-like EEG potential. This potential can be source localized to medial prefrontal regions (SMA, pre-SMA, ACC). Additionally, earlier (more uncertain) maintenance onset leads to a delay in the increase of alpha power - an oscillatory hallmark of memory maintenance. These findings suggest that surprising maintenance onset may trigger a later switch to memory maintenance, possibly guided by medial prefrontal regions.
Next, we recorded activity of single neurons in epilepsy patients as they completed a working memory task, where in 50% of trials the information of upcoming memory load was provided (load cue). In trials without load cue (uncertain maintenance onset) we observe that after maintenance onset average single unit effects are spatially correlated with source localized EEG uncertainty effects. On the other hand, during trials with a load cue, this spatial relationship shifts in time before the onset of maintenance. This shift may reflect utilization of load cue information to prepare for the anticipated encoding-maintenance switch.
In summary, these findings demonstrate that neurons within the medial prefrontal regions play a role in prospective executive control, and the impact of their activity can be detected in scalp EEG recordings.

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Poster

PSTR303: Executive Functions: Prefrontal Mechanisms in Humans

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR303.11/T2

Topic: H.04. Executive Functions

Title: Dissection of functional networks in the human brain using electrical stimulation and fMRI

Authors: *R. XU[1], A. TAKAHASHI[1], A. BUSH[2], A. WALTON[2,1], S. HUTCHINSON[1], N. JHINGAN[1], N. SISTERSON[2], V. KOKKINOS[2], C. J. VALENZUELA[1], A. MARVI[1], H. KEAN[1], R. PRAMOD[1], S. YEE[3], J. KIRSCH[4], N. G. KANWISHER[1], E. FEDORENKO[1], R. DESIMONE[1], M. RICHARDSON[2];
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Abstract: Understanding how neural circuits in the human brain anatomically connect is fundamental to understanding their function and dysfunction. The common noninvasive methods for mapping human brain connectivity, such as diffusion MRI and resting-state fMRI, have shown limited correlation with "ground-truth" anatomical tracing results in animal studies. We recently demonstrated in nonhuman primates (NHPs) that concurrent electrical stimulation and fMRI (ES-fMRI) can map connectivity patterns that well match tracing (Xu et al 2022, *Neuron*). Here, we report preliminary data on ES-fMRI connectivity in epilepsy patients.
We have developed a novel, IRB-approved ES-fMRI protocol that stimulates SEEG electrodes intracranially implanted for seizure mapping. This protocol, built upon previous studies demonstrating the feasibility, employs state-of-the-art MRI acquisition and has significantly improved signal quality and resolution while minimizing image artifacts and known risk factors, such as heating. Our findings show a remarkable multi-fold increase in statistical power (per t-statistic) compared to prior human ES-fMRI data, meeting the level deemed sufficient for reliable connectivity mapping in our NHP study. For most patients, we ran tailored fMRI and SEEG functional localizers to help plan stimulation sites and interpret results.
We had mapped ES-fMRI connectivity of five lateral prefrontal (lPFC; n = 1/4 by subject) and two anterior temporal (aTEMP; n = 1/1) sites in two subjects at the time of writing, which holds promising implications. Both lPFC and aTEMP are key areas for cognitive functions like executive control, social perception, and language. Consistent with our NHP work, we found predominately ipsilateral cortical and subcortical connections except in the cerebellum. The connections were strongest in lPFC/aTEMP, depending on the stimulation sites. Furthermore, the sites showed distinct, location-dependent connections in and outside of lPFC/aTEMP. We also found coarse resemblance in the connectivity patterns between pairs of NHP vs. human sites. Importantly, the connection zones of a stimulation site consistently showed similar functional mapping profiles. For example, a language processing site in aTEMP connects to patches that are distant from each other but all respond strongly to language tasks, whereas an executive control site in lPFC connects to executive control patches and high-level visual regions. These findings suggest that functionally informed human ES-fMRI could potentially unravel the organizing principles of the cognitive brain and decipher the interacting logic within and across functional networks.

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Poster

PSTR303: Executive Functions: Prefrontal Mechanisms in Humans

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR303.12/T3

Topic: H.04. Executive Functions

Support: TUBITAK 120K924

Title: It's not the rule! Rule representations are not present in multiple demand regions

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Abstract: Frontoparietal multiple-demand (MD) regions are thought to instantiate cognitive control by representing the rules for selecting the correct action based on the evidence that different rules elicit distinct activity patterns in these regions. However, in these studies, different rules correspond to distinct episodes with distinct task identities. This makes it unclear if the representations behind the distinct activity patterns in MD regions are rules or task episodes. Task episodes are not synonymous with rules because different task episodes may involve the same rule and vice-versa. We have previously shown that the beginnings of distinct task episodes that involve the same rule, nonetheless, elicit distinct MD activity patterns.

Here we investigated if rules elicit distinct activity patterns in MD regions after task-episode-related confounds are removed. In experiment 1, participants executed trials where one of two rules, parity (P) or value (V), could be applied to the displayed number to select the correct response. The relevant rule, conveyed by the context in which the number was displayed, switched or repeated across consecutive trials. This biased participants to carve trial runs into one of two rule-based task episodes, e.g., if the rule sequence across trials was P P P V V P⋯, the first three trials become parity episode, the next two trials become value episode, etc. Trials, where the rule changed from the previous trial (i.e., switch trials) would mark the beginning of these episodes, while trials where the rule repeated from the previous trial (i.e., repeat trials) would lie within these episodes.

If rules were represented in MD regions, then trials with distinct rules, whether switch or repeat, would elicit distinct activity patterns in them. But, if only the beginning of distinct episodes elicited distinct activity patterns, then only switch trials with distinct rules would elicit distinct activity patterns. Repeat trials with distinct rule would not elicit distinct patterns. Using three measures of pattern differences - MVPA decodability, correlation, and Mahalanobis distance, we found this was indeed the case.

In experiment 2, we forced participants to organize these same trials not into rule-based episodes but into arbitrary 4-trial long episodes. Since switch trials no longer marked the beginning of these episodes, different rule-switch trials no longer elicited distinct activity patterns.

We thus show that rule representation, as commonly understood, is not present in MD regions

and suggest that we need to think of a different principle governing control instantiation. This study was funded by TÜBİTAK grant number 120K924.

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Poster

PSTR303: Executive Functions: Prefrontal Mechanisms in Humans

Location: MCP Hall A

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Program #/Poster #: PSTR303.13/T4

Topic: H.04. Executive Functions

Title: Altered Cerebello-Frontal Connectivity in MS Patients and its Role in Processing Speed

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Abstract: Multiple sclerosis (MS) is an autoimmune, demyelinating disease of the central nervous system. While presentation of symptoms may vary, cognitive dysfunction and processing speed are some of the most prominent deficits in MS. Resting-state functional connectivity demonstrates the functional organization of spatially distributed regions that comprise different cognitive networks. MS patients are also known to exhibit altered connectivity networks compared to healthy controls (HC). Previous work in healthy samples has shown that connectivity between the cerebellum and frontal areas is associated with cognitive speed. In this study, we sought to use MS as a model for cognitive dysfunction to examine the role that cerebellar-frontal connectivity may play in processing speed. 20 participants (10 MS, 10 HC) who met inclusion criteria underwent resting-state functional magnetic resonance imaging (fMRI) using a 3T MRI scanner. Cerebellar and frontal ROIs were obtained from structural scans (MPRAGE) using FreeSurfer. Data were pre-processed within the CONN Toolbox. Subject-level connectivity values between the seed ROI and the peak voxel within the significant cluster were extracted for further analysis. Outside of the scanner, the Digit Symbol Substitution Test (DSST) was administered as a measure of information processing speed and cognitive dysfunction. A multilinear regression model to test the interaction between DSST scores and group effects (i.e., MS or HC) on connectivity measures was performed. MS patients exhibited higher connectivity between lobule IV/V of the cerebellum and the left frontal lobe compared to HC: $t(16)= -7.80$, $p< .001$. A significant disordinal interaction was found between DSST scores and group in predicting connectivity measures between lobule IV/V of the cerebellum and the left frontal lobe ($p< .001$). These results suggest that a cerebello-frontal network is vital to processing speed function, and changes to this network, as a result of MS pathophysiology, may contribute to the cognitive impairment observed in MS patients.

Disclosures: J. Ma: None. **M.D. Zuppichini:** None. **D. Okuda:** None. **B.P. Rypma:** None.

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

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Program #/Poster #: PSTR304.01/T5

Topic: H.06. Social Cognition

Support: NIH Grant MH128190
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Title: Geometry of social gaze in the primate orbitofrontal cortex and amygdala

Authors: *G. QI, S. W. CHANG;
Dept. of Psychology, Yale Univ., New Haven, CT

Abstract: Social interaction in primate species depends on the efficient and flexible neural representation of social gaze primitives. These encompass crucial components of social gaze interaction like social state (indicating your level of social engagement), gaze content (identifying the subject of your gaze), and gaze duration (measuring how long your gaze persists). How might the brain represent social gaze primitives through abstraction to reduce dimensionality and facilitate generalization? Our previous work reported widespread representations of multiple social gaze variables in the prefrontal-amygdala networks during naturalistic social gaze interaction. However, the representational geometry of social gaze primitives in these brain areas remains unknown. Furthermore, the stability of neural geometry in naturalistic behavioral contexts without any task structure remains unclear. To address these questions, we examined the representational geometry of social gaze primitives based on single-unit activity collected from the dorsomedial prefrontal cortex (dmPFC), orbitofrontal cortex (OFC), anterior cingulate gyrus (ACCg), and basolateral amygdala (BLA) while pairs of monkeys freely gazed at one another. Social state was operationally defined as the relative fractions of social and nonsocial gaze bouts, and a classification procedure (optimal k-means) resulted in high and low social states. Gaze content was categorized as looking at a face or an object, and gaze duration was classified into long and short. With representational similarity analysis and a general linear encoding model, we found that there are prevalent neural representations of social state in all examined brain regions. Conversely, gaze content was encoded in the OFC and BLA, but not in the ACCg and dmPFC. Furthermore, using population decoding and representational geometry analysis with a linear support vector machine, we found that only gaze state, but not social content, is represented in an abstract format in the BLA and OFC without decreasing their separability. Further single-neuron level analysis suggests that, although the selectivity of gaze content correlated between different social states, the neural information of gaze content was disjoint-mixed between social states in both BLA and OFC.

Both the neural representational geometry and single neuron findings are consistent in two individual monkeys. Our results reveal extensive neural representations of social state in the primate prefrontal-amygdala networks, abstraction of social state, and state-dependent content representation in the BLA and OFC during naturalistic social gaze interaction.

Disclosures: G. Qi: None. **S.W. Chang:** None.

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

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Program #/Poster #: PSTR304.02/T6

Topic: H.06. Social Cognition

Support: NIH Grant 1U01NS108680

Title: Effects of fairness during cooperation in freely moving non-human primates

Authors: *A. G. MCCONNELL[1,2], M. FRANCH[1], V. DRAGOI[1,2,3];
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Abstract: Advanced forms of cooperation, such as a sense of fairness, have been observed in macaques through experiments involving unequal rewards. Macaques sometimes refuse to cooperate when receiving a lesser reward despite having cooperated under identical circumstances previously. However, the neural mechanisms underlying these behaviors, particularly how these social variables are encoded within the visual-cortical network, remain poorly understood. To address this gap, we propose a novel approach to investigate the impact of fairness on neural correlates within the visual-cortical network. We hypothesize that fairness induces changes in the encoding of social variables before cooperation takes place. We aim to elucidate how the brain processes fairness-related information during cooperation. This research sheds light on the neural mechanisms of fairness and cooperation and offers insights into the broader mechanisms governing social behavior in primates.

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Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

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Program #/Poster #: PSTR304.03/T7

Topic: H.06. Social Cognition

Support: NIMH T32 AG049688
NINDS F99/K00 1F99NS125826

Title: Neuron population in anterior cingulate cortex gyrus dissociate informative from uninformative social cues

Authors: *J. SIMON IV[1], E. L. RICH[2];
[1]Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Neurosci., Mount Sinai Sch. of Med., New York, NY

Abstract: Social cognition includes the ability to interpret or predict the intentions of others, and is involved in most everyday decisions, such as who to befriend or avoid. Studies in social neuroscience have revealed that social information is encoded in many brain regions, including the anterior cingulate cortex gyrus (ACCg). We previously demonstrated that neurons in the ACCg can also encode the identity of conspecifics, even when not required to. However, it remained unclear how neuronal populations reflected social context. Here, we determined how the ACCg represents context at a population-level. To quantify this, we analyzed ACCg neuron population data (n = 215) collected from two rhesus monkeys that performed a reward localization task. We contrasted these responses with neuron population that were simultaneously recorded from the frontal eye fields (FEF)/area 8A (n = 228) , an area not strongly linked to social behavior. We assessed population activity within ACCg and FEF using representational similarity analysis (RSA). We demonstrate that population activity in ACCg exhibits stronger encoding of social context compared to the FEF. Additionally, during feedback, the ACCg dissociates informative from noninformative social cues. This study provides further evidence that the ACCg encodes social context at a population-level, as well as encodes relevant social context information when updating actions. Taken together, this shows that the ACCg encodes multifaceted social information, which may underlie its role in complex social reasoning.

Disclosures: J. Simon: None. **E.L. Rich:** None.

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

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Program #/Poster #: PSTR304.04/T8

Topic: H.06. Social Cognition

Support: Kavli Neuroscience Discovery Institute Fellowship

Title: Neural representation of social information in amygdala of rhesus macaques

Authors: *J. HWANG;
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Abstract: Social knowledge is vital for humans and other primates, enabling them to navigate complex social dynamics, forge alliances, and adapt effectively to their surroundings. Here, we investigated the neural representation of social network information in amygdala of macaque monkeys. To characterize social network knowledge for individual monkeys, we collected surveillance videos of their home, neighboring, and unfamiliar social groups at the Johns Hopkins breeding farm. Videos collected across a three-month time frame were analyzed for interactive and solitary behaviors in four stable social groups, ranging in size from 7-18 individuals. We used this behavioral data to construct multi-edge social network graphs based on dominance relationships, facial threat behavior, physical aggression, dominance mounting, stealing, submissive facial signals, physical submission, grooming, physical proximity, social play, and knowable genetic relationships. Two subject monkeys from the same group were studied with linear array probe recording in amygdala while viewing photographs of monkeys from the home, neighboring, and unfamiliar groups. We analyzed neural coding of personal social knowledge about home and neighboring groups, using unfamiliar monkeys as a control. We found that many neurons in amygdala encode personal social knowledge about relationships involving the subject monkey, relationships involving other monkeys, and relationships across the entire groups (home and neighboring), including dominance hierarchies, physical and symbolic agonism, physical and symbolic affiliation, and the knowable tree of genetic relationships.

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Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

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Topic: H.04. Executive Functions

Support: "FOGASSI_PRIN2020" (CUP: D95F22000410001)

Title: Role of ventral prefrontal and premotor cortex in exploiting social and non social information for action organization

Authors: *C. BASILE[1], M. GERBELLA[1], A. GRAVANTE[2], M. SOMMA[1], A. LAPADULA[1], L. FOGASSI[1], S. ROZZI[1];
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Abstract: The ability to use contextual (social and non-social) cues for the execution of appropriate behavioral responses is critical for allowing us to interact with the environment. Recent studies suggested that a key role in this process could be played by a network of interconnected ventrolateral prefrontal (VLPF) and parieto-frontal areas (Borra et al., 2017). In fact, it has been demonstrated that all the areas of this network are involved both in the execution of hand actions and in their observation and, further, that abstract task instructions are encoded by the VLPF areas of this circuit in the "pragmatic" format of the goals to reach (Simone et al., 2017, Rozzi et al., 2023). However, little is known about the possible role of these regions in exploiting social cues to select and guide appropriate behaviors. In the current study, we aimed to assess the possible involvement of VLPF areas 46v and 12r and of ventral premotor area F5 in the organization of the action based on social and non-social information.To address this issue, we simultaneously recorded neural activity using multi-electrode arrays implanted in these cortical sectors, in one monkey, during a task in which it had to perform appropriate hand actions based on different colored cues and on the observation of specific social and non-social visual stimuli. Single and multi-units were sorted using Mountainsort5. Task related units, identified by means of a threshold analysis (Hoshi e Tanji 1998), were analyzed using population approaches such as the Demixed Principal Component (dPCA) and decoding analyses. We identified 1156 task related units, of which 445 and 407 were recorded in two VLPF sectors, respectively, and 304 in F5. Our results showed that the VLPF units are, in virtually all the task phases, strongly modulated by the behavioral rule instructed by the colored cues and, more weakly, by the type of stimulus, regardless of whether it belongs to the social domain or not. F5 units were modulated by the behavioral rule mostly during action execution, although showing a response during the instructing cue phase, while they were not modulated at all by the type of visual stimuli. Overall, our observations suggest that the VLPF could be involved in action organization based on the type of rules and stimuli and, more in particular, could encode the contextual information in terms of the behavioral goal to reach, while F5 could be mostly involved in integrating and exploiting information received from VLPF, which is essential for the execution of the appropriate response, based on the current task rule and regardless of the presented stimulus.

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Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

Location: MCP Hall A

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Program #/Poster #: PSTR304.06/T10

Topic: H.06. Social Cognition

Title: Flexible social attention allocation in orbitofrontal cortex

Authors: *X. ZHOU[1,2], L. MA[1,2], Z. ZHANG[1,2], L. WEI[1], Z. WANG[1,2];
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Abstract: In social settings, animals demonstrate the ability to flexibly allocate social attention according to their individual needs. Social Place Cells, which encode the position of observed conspecifics, have been identified in the dorsal CA1 region of the hippocampus in bats and rats. However, the mechanism underlying flexible social attention allocation is largely unknown. Here, we designed a pair of tasks involving social and non-social navigation, wherein each pair of rats alternated roles as observer and demonstrator in an elevated polygonal maze. Rats were trained to obtain rewards in the two context-dependent tasks. In the non-social task, the demonstrator rat performed classic goal-directed navigation behavior, while in the social task, the observer rat followed and mimicked the demonstrator rat. Once the rats exchanged their roles, they were capable of adapting their behavior rapidly and changing strategies in seconds. The results of our in vivo recording experiments show that neuronal dynamics in the orbitofrontal cortex (OFC) are highly correlated with multiple aspects of social cues during social tasks rather than non-social tasks, supporting flexible behavior adaptation. Most OFC neurons switch between encoding different task-relevant information in the two tasks, while a neuronal subpopulation only represented multiplexed social attentional behavior. Moreover, results of dimensional reduction analysis showed that social information and navigational information are orthogonally encoded in OFC populations, suggesting that social attentional behavior and goal-directed navigation are supported by two different coding modes. Inhibition of OFC activity disturbed both social and non-social navigation behavior. Taken together, our findings demonstrate that OFC dynamics explicitly encoded multiplexed social information during a social-dependent task, mediating flexible social attention allocation.

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Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

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Klingenstein-Simons

Title: Hormone-mediated reorganization of cortical dynamics during female social choice

Authors: *M. M. ASOKAN, L. SIRRS, S. N. OLINE, A. L. FALKNER;
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Abstract: Sex steroid hormones such as estrogen and testosterone powerfully regulate internal state, mood and social drives. For instance, in many species, including mice, females are sexually receptive only in the peri-ovulatory phase known as estrus that follows a surge in their hormone levels. Yet, we still have a major gap in our understanding of how hormones drive changes in neural circuits to ultimately regulate social behaviors. In particular, we know very little about whether and how hormonal changes alter neural coding of social interactions in crucial hubs for top-down control of sociability, such as the medial prefrontal cortex (mPFC). Here, we test for changes in mPFC circuit representations of social motivation and preference in female mice for male or female conspecifics across different hormone states in naturally cycling females as well as estradiol and/or progesterone primed ovariectomized mice. We quantify moment-to-moment changes in behavioral state based on features from multi-animal pose tracking using the deep-learning based framework SLEAP, and use Neuropixels probes to record from all mPFC subdivisions longitudinally across changes in hormone state. We find female subjects reversibly change their social preference from females to males following hormonal surge. Further, we find single-units in both anterior cingulate (ACC) and prelimbic cortex (PL) that respond in varying magnitudes to male and female interactions as well as asocial behaviors. Population analyses suggest largely non-overlapping ensembles encode for these different behavioral states. Interestingly, we find subregion-specific change in encoding across hormone states. In ACC, we see an increase in the number of units tuned to male or female social interactions relative to asocial behaviors following estrogen surge, suggesting an enhanced representation of other vs self. Whereas in PL, we see a decrease in the number of units tuned to male relative to female following estrogen surge, suggesting a suppression of response to the preferred social stimulus. Further, preliminary data from longitudinally tracked units reveal a reversible increase in spike amplitude and firing rate during estrogen surge suggesting that there are hormone-dependent changes in intrinsic cellular properties within mPFC. To test if changes in local mPFC hormone signaling mediate the changes in behavior, we are testing the behavior following mPFC-specific knock-out of estrogen receptors. Together, our work demonstrates that sex steroid hormones drive changes in female social preference and remodel mPFC cellular physiology and neural coding of social interactions.

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Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

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Topic: H.06. Social Cognition

Support: NARSAD young investigator grant 2022
US Army AWARD DEVELOPMENT CAREER

Title: Brain wide network within and between naturally socializing mice

Authors: *O. MARMOR[1], A. GILAD[2];
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Abstract: Social interaction is one of the crucial and versatile human abilities, enabling us to live in society. Growing evidence indicates that the social information is encoded in a brain-wide manner rather than within a specific region. But the exact brain-wide dynamics between and within two socializing subjects has been largely unknown. To better understand the social brain network, we use a novel state-of-the-art multi-fiber method to measure the brain-wide network from two mice engaging in freely moving and natural social interactions. This method enables us to chronically and simultaneously record neuronal activity from 24 recording sites brain-wide, including social related areas such as the prefrontal cortex, amygdala, ventral striatum, hippocampus, thalamus and more. Our results of four groups of mice (5 mice in each group), show that social interactions evoke most of our recorded brain areas and brain activity can reliably decode social contact. Interestingly, we find that during social contact the functional correlations between the two brain increase while correlations within each brain decrease. A further dissection into distinct subnetworks highlight prelimbic-hippocampus-thalamus inter subnetwork and a parallel accumbens-amygdala subnetwork. We further find that the former subnetwork is related to social dominance whereas the latter subnetwork is more related to interaction initiation. These results pave the way to understanding how brains communicates during social interaction.

Disclosures: O. Marmor: None. **A. gilad:** None.

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

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Title: Neural basis of collective behavior during environmental challenge

Authors: *T. RAAM[1], Q. LI[2], L. GU[3], K. Y. LIM[3], G. ELAGIO[1], S. CORREA[4], W. HONG[5]; [1]UCLA, Los Angeles, CA; [2]Bioengineering, UCLA, LOS ANGELES, CA; [3]Biol. Chem., [4]Dept. Of Integrative Biol. and Physiol., [5]Neurobio. and Biol. Chem., UCLA, Los Angeles, CA

Abstract: Social interactions are critical to the well-being of a wide variety of species. While a growing body of literature has identified neural circuits for dyadic social interactions between two animals, our understanding of higher order interactions at the level of larger groups is weak. Many species organize into social groups, in which the individual contributes to and benefits from the well-being of the whole. However, little is known about the neural basis of collective behaviors in response to environmental stressors. To address this gap, we are using a novel approach to study how groups of mice self-organize into huddles in response to thermal cold challenge. Here, we used computer-vision based multi-animal pose estimation tools to identify five unique huddling states in groups of four mice. We found that huddling behavior is modulated by group size--individual mice huddle more in groups than in pairs, suggesting that social groups have emergent properties that dyads do not have. When assessing behavior at the level of the individual, we found that individual mice demonstrate active (self-initiated) and passive (partner-initiated) behavioral strategies to engage or disengage in huddles with other animals. Interestingly, these active and passive strategies are heavily dependent on the size of the huddle being entered or exited. We then asked which neural circuits coordinate the decisions to huddle in response to cold stress. Previous work suggests that medial prefrontal cortex (mPFC) is a critical node for regulating dyadic and group level social behaviors, as well as decision making more broadly. Using Miniscope calcium imaging, we found unique populations of cells in mPFC that encode active and passive decisions to engage or disengage from a huddle, but do not encode other social behaviors. Using mPFC population activity, we found that huddling behaviors are separable from other behaviors in population space, and can be accurately decoded from other behaviors using SVM decoders. We also found that mPFC neurons encode the social identity of huddling partners. Finally, we used chemogenetics to silence mPFC and found that mPFC activity is required for active decision making in the context of a group. Together, these data suggest a critical role for mPFC in encoding group-level responses to environmental changes and present a novel avenue towards studying social interactions in larger groups.

Disclosures: T. Raam: None. **Q. Li:** None. **L. Gu:** None. **K.Y. Lim:** None. **G. Elagio:** None. **S. Correa:** None. **W. Hong:** None.

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR304.10/U2

Topic: H.06. Social Cognition

Support: Institutional grant (IBS-R001-D2)

Title: Cortical Representations of Pain Affect Embody Empathic Fear

Authors: *S. KEUM;
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Abstract: Affect sharing — the ability to vicariously feel others' emotions — is the primary component of empathy. However, the neural encoding of the perception of others' distress and representation of shared affective experiences remain poorly understood. Here, using miniature endoscopic calcium imaging, we identify distinct and dynamic neural ensembles in the anterior cingulate cortex (ACC) that represent observational fear across both excitatory and inhibitory neurons. Notably, the population dynamics encoding vicarious freezing information is preserved in ACC pyramidal neurons and selectively represented by emotional responses, but not sensory responses, after experiencing direct pain. Furthermore, using circuit-specific imaging and optogenetic manipulations, we demonstrate that specific populations of ACC neurons that project to the periaqueductal gray, but not to the basolateral amygdala, selectively convey affective pain information to modulate observational fear. Collectively, our findings establish a functional role for ACC neural representations in shaping observational fear through the encoding of affective pain.

Disclosures: S. Keum: None.

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR304.11/U3

Topic: H.06. Social Cognition

Support: NSER, RGPIN-2018-04699

Title: The medial prefrontal cortex and the rapid regulation of sex hormones on social and non-social cognition in male mice

Authors: *S. E. MCGUINNESS, E. CHOLERIS;
Psychology, Univ. of Guelph, Guelph, ON, Canada

Abstract: Sex hormones are involved in regulating social behavior via rapid or genomic effects, with their rapid effects of interest to the present study. Estrogens and androgens, namely 17-b estradiol (E2) and dihydrotestosterone (DHT), are implicated in medial prefrontal cortex (mPFC) neural alterations when administered directly in the mPFC or indirectly in the hippocampus. The mPFC is known for mediating higher order cognitive processes and evidence suggests that sex hormones in the mPFC rapidly modulate short-term social but not non-social cognition. A study using female mice found that when E2 was administered into the mPFC, two social cognitive processes were facilitated (social recognition (SR) and social learning), however two non-social cognitive processes were not (object recognition (OR) and object placement (OP)). Although literature has previously demonstrated that administration of E2 in the mPFC facilitates OR and OP, these results seem to be time dependent, with E2 in the mPFC having a role in long-term but not short-term facilitation of non-social cognition. As well, it is unknown how social and non-social cognition is regulated by sex hormones in the mPFC of males. The present study is investigating the rapid effects of both E2 and DHT in the mPFC of male mice on short-term social and non-social cognition. Following castration and cannulation surgeries, mice receive an E2 or DHT infusion into the mPFC and perform in either a SR, social learning, OR or OP behavioral paradigm. It is predicted that, like in females, E2 in the mPFC of males will facilitate short-term SR and social learning, but not OR and OP. It is also predicted that since DHT has been found to facilitate SR in other brain regions involved in social cognition (dorsal hippocampus and BNST), DHT in the mPFC will facilitate SR and social learning, but not OR and OP.

Disclosures: S.E. McGuinness: None. **E. Choleris:** None.

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR304.12/U4

Topic: H.06. Social Cognition

Title: A feedforward circuit mediated by hypocretinergic neurons encodes social cognition and social affiliation

Authors: *Y. WANG, C. YE, Z. LIU, N. N. GUAN, J. SONG;
Sch. of Med., Tongji Univ., Shanghai, China

Abstract: Social affiliation is highly conserved across many vertebrates and the sensory detection of social signals is critical for driving such a behavior. Social perception in human largely requires visual social signals. It remains elusive how the brain integrates visual social cognition and transform it into social affiliative drive. Here we employed an unsupervised deep learning method to characterize the social affiliative behaviors in zebrafish, which suggests that

visual-social stimulation triggers social preference in zebrafish. Calcium dynamics across the whole brain acquired by two-photon microscope during zebrafish visual social events revealed a tight relationship between neuronal activity in the rostral zone of hypothalamus, optic tectum as well as thalamus and social preference. The hypocretin neurons in the rostral zone of hypothalamus mediated a feedforward excitatory circuit recruited by the synaptic input from retinal ganglion cells (RGCs) and driving the neurons in the optic tectum to reinforce visual social signals. Further investigation revealed that hypocretin neurons were activated by RGCs terminals via glutamatergic receptors, and therefore excited the visual social neurons in optic tectum via both glutamatergic and hypocretinergic receptors. Early-life social isolation significantly reduced the number and excitability of the remaining hypocretin neurons, which led to a strong anti-social behavior. The consequence of social isolation was partially rescued by the administration of hypocretin receptor 2 (HCRTR2) agonist or chemogenetic activation of hypocretin neurons. Overall, we discovered a feedforward hypocretinergic circuit that reinforces the visual social cognition and encodes social preference. This study also indicates potential therapeutic targets for treating diseases related to social isolation.

Disclosures: Y. wang: None. **C. Ye:** None. **Z. liu:** None. **N.N. Guan:** None. **J. Song:** None.

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR304.13/U5

Topic: H.06. Social Cognition

Title: Unravel the role of prefrontal cortex noradrenergic circuit in social cognition

Authors: *R. WALLE[1], C. SENDRA[2], M. NIELLO[1], F. MANAGO'[1], Z. BIMPISIDIS[1], G. PACINELLI[1], A. BENEDETTI[1], F. ANTONELLI[1], A. MONAI[1], C. MOLENT[1], I. CARTA[1], C. STUBBENDORFF[1], F. PAPALEO[1];
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Abstract: Deficits in social-emotional skills are fundamental characteristics of various neurodevelopmental disorders (NDD) such as schizophrenia (SCZ) and autism spectrum disorder (ASD). Research has characterized social behavior as a complex activity mediated by limbic and cortical neural systems, with the prefrontal cortex (PFC) being a crucial component in both humans and rodents (Scheggia et al., 2020). However, the pathophysiological circuit mechanisms that underlie socio-cognitive abnormalities relevant to NDD remain largely unknown. Monoaminergic neuromodulations, particularly focusing on dopamine transmission, have been emphasized in studies concerning social processes. Nonetheless, many studies have identified the primary importance of another brain catecholamine, namely noradrenergic transmission (Li et al., 2014; Lu et al., 2017). In this study, we aimed to determine whether and

how noradrenergic neurotransmission could contribute to appropriate socio-cognitive behaviors, with a specific emphasis on emotional discrimination. By integrating in vivo fiber-photometry, optogenetics, and molecular biology techniques in mice, we discovered that optogenetic stimulation of the Locus Coeruleus noradrenergic terminals in the medial prefrontal cortex (mPFC) impaired emotion recognition processes by modulating the activity of SOM+ interneurons. This research could uncover adaptive mechanisms involved in social impairment and might offer new therapeutic approaches for psychiatric diseases.

Disclosures: R. Walle: None. **C. Sendra:** None. **M. Niello:** None. **F. Manago':** None. **Z. Bimpisidis:** None. **G. Pacinelli:** None. **A. Benedetti:** None. **F. Antonelli:** None. **A. Monai:** None. **C. Molent:** None. **I. Carta:** None. **C. Stubbendorff:** None. **F. Papaleo:** None.

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

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Program #/Poster #: PSTR304.14/U6

Topic: H.06. Social Cognition

Support: PID2022-141733NB-I00/MCIU/AEI 10 13039 501100011033/FEDER UE

Title: Vomeronasal amygdala neurons encode individual recognition in mice

Authors: M. VILA-MARTÍN[1], A. TERUEL SANCHIS[1], C. SAVARELLI BALSAMO[2], L. JIMÉNEZ[2], J. MARTÍNEZ -RICÓS[2], ***E. LANUZA**[3], V. TERUEL-MARTI[2];
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Abstract: Mice are territorial animals, and males mark their territory with urine spots. During spatial navigation, the information in these urinary marks allow the recognition of the territory owner. This information is derived from the variable patterns of major urinary proteins found in the urine marks. Major urinary proteins are detected by the vomeronasal organ, but it is unknown how this social information is integrated in the brain. We have previously shown that the only cortical vomeronasal structure is the posteromedial cortical amygdaloid nucleus, and therefore we hypothesize that this amygdaloid structure plays a key role in the recognition of the particular pattern of urinary proteins allowing the recognition of individual animals. To test this hypothesis, we recorded single-unit extracellular activity within this nucleus in awake, head-fixed female mice presented with various male-derived stimuli. These stimuli included the urine of two different males, a blend of these urines, urine from one male on top of that of another (simulating a countermarking scenario), and the urine of the female mouse under examination. The selection of these stimuli aims to unravel the encoding mechanism of these natural stimuli, allowing

individual recognition, countermarking, and self-perception. To analyze our large-scale dataset, we applied tensor decomposition methods for neural activity analysis, supplemented by CEBRA low-dimensional embeddings and decoding accuracy techniques. Tensor decomposition analysis uncovered that within the cortical amygdala, there exists a dual mechanism: one that responds to a wide spectrum of stimuli and another that is activated specifically by certain stimuli, illustrating a balance between generalization and precise recognition. Likewise, CEBRA embeddings revealed distinct global activity patterns corresponding to each type of urine, enabling the discrimination between individuals. Furthermore, decoding accuracy examination demonstrated that neurons responding to specific individual stimuli subtly alter their responses upon repeated exposures to the same urine, indicating the presence of a learning process within the cortical amygdala. In summary, neural responses in the vomeronasal cortical amygdala encode individual recognition based on these chemosensory cues, and allow the distinction between the own signals of the experimental subjects and those from others.

Disclosures: M. Vila-Martín: None. **A. Teruel Sanchis:** None. **C. Savarelli Balsamo:** None. **L. Jiménez:** None. **J. Martínez -Ricós:** None. **E. Lanuza:** None. **V. Teruel-Marti:** None.

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

Location: MCP Hall A

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Topic: H.06. Social Cognition

Support: LKC-LEARN grant
Singapore Ministry of Education Academic Research Fund Tier 1 RT11/19
Singapore Ministry of Education Academic Research Fund Tier 1 2018-T1-001-032

Title: Emergence of social value representations in the mouse prefrontal cortex during cooperative learning

Authors: *C. LI, H. MAKINO;
Lee Kong Chian Sch. of Med., Nanyang Technological Univ., Singapore, Singapore

Abstract: Cooperation, where individuals coordinate their actions to obtain shared rewards, is a prevalent behavior observed across species. While artificial intelligence has successfully modeled social behavior using deep reinforcement learning algorithms, the neural basis of cooperation in biological systems remains elusive. Here, we trained mice and artificial agents to perform the same cooperative task, where a pair of head-restrained mice or agents were required

to simultaneously turn wheels to receive rewards. Over the course of learning, representations of social values - those that arise through interactions with others - emerged in artificial agents. Remarkably, by comparing neural representations between artificial and biological neurons in the medial prefrontal cortex (mPFC) of mice, we found that similar representations emerged in mice during learning. Importantly, optogenetic inhibition of the mPFC or disrupting the visual access to partner's movement significantly impaired task performance in mice. Furthermore, manipulating the reward structure altered the representations in the mPFC, suggesting that these representations were tightly linked to values associated with cooperation. Together, our study reveals the learning-related emergence of social value representations in the mPFC, providing insights into the neural mechanisms underlying cooperation in biological systems.

Disclosures: C. Li: None. **H. Makino:** None.

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR304.16/U8

Topic: H.06. Social Cognition

Support: The Francis Crick Institute

Title: The neural circuit basis of cooperative social behaviour

Authors: *L. KIMBLEY, L. COCHRANE, C. SHAND, X. CANO FERRER, A. IMBERT, A. STRANGE, M. WINDING;
The Francis Crick Inst., London, United Kingdom

Abstract: Social interactions are important across the animal kingdom. In group settings, these networks of interactions lead to increasingly complex dynamics between animals. Despite social behaviours playing an integral role in health and disease, the neuronal circuits involved are not well understood. Studying these neurons is challenging in humans or mammals, due to the complexity of their brain circuitry. We therefore use the larva of the fruit fly as a model for social behaviour. Larvae engage in social interactions including cooperative digging, where individuals group together to more effectively forage for food. Taking advantage of the larva's compact nervous system, we have previously mapped the connectome of the larval brain, including all neurons and their synaptic connections, providing the required structural information to study the circuits underlying social behaviour. Individual circuit elements from the connectome were then linked to genetic tools allowing manipulation of individual neurons. Using these tools, this project aims to identify the neurons involved in cooperative digging behaviour using a high-throughput inactivation screen. We have developed a novel behavioural rig that enables cooperative behaviour to be recorded in a naturalistic context, as larval groups

form holes in food. Additionally we have developed an automated detection pipeline to identify cooperative groups from imaging data, facilitating analyses of how specific neuronal inactivations modulate cooperative behaviour. Overall, this study will link social behaviours to synaptic structures of the larval brain, extensively characterising the neural circuit basis of a social behaviour.

Disclosures: L. Kimbley: None. **L. Cochrane:** None. **C. Shand:** None. **X. Cano Ferrer:** None. **A. Imbert:** None. **A. Strange:** None. **M. Winding:** None.

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR304.17/U9

Topic: H.06. Social Cognition

Title: Neural mechanism regulating rat cooperative behavior by oxytocin

Authors: *Y. LIN[1], Z. WANG[2];
[1]Inst. of Neurosci., Shanghai, China; [2]Inst. of Neurosciences, CAS, Shanghai, China

Abstract: Cooperation is affiliative social behaviors that widely exist in nature and play a key role in the lives of animals. However, the neural mechanisms underlying cooperative behavior are less studied. Oxytocin (OXT) is a neuromodulator that regulates many social and non-social behaviors, including cooperative behavior in humans. Nevertheless, its mechanisms regulating social behaviors remain to be fully understood. To dissect the underlying neural mechanisms of oxytocin in regulating cooperative behaviors, we manipulated the oxytocin system in rats during a cooperative task based on temporal coordination. We found that rats can acquire cooperation and show flexible choices in a cooperation preference task. In $Oxt^{-/-}$ rats, the acquisition of cooperation, but not the execution or preference, is impaired. By using fiber photometry recording of neuronal signals, we found that the activities of oxytocinergic neurons in the paraventricular nucleus (PVN) are correlated with cooperative task performance. Inhibition of these neurons in PVN (PVN^{OXT}) reduced cooperation, while activation of PVN^{OXT} perturbed cooperation. However, both inhibition and activation of PVN^{OXT} had no effect on cooperation preference. These results provide new insight to our understanding of the neural mechanisms of oxytocin in regulating cooperative behavior, and clues for using oxytocin to treat psychiatric disorders, for example, autism spectrum disorder and social anxiety disorder.

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

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

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Program #/Poster #: PSTR304.18/U10

Topic: H.06. Social Cognition

Support: NIH Grant R01MH112788
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Title: Selective inhibition of oxytocin receptor expressing neurons in anterior cingulate cortex disrupts consoling in male and female prairie voles

Authors: *S. BLUMENTHAL[1], K. INOUE[2], J. GUO[3], N. RIGNEY[1], L. J. YOUNG[4]; [1]Emory Univ., Atlanta, GA; [2]Dept. of Psychiatry, Emory Univ., Atlanta, GA; [3]Psychiatry, Emory Univ., Atlanta, GA; [4]Ctr. for Translational Social Neurosci., Emory Univ., Decatur, GA

Abstract: Consolation, or comforting contact directed toward a distressed party, is an empathy-like response observed across species including in monogamous prairie voles (*microtus orchogaster*). Consolation was found to induce neural activity in the anterior cingulate cortex (ACC) of prairie voles and the ACC is also implicated in empathy in humans, suggesting conserved neurobiological mechanisms across species. As a first step towards cell-type specific manipulation of consolation, we used designer receptors exclusively activated by designer drugs (DREADDs) to inhibit general activity in the ACC. Voles of both sexes received a control (AAV8-hSyn-mCherry; N=11) or inhibitory DREADD (AAV8-hSyn-hm4di-mCherry; N=11) virus bilaterally to the ACC (500 nL/side) and were later paired with an opposite-sex partner. Experimental animals underwent a multi-day consolation test where on subsequent days their partner was either separated for 30 min or separated and administered footshocks (30 min: 5x, 0.8mA, 0.5s) before being reunited with the experimental animal. Experimental animals received 3mg/kg injections of clozapine-N-oxide (CNO) 30 min prior to partner reunion and behaviors exhibited by the experimental vole towards the partner during the reunion were scored. Voles expressing the control virus displayed an increase in allogrooming towards their partner after their partner was separated and shocked increase allogrooming in response to their partner's distress ($p > 0.05$) and ex-vivo slice electrophysiology revealed that CNO application induced membrane hyperpolarization in cells expressing hm4di. Next, *in situ* hybridization determined that OXTRs in the prairie vole ACC, and are expressed primarily on glutamatergic, but not GABAergic, cells. Finally, *Oxtr-P2A-Cre* voles were generated using CRISPR/ Cas9 to allow for selective manipulation of Oxtr-containing cells. Male and female voles received a cre-dependent control (AAV8-hSyn-DIO-mCherry; N=11 per sex) or inhibitory DREADD (AAV8-hSyn-DIO-hm4di-mCherry; N=11 per sex) virus bilaterally to the ACC (500 nL/side). Experimental animals were subsequently paired with an opposite-sex partner and underwent the consoling test with 3 mg/kg CNO administration. Animals expressing the control virus increased allogrooming in response to partner distress (male: $p < .05$; female: $p < .01$). Chemogenomic inhibition of ACC^{Oxtr} cells disrupted distress-induced allogrooming in both male ($p > .05$) and female ($p >$

.05) voles. Future experiments will utilize *Oxtr*-Cre voles for visualization and manipulation of ACC^{Oxtr} circuits involved in rodent empathy-like behavior.

Disclosures: S. Blumenthal: None. **K. Inoue:** None. **J. Guo:** None. **N. Rigney:** None. **L.J. Young:** None.

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR304.19/U11

Topic: H.06. Social Cognition

Title: Eliciting Cooperative Dynamics with a Computer Agent Simulating Theory of Mind

Authors: L. O. JIMENEZ[1], J. O. GARCIA[2], S. NGUYEN[3], J. L. KRICHMAR[4], *E. D. GROSSMAN[5];
[1]Humans in Complex Systems Div., DEVCOM Army Res. Lab., San Francisco, CA; [2]Humans in Complex Systems Div., US DEVCOM Army Res. Lab., San Francisco, CA; [3]Cognitive Sci., Univ. of California Irvine, Irvine, CA; [4]COGNITIVE Sci., Univ. of California, Irvine, Irvine, CA; [5]Univ. of California-Irvine, Irvine, CA

Abstract: Although humans tend to be more cooperative than selfish, ultimately decision-making in social and interactive situations depends heavily on the behaviors of the others involved. The tendency to try and elicit a cooperative dyadic interaction can be induced using computer-simulated agents with behavior driven by models that simulate theory of mind (ToM). In this study, we examine how participants navigate collaborative and non-collaborative scenarios with these agents, focusing on their strategic decisions. Specifically, we analyze key behavioral markers such as choice variability and decision-making speed, and explore their neural correlates in critical areas including the dorsomedial prefrontal cortex and temporo-parietal junction. Twenty-eight subjects played the stag hunt game in the fMRI scanner against three types of computer agents: an adaptive agent with simulated ToM capabilities that dynamically adjusted its strategies based on the players' moves, a fixed-strategy agent that enacted strategies determined by the outcome of the previous game, and an agent that selected strategies randomly at the game's start. Human players showed more frequent movement, larger variation in movement times, clearer signs of cooperative intent, and a preference for collaboration over defection, particularly when interacting with the ToM agent. Games involving defection against the ToM agent tended to last longer, whereas those against fixed-strategy agents were notably shorter. With ongoing interaction, participants demonstrated a growing reluctance to select defection when playing against the ToM agent. Interactions with the ToM agent were associated with stronger neural activity in social and executive brain systems, namely the right temporo-parietal junction, superior temporal sulcus, bilateral dorsomedial prefrontal

cortex, bilateral insula, and right dorsolateral prefrontal cortex. Additionally, network analysis showed that players who were flexible in adjusting their strategies upon encountering a new agent also exhibited greater reluctance to defect against the ToM agent and had functional brain networks with longer paths.These findings suggest that adaptive agents with ToM features not only enhance engagement and collaboration but also stimulate social and executive brain functions differently than static agents, potentially influencing neural communication in a subject-specific manner

Disclosures: L.O. Jimenez: None. **J.O. Garcia:** None. **S. Nguyen:** None. **J.L. Krichmar:** None. **E.D. Grossman:** None.

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR304.20/U12

Topic: H.06. Social Cognition

Title: Nucleus reuniens mediates mouse social investigation behaviors

Authors: *Y. CHENG[1], Q. JING[1], G.-Q. BI[2], P. LAU[2];
[1]Univ. of Sci. and Technol. of China, Hefei, China; [2]Univ. of Sci. and Technol. of China, Anhui, China

Abstract: Sociability is fundamental for daily life, and social novelty preferences play a key role in the drive of social behavior. Impaired social preference and cognitive decline is compromised in major neuropsychiatric disorders. In our study, we used a modified social paradigm, resident-intruder test, to establish different social occasions in mice, then used Volumetric Imaging with Synchronized on-the-fly-scan and Readout system (VISoR) to map the whole brain neuronal activity trace with the c-Fos immunofluorescence staining. We found that the activity of the nucleus reuniens (RE) was altered during novel social interaction, suggesting that the RE may carry information important for social recognition. However, the functional role of the RE in social behavior and its neuronal circuit has not been fully understood. Firstly, we used retrograde tracing and anterograde tracing to map upstream and downstream brain areas of the RE, and mapped part of the excitatory and inhibitory afferent connectivity of the RE with RNA-FISH. It was found that the RE had dense connections with the mPFC and HC in a reciprocal manner. To investigate how the RE participates in social recognition, we found that the RE neurons were not activated during interaction with novel juvenile intruder. Optogenetic and chemogenetic activation of the RE neurons abolished social novelty preference. To investigate whether the RE-dependent coordinated prefrontal-hippocampal activity participates in social novelty, we found that activity of the prefrontal-hippocampal circuit altered during novel social. Our study revealed the fundamental role of the RE in social recognition behavior in which the classic prefrontal-

hippocampal neural circuit also participated, providing new insights for understanding the circuit level mechanisms that underlie social novelty preference and social deficits related neuropsychiatric disorders.

Disclosures: Y. Cheng: None. **Q. Jing:** None. **G. Bi:** None. **P. Lau:** None.

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR304.21/U13

Topic: H.06. Social Cognition

Title: Prelimbic versus anterior cingulate cortex impacts in emotion recognition in mice

Authors: *A. MONAI, F. ANTONELLI, C. MOLENT, G. PACINELLI, A. BENEDETTI, R. WALLE, M. NIELLO, C. STUBBENDORFF, Z. BIMPISIDIS, I. CARTA, F. MANAGO', F. PAPALEO;
Inst. Italiano di Tecnologia, Genova, Italy

Abstract: Social life is determined by the ability to perceive, process, and react to emotions in others. In the past, we have already shown that the prelimbic cortex (mPFC) has a pivotal role in modulating emotion recognition (Scheggia. et al., 2020). However, previous studies reported that the Anterior Cingulate Cortex (ACC) is also important for the ability to acknowledge and understand another individual's emotions (Post SG et al., 2014; Mercer SW and Reynolds WJ, 2002; Soanes C and Stevenson A., 2010), both in humans (Arioli M et al., 2021) and mice (Smith ML, et al., 2022). In mice, this brain area is also involved in the social transfer of fear (Keum et al., 2018). In this study, we adopted a validated behavioral test designed to measure the ability of mice to recognize conspecifics based on their affective state (Scheggia. et al., 2020). Through optogenetic manipulations done on Sa-Cre freely moving mice, we found that ACC SOM+ neurons have a crucial role in emotion recognition, similar to what has been reported in the medial prefrontal cortex (mPFC) (Scheggia et al., 2020). We found that their inhibition in mPFC impairs the mice's emotion recognition, but causes no effect when are involved with the ACC ones. Our data show that the stimulation of SOM+ neurons in ACC causes an impairment in emotion recognition as well. We injected DIO-hChR2 for the stimulation of SOM+ neurons or DIO-eNpHR 3.0 for their inhibition. For the stimulation, we used a 473nm CNI laser with an intensity of 5mW and a pulse of 5Hz, for a maximum of 6 minutes. For inhibition, we used a 532nm CNI laser with continuous light, for a maximum of 6 minutes. Twenty adult mice were tested for stimulation (aged 3-6 months), and twenty-three adult mice were tested for inhibition. No sex differences were noticed. By creating an excitatory/inhibitory imbalance, we were able to impair such a crucial social ability, indicating the necessity to examine this phenomenon at a more circuit level. Anatomical tracing has shown that these two areas each have a subclass of

SOM+ neurons that project to different parts of the brain. Our aim now is to study such differences to define their roles in emotion recognition and discrimination and provide new targets for potential treatments.

Disclosures: A. Monai: None. **F. Antonelli:** None. **C. Molent:** None. **G. Pacinelli:** None. **A. Benedetti:** None. **R. Walle:** None. **M. Niello:** None. **C. Stubbendorff:** None. **Z. Bimpisidis:** None. **I. Carta:** None. **F. Manago':** None. **F. Papaleo:** None.

Poster

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Program #/Poster #: PSTR304.22/U14

Topic: H.06. Social Cognition

Title: Silencing of cerebellar projections shapes subtle aspects of social interactions in mice

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Abstract: The cerebellum is a unique part of the central nervous system, with distinct, evolutionarily preserved architecture. Cerebellar damage has been known for centuries to induce motor deficits in both humans and animals. It was therefore traditionally seen as a motor center and gained little notice in the neuropsychiatric community. However, recent studies suggest that cerebellar abnormalities in humans and rodents result in deficits in social and other higher order behaviour. This goes hand in hand with recent research showing that the cerebellum projects to the medial prefrontal cortex (mPFC), an important hub controlling emotional behaviours. How these cerebellar projections influence fine aspects of behaviour and the role they play in shaping the representation of social stimuli within the mPFC remain unclear.
Here, we hypothesized that cerebellar activity participates in the control of social behaviours by changing mPFC activity. We hypothesize that cerebellar activity shapes the cortical representation of social stimuli and behaviours in the mPFC.
We observed subtle but significant changes in mouse behaviour during silencing of the dentate nucleus. During social interaction with an unfamiliar conspecific, mice demonstrated significantly more allogrooming after injection of the HM4Di synthetic ligand, C21, in comparison with control injection of PBS. On the other hand, self-grooming in the presence of a familiar conspecific showed a reverse trend. This suggests that the cerebellum is involved in shaping specific aspects of social behaviour.
These results demonstrate that the cerebellum shapes the subtle aspects of social behaviour. To understand better how cerebellar activity shapes mPFC activity, we used calcium imaging of

mPFC neurons in a single cell resolution. This was done using miniaturized cameras mounted on an implanted gradient index (GRIN) lens. Again, this was repeated with and without silencing of the dentate nucleus chemogenetically. The subtle previously mentioned behavioural changes we observed were accompanied by subtle changes in changes in neuronal activity. This suggests that the cerebellum shapes mice's behaviour during social interaction by shaping the mPFC activity.

Disclosures: R. Mitelman: None. **O. Skorinskaya:** None. **C. Chipak:** None. **J.S. Bains:** None. **J. Guo:** None.

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR304.23/U15

Topic: H.06. Social Cognition

Support: Oxytocin U19 BRAIN Initiative Grant, National Institute of Neurological Disorders and Stroke, National Institutes of Health (2U19NS107616)

Title: Neural basis of parental care of sick offspring in mice

Authors: *A. CASLIN[1], G. KAUR[2], K. QUIÑONES-LARACUENTE[3], R. C. FROEMKE[4]; [1]New York Univ., New York, NY; [2]NYU Langone, New York, NY; [3]Psychiatry, NYU Sch. of Med., NY, NY; [4]Otolaryngology, NYU Med., New York, NY

Abstract: Maternal care is critical for offspring well-being, and must be directed and flexible depending on the perceived conditions and needs of offspring (Insel & Young, Nat Rev Neuro 2001; Opendak et al. Dev Cog Neuro, 2017). Sickness behaviors may serve as socially-useful signals that solicit comfort or caregiving, and the context of maternal care may reinforce prosocial behaviors. Here we aimed to determine which neural systems and mechanisms are important for mouse mothers to sense and respond to infant needs. We specifically asked if and how the oxytocin neuropeptide central modulatory system (Froemke and Young, Annu Rev Neurosci 2021) might be recruited to help mothers recognize and respond to sick juveniles. We used long-term behavioral recordings combined with optically-tagged in vivo electrophysiological recordings and photometry to test whether the maternal mouse oxytocin system contributes to caregiving (Carcea et al. Nature 2021). Using a multi-camera longitudinal behavioral monitoring system developed in the lab (Schuster et al. bioRxiv 2023), we characterized and compared maternal and caregiving behaviors of a mouse dam toward offspring injected with lipopolysaccharides (LPS) vs. saline controls over the course of up to 72 hours. Time spent moving or huddling near juveniles was quantified with DeepLabCut (Mathis et al. Nat Neurosci 2018). Dams were also tested in a three-chambered social preference assay (Kaur et al. SFN Abstracts 2024). Using silicon probe implants coupled with a fiber in the

oxytocinergic paraventricular nucleus of the hypothalamus (PVN), we examined how dam oxytocin and non-oxytocin neurons respond to sick vs. healthy pups. Dams displayed persistent increased physical contact (i.e., huddling behavior) toward LPS-injected offspring and non-offspring vs. saline controls after injection with huddling returning to baseline levels 48h post injection. Dams also spent more time in the chamber with LPS-injected offspring than saline-injected offspring in a three-chamber social preference test. However, there was no preference for LPS-injected non-offspring. Analysis of in vivo PVN recordings indicated that a subset of neurons selectively responded to interactions with pups. Pup-responsive neurons were significantly modulated by interactions with LPS-injected instead of saline-injected pups. These data suggest that dams exhibit increased caregiving behavior and approach toward sick vs. healthy pups which may be mediated by differential PVN activity.

Disclosures: A. Caslin: None. **G. Kaur:** None. **K. Quiñones-Laracuente:** None. **R.C. Froemke:** None.

Poster

PSTR304: Circuits and Neural Mechanisms of Social Cognition II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR304.24/U16

Topic: H.06. Social Cognition

Support: HFSP Grant RGP0019

Title: How has sociality shaped the cetacean brain? A white matter tractography analysis

Authors: *I. ORIGLIO, P. COOK;
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Abstract: Although dolphins and whales share a common terrestrial ancestor and inhabit overlapping ecological niches in the aquatic environment, they exhibit wide variability in social organizations. Prominent theories of brain evolution emphasize the role of social pressures in driving adaptations in brain size and organization. Exploring the brains of multiple cetacean species can reveal patterns between neural connectivity and observable social behaviors. Using postmortem diffusion tensor imaging (DTI), we mapped the white matter connectivity of the amygdala in four cetacean specimens, from species with different social organizations. Amygdala connectivity was found to vary with social complexity of the species. Pilot whales are the most socially complex of the four species in this analysis, forming stable matrilineal pods, and exhibit the strongest amygdala connectivity overall. Sei whales are mostly solitary and show the weakest amygdala connectivity. Atlantic white-sided dolphins and common dolphins both form flexible short-term bonds within a larger group, and their amygdala connectivity tended to fall between the pilot whale and sei whale. An exploratory analysis of white matter connectivity

between the anatomical insular cortex and subcortical regions of a pilot whale brain was also conducted. This investigation was based on published research on white matter connectivity of the human insula. Comparable but shifted patterns of connectivity between the pilot whale and the human insula were found. This preliminary assessment suggests that the anatomically insular region of this cetacean might perform similar basic functions to the human insula, though the relative predominance of these functions likely differs. Our findings of variable connectivity with subcortical and cortical brain regions in cetaceans emphasize the potential of social ecology to reshape the nervous system over evolutionary timescales.

Disclosures: I. Origlio: None. **P. Cook:** None.

Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR305.01/U17

Topic: H.07. Long-Term Memory

Support: ZIA MH002588

Title: Associating BOLD repetition suppression, behavioral repetition priming, and repetition-related changes in EEG power during overt object naming

Authors: *A. W. GILMORE[1], L. CLAUDINO[1], C. M. LEVESQUE[1], A. M. AGRON[1], P. J. MOLFESE[1], V. ROOPCHANSINGH[1], M. D. RUGG[2], S. J. GOTTS[1], A. MARTIN[1]; [1]NIMH/NIH, Bethesda, MD; [2]Ctr. for Vital Longevity, Univ. of Texas at Dallas, Dallas, TX

Abstract: Repeatedly processing a stimulus results in faster response times (repetition priming) along with reductions in neural activity (repetition suppression). How and why behavioral improvements can accompany reduced neural responses remains an open question, although certain theoretical models make testable predictions regarding repetition-related changes in the initial latency or coordination of neural responses to stimulus processing (referred to as the "Facilitation" and "Synchrony" models, respectively). Here, we attempted to address this question using a simultaneous EEG-fMRI approach to maximize both temporal and spatial resolution during a repeat object naming task. Prior to scanning, 40 participants viewed and verbally identified 100 images. These same images ("repeat" stimuli), as well as 100 new ("novel") images, were then presented and verbally identified while EEG and fMRI data were recorded concurrently. Naming response times were ~100 ms faster for repeat stimuli. In fMRI BOLD data, significant repetition suppression was observed in regions including left frontal and bilateral fusiform cortex. Induced EEG source power in these fMRI-defined regions differed between conditions, such that repeat stimuli tended to show more rapid increases and earlier peaks than were observed for novel stimuli. Critically, repetition-related differences in EEG

power correlated across participants with behavior, in the form of repetition priming, and fMRI data, in the form of BOLD repetition suppression. Taken as a whole, results support a mix of the Facilitation and Synchrony models: improved stimulus processing may result from earlier, more coordinated neural responses for repeat, as compared to novel stimuli. More generally, these data also highlight the utility of multimodal EEG-fMRI approaches in the study of brain-behavior relationships.

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Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR305.02/U18

Topic: H.07. Long-Term Memory

Support: R00—MH122663

Title: Initial encoding strength determines the effectiveness of targeted memory reactivation with odor cues

Authors: *G. NARAYAN[1], G. BABINEAUX III[2], M. CHO[1], S. MURUGAVEL[1], T. LU[1], N. J. LEW[1], S. AQUINO ARGUETA[1], E. SCHECHTMAN[1];
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Abstract: Sleep plays an active role in the consolidation of memories that were encoded during the day. Targeted memory reactivation is a technique that uses non-invasive sensory cues, such as odors, to preferentially reactivate associated memories during sleep. In a pioneering study, Rasch et al. (2007) showed that odor reactivation during sleep improved memory for all learned items in a spatial task. Since the design included only one odor and a single learning block, it remains unclear whether the odor cues benefited consolidation of the learning context as a whole or selectively targeted the learned associations. We used a within-subjects design to test whether the presentation of odor cues during sleep would selectively prioritize consolidation for one category of objects over another. Participants (N=32) were trained on a 2D object location task, where each category was paired with a distinct odor, before taking a pre-nap test. During non-REM sleep, participants were re-exposed to one of the odors. After their nap, participants completed a test of the object locations. Our results showed a benefit of cueing, but only when accounting for pre-sleep memory performance. Cueing benefited memory uniformly, but this benefit was stronger for weakly encoded memories. These results provide a conceptual

replication of Rasch et al. (2007), suggesting that odor-cueing can be used to selectively reactivate sets of memories with a task rather than the learning context as a whole. Moreover, our results provide more evidence that initial encoding strength dictates the extent of reactivation effectiveness.

Disclosures: G. Narayan: None. **G. Babineaux III:** None. **M. Cho:** None. **S. Murugavel:** None. **T. Lu:** None. **N.J. Lew:** None. **S. Aquino Argueta:** None. **E. Schechtman:** None.

Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

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Program #/Poster #: PSTR305.03/U19

Topic: H.07. Long-Term Memory

Support: Veterans Affairs Merit Award I01CX001375
NIA R25 AG066594-01

Title: Structural Neuroanatomy of Semantic Retrograde Memory in Older Adults with Variable Risk for Alzheimer's Disease

Authors: S. TAN[1,3], J. C. BANOS[1], I. E. ASP[1], J. SNYTTE[4], M. W. BONDI[5,2], *C. N. SMITH[1,5,6];
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Abstract: Lesion studies indicate that the integrity of medial and lateral temporal lobe cortices are important for news event memory accuracy, but the relationship between cortical thickness and news event memory accuracy has not yet been investigated in individuals with variable risk for Alzheimer's disease (AD). In older adults (N=70) with variable risk for Alzheimer's disease (34 with normal cognition, NC, and 36 with mild cognitive impairment, MCI), we investigated the relationship between cortical thickness or brain volumes and news event recognition memory accuracy across the entire adult lifespan using the Retrograde Memory News Events Test (RM-NET). Partial Least Squares analysis was used to identify brain regions where news event memory accuracy significantly correlated with cortical thickness, cortical volumes, and hippocampal volumes. We found that mean news event memory accuracy significantly predicted hippocampal volume and cortical thickness in the medial/lateral temporal lobe, medial/lateral parietal lobe, and specific areas within the medial/lateral prefrontal cortex. Many of these regions exhibited decreasing correlations with news event memory as the age of the memory increased. Poorer performance was associated with a thinner cortex (or smaller volumes). Many of the

regions identified were unique to the RM-NET and were not identified by the RM-NET post-test (a measure of episodic anterograde memory for the content of the RM-NET) or traditional neuropsychological tests. The regions identified as uniquely contributing to news event memory overlap with regions known to exhibit increasing AD pathology and cortical thinning when pathology begins to spread outside of the medial temporal lobe.

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Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR305.04/U20

Topic: H.07. Long-Term Memory

Title: Aging and the Role of Prior Knowledge in Neural Discrimination of Scene Images

Authors: *K. KIMURA, Y. HONG, W. C. ALLEN, S. BIRR, C. HAWKINS LUKEN, C. R. BOWMAN;
UW-Milwaukee, Milwaukee, WI

Abstract: It is well established that the detail and specificity of episodic memory declines in older age, which can make it more difficult for older adults to distinguish between old and new information when they share overlapping elements. Semantic memory - general world knowledge - declines less substantially with age. Prior work has shown that using prior semantic knowledge can sometimes help older adults successfully encode new information. However, relying on semantic knowledge can also sometimes lead to increases in false recognition. It has become increasingly common in memory and aging research to use neural pattern information as a window into the contents of memory, but it is not known how prior knowledge of to-be-learned stimuli affects the discriminability of neural patterns in older adults. The present study investigated how using images of famous versus non-famous locations affected discriminability of their neural patterns during perception/encoding and memory retrieval. In this experiment, both young (18-30 years old) and older adults (60-80 years old) viewed a set of scene images while undergoing fMRI. Some scenes were well-known landmarks and others were less well-known. After viewing those scenes, participants were asked to vividly recall the images from memory also while undergoing fMRI. Post-scan activities included a forced-choice recognition task and free recall test. Behavioral results reveled that while older adults' recognition performance was comparable to young adults' for famous scenes, there was an age deficit in recognition performance of non-famous scenes. Imaging data show that the detectability of category-level information in neural patterns across several regions of interest (temporal pole, medial PFC, parahippocampal cortex, and hippocampus) was positively associated with memory

for famous scenes. In conclusion, our preliminary data demonstrate that prior knowledge of scenes seem to reduce age deficits in memory.

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Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

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Topic: H.07. Long-Term Memory

Support: This work was supported by a Max Planck Research Group awarded to R.G.B.

Title: Concurrent representations of reinstated and transformed memories and their modulation by reward

Authors: *H. SCHULTZ[1,2], H. STOFFREGEN[3], A. DABAS[3], M. ALCOBENDAS[4,2], R. G. BENOIT[5,2];
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Abstract: An integral part of episodic retrieval is the reinstatement of neural activity that was present in the medial temporal lobe during encoding. However, neural memory representations do not remain static. Consolidation promotes the transformation of representations that are specific to individual episodes towards more generalized representations that reflect commonalities across episodes. Moreover, reward has been shown to augment episodic memory by enhancing consolidation, and it may accelerate the transformation of neural memory representations. We investigated this account with n=40 human participants (19-35 years; 28 women, 12 men) using fMRI and an associative memory task. They encoded pictures of objects, each with one of four recurring scenes. Two scenes led to high reward, two led to low reward. The next day, participants encountered the objects again and retrieved the scenes from memory. Using representational similarity analysis, we demonstrate that retrieval is concurrently accompanied by the reinstatement of original neural representations and the activation of transformed, more generalized memories. Specifically, the parahippocampal cortex reinstates scene-specific patterns from the encoding phase during successful retrieval. In contrast, activity patterns in the medial prefrontal cortex and anterior hippocampus reflect transformed memories: They become more similar to each other for memories sharing the same scene, independent of

memory success. Importantly, high reward enhances memory transformation in the anterior hippocampus. The brain thus maintains complementary memory representations: An episodic representation that resembles the original encoding pattern, and a generalized representation that summarizes commonalities across memories - in part for particularly valuable information.

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Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR305.06/U22

Topic: H.07. Long-Term Memory

Support: RS-2023-00217361

Title: Work Together, Work Alone: The Dynamics of DMN Activity in Narrative Model Updating

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Abstract: The default mode network (DMN) plays a crucial role in forming and updating narrative models by integrating new, relevant information (Lee & Chen, 2022; Zadbood et al., 2022). To identify this dynamic process, we devised a narrative comprehension task where participants not only formed narrative models but also updated them in an fMRI scanner. Specifically, we extracted visual and auditory components from four audio-visual clips to prepare separate visual and auditory stimuli for a common narrative. In the first encoding session, participants only watched the visual stimuli without sound. In the second encoding session, they only listened to the auditory stimuli with an explicit instruction of integrating new auditory information with previously encountered visual information. Following each encoding session, participants were asked to recall each story in as much detail as possible. We created visual and auditory memory test rubrics, respectively, to evaluate participants' recall data. Each rubric probed information that could be obtained from either the visual or auditory stimulus. Overall, recall performance increased after the auditory encoding session compared to the initial visual encoding session ($F(1, 77) = 389.37$, $p < 0.001$). In addition, recall performance from the two encoding sessions increased when evaluated by the auditory rubric but did not change when evaluated by the visual rubric ($F(1, 77) = 320.88$, $p < 0.001$). These results suggest that participants enriched the narrative models by integrating auditory information to the models

created when watching visual stimuli. Inter-subject correlation for both encoding sessions revealed a reliable response in subregions of the DMN, the temporal parietal junction (TPJ) and posterior cingulate cortex (PCC), suggesting that both are implicated in building and holding similar narrative models across individuals. However, these two areas are distinguished by intra-subject correlation. We found that the intra-subject correlation between the visual and auditory encoding sessions was positive and comparable across all four stories in TPJ ($F(3,120) = 0.24$, $p = 0.87$). On the other hand, the correlation differed across the four stories even including one negative correlation in PCC ($F(3,120) = 8.66$, $p < 0.001$). Together, these results suggest that TPJ and PCC play different roles in maintaining and updating narrative models.

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Poster

PSTR305: Human LTM: Encoding and Retrieval II

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Program #/Poster #: PSTR305.07/U23

Topic: H.07. Long-Term Memory

Support: NIH Grant R01MH133732
Office of Naval Research MURI Award 90103554

Title: Transitions in Emotional Context Shape Temporal Order Memory of Events

Authors: *S. TAVASSOLI[1], J. CHEN[2];
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Abstract: Studies on human episodic memory have demonstrated that emotional context shapes how we recall events. For instance, highly negatively valenced contexts enhance temporal order memory of events by more strongly binding them to a distinct point in time (Dev et al., 2022; Cliver et al., 2024). In this study, we explored how the emotional flow of a series of events—i.e., the speed of inter-event shifts in emotional valence—affects event order memory. One possibility is that slow emotional flows, or gradual changes in valence, lead to more stable emotional contexts, creating stronger event-order bindings. Alternatively, fast emotional flows, or rapid switches between valence levels, might allow for better differentiation of events, enhancing temporal order memory. To test these ideas, we created three different versions of a story by re-ordering the sentences such that they had “fast,” “medium,” and “slow” emotional flows, respectively, while keeping the literal words the same. To confirm the emotional flow of each story version, independent raters assessed story events’ emotional valence on a scale of 1 (negative) to 5 (positive) for each condition (N = 18). We then measured the degree of fluctuation in emotional valence ratings across events per condition by calculating the lag-1 difference of the time series of all events’ average valence ratings. As expected, we found overall

between-condition differences (max T = 3.91, $p < 0.0001$), with the valence fluctuations being greatest for the fast flow condition ($\bar{x} = 0.589$), followed by medium ($\bar{x} = 0.493$) and slow flow ($\bar{x} = 0.419$). For our main experiment, subjects assigned to each condition read the story, then completed two temporal order memory tasks: an event timing estimation task (N = 33) and a modified free recall task (N = 34). We found that subjects in the fast flow group showed less error in estimating event timings than the slow flow group (T = 2.00, $p = 0.049$), and their free recall resembled the chronology of their respective story version significantly more than the medium (T = -2.97, $p = 0.006$) and slow flow groups (T = -3.64, $p = 0.001$). Overall, these findings suggest that rapidly shifting emotional contexts can enhance temporal order memory by better binding events to distinguishable timepoints. In the future, we plan to investigate if neural representations of events are better distinguished when they are presented in rapidly shifting vs. gradually shifting emotional contexts.

Disclosures: S. Tavassoli: None. **J. Chen:** None.

Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

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Program #/Poster #: PSTR305.08/U24

Topic: H.07. Long-Term Memory

Support: ERC-2019-CoG 864353
Tel Aviv University Sagol School of Neuroscience Postdoctoral Fellowship

Title: Dissociating language and memory: Neuronal correlates of episodic memories independent of verbal report via anticipatory gaze

Authors: *F. SCHMIDIG[1], D. YAMIN[2], O. SHARON[3], C. RANGANATH[4], Y. NIR[5]; [1]Tel Aviv Univ., Tel Aviv - Jaffa, Israel; [2]Univ. of Tel Aviv, Tel Aviv, Israel; [3]Psychology, Univ. of California, Berkeley, CA; [4]UC Davis, Davis, CA; [5]Tel Aviv Univ., Tel Aviv, Israel

Abstract: Asking a participant to report whether they remember is a high threshold that confounds the presence of episodic memory with its measurement, and neglects its presence in populations that may not be able to report (e.g. patients, infants, non-human primates). We propose a novel "no-report paradigm" using anticipatory gaze to assess episodic-like memory during repeated viewings of movie clips. By watching movie clips twice, participants encoded and retrieved a narrative including a surprising event. The gaze patterns anticipating these events reveal memory-guided viewing behavior towards specific times and locations, seconds before these events appear on the screen. In a series of studies (4 studies, 126 participants), we established the reliability of anticipatory gaze in successfully quantifying memory without

report, being robust across stimulus types (animations vs. real-world movies), experimental settings, and different naïve groups of participants. Detailed comparison to an array of verbal reports confirms that anticipatory gaze marks declarative recollection of episodic events, and reveals dissociation from pupil metrics that index context familiarity. Notably, gaze proximity provides a continuous metric that is highly correlated with the precision of the explicitly recalled event location. Lastly, we illustrate one potential application of anticipatory gaze by demonstrating beneficial sleep effects on consolidation independent of language. This demonstrates that sleep enhances episodic-like memory beyond simply improving memory accessibility and reporting abilities. Anticipatory gaze represents a novel and robust language-independent tool to reliably measure episodic-like memory with broad applicability across cognitive research and clinical domains.

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Poster

PSTR305: Human LTM: Encoding and Retrieval II

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Program #/Poster #: PSTR305.09/U25

Topic: H.07. Long-Term Memory

Support: NSERC Discovery Grant
Canada Foundation for Innovation JELF
Ontario Research Fund
University of Toronto funds to MLS

Title: Memory reinstatement at item and category levels differs according to retrieval specificity demands in adolescents and adults

Authors: *M. WOODBURY[1], S. VIJAYARAJAH[2], M. L. SCHLICHTING[3];
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Abstract: Over time we accumulate memories that overlap through shared features. However, the neural mechanisms that allow us to select among similar memories at retrieval are still maturing into adolescence. Here, we asked how this ongoing maturation influences the information that adolescents retrieve when recalling overlapping memories along with the level of memory specificity required behaviourally. Adolescents (12-13 years) and adults learned to associate unique artifacts with natural objects from four categories (e.g., apples, rocks), yielding object pairs that overlapped at the category level. During subsequent magnetic resonance

imaging (fMRI) scanning, they were then shown artifacts and prompted to retrieve the paired natural objects in preparation for questions which probed memory at different levels of specificity. We used representational similarity analysis (RSA) to measure neocortical reinstatement at both item and category levels during this preparatory retrieval period. Both adolescents and adults reinstated at item and category levels when preparing for memory questions that could be answered using multiple levels of specificity. However, age differences emerged when preparing for questions that required either general (apple vs. rock) or specific (apple 1 vs. apple 2) memories. Both age groups reinstated at the category level for both general and specific questions, however, only adults modulated item reinstatement according to the question specificity. Specifically, adults reinstated at the item level while preparing for specific questions but showed item suppression prior to general questions. While adolescents did not modulate item reinstatement, they did show that decreased category reinstatement was advantageous for specific question performance (i.e. decision speed) on a trial-by-trial basis. Moreover, trials with reduced category reinstatement were associated with greater inferior frontal gyrus (IFG) engagement, suggesting that adolescents may engage this region's memory selection mechanisms to reduce interference from overlapping category information. Together, these results highlight that differences between adolescents and adults in the contents of memory retrieval may be most prominent under demands for high memory precision. Further, the neural mechanisms supporting retrieval of specific information among overlapping memories continue to mature into adolescence.

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Poster

PSTR305: Human LTM: Encoding and Retrieval II

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Program #/Poster #: PSTR305.10/U26

Topic: H.07. Long-Term Memory

Support: ZIA NS03144

Title: Characterizing the neural electrical signals that support episodic memory function with dimensionality reduction techniques

Authors: *R. KIRKPATRICK[1], M. BAUMHAUER[2], S. INATI[1], K. A. ZAGHLOUL[1]; [1]NIH/NINDS, Bethesda, MD; [2]Univ. of Michigan, Ann Arbor, MI

Abstract: A significant line of scientific inquiry has involved investigating the neural mechanisms that support healthy episodic memory function. Previous investigations into these neural mechanisms have identified relevant neural patterns by averaging across electrodes or separating electrode clusters into distinct functionally relevant spaces. Given this foundational

knowledge, an open question is: how are the dynamics at various spatial scales related to memory performance? We address this question with electrical recordings collected from human epilepsy patients with electrodes implanted subdurally for seizure monitoring who performed an associative memory task. This population affords us the unique opportunity to investigate electrical recordings simultaneously collected at the level of microelectrodes and the level of macroelectrodes. We perform principal components analysis to identify the latent neural trajectories at each of these levels of spatial granularity and characterize how the neural trajectories differ between trials where the patients engaged in successful and unsuccessful memory retrieval. Ultimately, through our analyses, we show how the neural dynamics change across different spatial scales within the same patients and how the changes in neural dynamics support episodic memory function.

Disclosures: R. Kirkpatrick: None. **M. Baumhauer:** None. **S. Inati:** None. **K.A. Zaghloul:** None.

Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR305.11/U27

Topic: H.07. Long-Term Memory

Title: Temporal dynamics of microstructural plasticity in the human brain

Authors: *D. KUMRAL, A. LENDERS, M. SCHÖNAUER;
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Abstract: Neuroplasticity, the brain’s capacity for functional and structural changes following a learning experience, allows humans to adapt behavior and form new memories. With new neuroscientific methods, such as diffusion-weighted MRI (DW-MRI), we can now characterize brain microstructural changes that reflect neuroplasticity by assessing motion profiles of water molecules (Sagi et al., 2012). Recent studies have shown that during repeated encoding and retrieval of an object-location learning task, a physical memory trace is formed rapidly in the parietal cortex, which can be observed already 90 minutes after training (Brodt et al., 2018). The exact temporal dynamics of microstructural changes following a learning experience are, however, unclear. In the present study, we investigated functional and structural changes in the brain over the course of learning. Seventy-nine participants completed an object-location learning task with repeated encoding and retrieval of image pairs and their locations, while a no-learning control group (N=38) was tested at corresponding times. To assess structural changes, DW-MRI was acquired at 22 time points across three hours: just before learning, during the learning task, and over a one-hour-long wake rest phase following the task. We observe a decrease in mean diffusivity (MD), reflecting neural plasticity, immediately after learning in

memory-related areas such as the precuneus and the cingulate gyrus, the thalamus, as well as in visual processing regions including the lateral occipital cortex and the cuneus. We did not find structural changes in the control group.Importantly, the MD decreases observed immediately after learning further develop and increase in size during the ensuing awake rest phase, and are linked to how well participants retain the memory.Finally, we confirm our results using an independent measurement of structural MRI through voxel-based morphometry. The simultaneous investigation of functional and structural changes confirms the rapid formation of long-term memory representations in the human brain, highlighting the dynamic nature of neuroplasticity and its crucial role in memory maintenance.

Disclosures: D. Kumral: None. **A. Lenders:** None. **M. Schönauer:** None.

Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR305.12/U28

Topic: H.07. Long-Term Memory

Support: NSF CAREER Award BCS-1844241 to M.A.

Title: Cooperation vs. competition between encoding and retrieval in behavior and in the hippocampus

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Abstract: Forming new memories and retrieving existing memories rely on opposing computational processes in the hippocampus. Evidence from rodent work, computational models, and neuroimaging in humans suggests that the hippocampus balances these processes by alternating between states in which it prioritizes memory encoding and states in which it prioritizes memory retrieval. Despite this work, there has been a lack of evidence as to whether encoding and prediction trade off with each other in behavior, on a trial-by-trial basis. We designed complementary human behavioral experiments and computational modeling to test whether encoding new memories competes with the use of existing memories to generate predictions. In our behavioral experiments, 93 human participants learned a sequence of scene categories (e.g., beaches, castles, forests). After sequence learning, they completed a simultaneous encoding and prediction task. They were shown trial-unique category images and asked to make predictions about upcoming scene categories. Finally, they were given a surprise memory test for the trial-unique images. We were thus able to measure both prediction success and encoding success for each trial-unique stimulus. Across two behavioral studies, we found no

evidence that encoding and prediction competed: instead, when prediction suffered, encoding also suffered. We then implemented the same task in a previously developed neural network model that is constrained by the known properties of the hippocampus. In our model, as in our behavioral data, we found no evidence for a trade-off between encoding and prediction. Ongoing work will modify the properties of the hippocampal model to determine when and how encoding and prediction compete vs. cooperate. Together, our work offers important constraints for models of hippocampal function that propose a tension between encoding and retrieval.

Disclosures: C. Poskanzer: None. **R. Javid:** None. **H. Tarder-Stoll:** None. **M. Aly:** None.

Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR305.13/U29

Topic: H.07. Long-Term Memory

Title: Looking at concepts: Neural underpinnings of non-visual eye movements during thinking

Authors: *A. PILACINSKI[1], L. WAGNER[1], M. MATOS[2], E. ARAÚJO[2], G. BESSON[3], C. KLAES[4];
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Abstract: What is the biggest country in Africa? Why should we pay taxes? Ask someone these questions and you will probably notice how their eyes move while they are trying to find the right answer. Although there is clearly a link between eye movements and memory, the neural basis of this link is not yet well understood. It was recently proposed that our brain uses the neural systems responsible for spatial navigation to also organize semantic spaces. That means, organizing information in long term memory might have evolved from the circuitry responsible for navigating the environment. When thinking, task-relevant information is activated and manipulated within those spaces. It is unclear, however, why would eye movements accompany this mnemonic information manipulation process. To tackle this, we studied non-visual eye movements (NEMs) in verbal long term memory retrieval (thinking), and their underlying neural patterns recorded with human EEG. Our results suggest that NEMs are a byproduct of attentional shifts that engage parieto-frontal saccade planning network during semantic memory search. It appears that, when thinking, we scan the activated concept space similarly to as if we were looking at the objects. This is the first study on the neural underpinnings of the phenomenon of eye movements we make while thinking. Its implications yield a new perspective on the evolution of human intelligence from sensorimotor systems.

Disclosures: A. Pilacinski: None. **L. Wagner:** None. **M. Matos:** None. **E. Araújo:** None. **G. Besson:** None. **C. Klaes:** None.

Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR305.14/U30

Topic: H.07. Long-Term Memory

Support: NIA grant RF1AG039103

Title: Age-invariancy of increased functional connectivity in brain regions demonstrating sustained recollection effects

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Abstract: We previously reported that in both young and older adults members of the 'core recollection network' dissociate according to the time course of their respective recollection effects: whereas the effects are transient in the hippocampus and midline cortical regions, in left angular gyrus (AG) and middle temporal cortex (MTC) they track the delay over which recollected content is maintained. Here, we further examined recollection-related changes in connectivity in these regions. Young (N = 23, 11 female, mean age 22 yrs) and older participants (N = 22, 15 female, mean age 70 yrs) encoded a series of word-picture pairs, judging which of the denoted objects was the smaller. In a subsequent scanned test phase, studied and unstudied words were presented. Participants first judged whether a test word was old or new. For items judged old, instructions were to recall the associated picture and hold it in mind over a delay that varied randomly from 2 to 8 s. A cue denoted which of three judgments should be made on the retrieved picture. Additional responses were available for words deemed new or when an associate could not be retrieved. fMRI recollection effects were operationalized as greater neural activity elicited by test words for which the associated picture was successfully retrieved relative to words for which recollection of the associate picture failed. Transient and sustained effects were identified according to whether the recollection-related activity was unaffected by, or covaried with the length of the delay. To examine transient and sustained recollection-related changes in connectivity, we conducted a psychophysiological interaction (PPI) analysis employing each of the above-mentioned regions as the seed region. No reliable connectivity changes could be identified with the regions demonstrating transient effects. Robust changes were evident however with regions demonstrating sustained effects. These included, most prominently, connectivity increases between the AG and a variety of other regions, including left

lateral prefrontal cortex, dorsomedial prefrontal cortex, middle cingulate cortex, and dorsal and medial parietal cortex. Additionally, connectivity increases were identified between the MTC and, among other regions, the left anterior and lateral prefrontal cortex, and precuneus. Of importance, none of these connectivity effects differed reliably according to age group. These results suggest that sustained recollection-related connectivity increases between the left AG and MTC and a distributed set of other brain regions are largely stable across the healthy adult span.

Disclosures: M. Hou: None. **M. De Chastelaine:** None. **M.D. Rugg:** None.

Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR305.15/U31

Topic: H.07. Long-Term Memory

Support: R01AG065255
NSF GRF (DGE-2146755)

Title: Mnemonic prediction error strength modulates pupil-linked arousal

Authors: *A. M. XUE[1], A. M. NORCIA[2], A. D. WAGNER[3];
[1]Stanford Univ., Stanford, CA; [2]Wu Tsai Neurosciences Inst., Stanford Univ., Stanford, CA;
[3]Dept. of Psychology, Stanford Univ., Stanford, CA

Abstract: Memories of past experiences can be used to generate predictions about future events. When ongoing experience diverges from memory-based predictions, it is adaptive for the brain to prioritize processing the external world in service of encoding novel, incoming information into memory. These “mnemonic prediction errors” may consequently evoke changes in neural activity and pupil-linked arousal to support learning of new information. Moreover, the extent to which these errors promote memory encoding may depend on the strength of the initial memory prediction, such that stronger prediction errors may promote better encoding. We recorded pupillometry and scalp EEG as 50 young, healthy human adults performed an associative novelty task, wherein they encoded strong (studied 4x) and weak (studied 1x) verb-image pairings and then performed a cued associative match/mismatch retrieval task. Analyses revealed effects of memory strength and mnemonic prediction errors on behavioral and pupil metrics. First, memory performance was higher for strong compared to weak associations. Second, in a surprise subsequent recognition memory test, memory for expectation-violating stimuli (i.e., mismatch items) was above chance and similar for strong and weak mismatch stimuli, showing that stronger prediction errors did not additionally enhance memory encoding. However, between-subject analyses revealed that participants with substantially better associative memory for strong pairings compared to weak pairings were less likely to remember strong mismatch stimuli

compared to weak mismatch stimuli, suggesting that prediction strength trades off with encoding. Third, whereas strong prediction errors evoked greater pupil dilation than strong matches, weak prediction errors resulted in more pupil constriction than weak matches. Fourth, we observed a positive subsequent memory effect in the pupil data such that larger pupil size throughout the weak match/mismatch trials was associated with better later memory for weak mismatch stimuli; no subsequent memory effect was observed for strong mismatch stimuli. Fifth, we deconstructed the pupil time series using temporal principal components analysis and identified features that differed as a function of prediction error detection, prediction error strength, and subsequent memory for mismatch probes. Future analyses will further characterize how these changes in pupil-linked responses to mnemonic prediction errors relate to changes in neural activity and cortical representations of predicted and unpredicted information.

Disclosures: A.M. Xue: None. **A.M. Norcia:** None. **A.D. Wagner:** None.

Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR305.16/U32

Topic: H.07. Long-Term Memory

Support: DFG grant SCHO 1820/2-1

Title: Rapid formation of a memory engram for complex episodic narratives in the parietal cortex

Authors: *A. LENDERS[1], K. KLEESPIES[1], M. SUMNER[3], S. BRODT[4], E. A. MCDEVITT[5], C. BALDASSANO[7], U. HASSON[8], K. NORMAN[6], M. SCHÖNAUER[2];
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Abstract: The standard model of systems memory consolidation postulates two interacting memory stores: a hippocampal store that rapidly encodes new information and a neocortical store where enduring representations develop gradually over time. These theories assume that systems memory consolidation requires weeks, months, or even years to form stable neocortical engrams. Recent studies have shown that with repeated learning, the uncoupling from the hippocampus and formation of an enduring neocortical engram can ensue within a single learning session, especially if the learning material is already embedded into pre-existing schemas (Brodt et al., 2016, 2018). In the present study, we used functional and diffusion-weighted MRI to investigate where and when participants form content-specific cortical memory engrams of complex

naturalistic stimulus material. To this end, 40 healthy participants repeatedly watched and freely recounted four movie clips that were either set in a restaurant or at an airport. Using representational similarity analysis, we were able to discriminate the different narrative contexts in functional MRI data recorded from the posterior parietal cortex (PPC) and the hippocampus. Moreover, with repeated encoding and retrieval, the PPC showed increased activity in univariate analyses when processing the memory content. Notably, we observed an increase in content-specific discriminability over learning repetitions in the precuneus, indicating a gradual strengthening of stable neocortical mnemonic representations and a decrease in content separability in the hippocampus. In diffusion MRI data, which we used to image microstructural brain plasticity in human grey matter (Sagi et al, 2012), we found that a physical memory trace formed rapidly in the parietal cortex during repeated encoding and retrieval of the complex episodic narratives and was maintained for at least 24 hours after learning. Remarkably, we could discriminate the narrative contexts in which the movies were set based on these microstructural changes in the PPC. Both functional and structural brain changes were related to how many details participants remembered about the narratives. Thus, our findings suggest that the PPC rapidly forms stable content-specific memory traces of complex narratives from the outset of learning fulfilling all criteria for a memory engram: they (i) directly relate to the learnt content, (ii) cause a change in the underlying neural substrate, (iii) predict memory retention at the behavioural level, and (iv) can be observed while lying dormant between times of active processing (Josselyn et al., 2015; Semon, 1921).

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Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

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Program #/Poster #: PSTR305.17/U33

Topic: H.07. Long-Term Memory

Support: ERC-2018-COG819814

Title: Focal human left temporal pole damage produces emotional amnesia

Authors: *R. HELLERSTEDT[1], M. COSTA[1], R. TOLEDANO[2], A. GIL-NAGEL[2], C. G. BIEN[3], P. GREWE[4], J. KISSLER[3], B. A. STRANGE[1];
[1]Univ. Politecnica de Madrid, Madrid, Spain; [2]Ruber Intl. Hosp., Madrid, Spain; [4]Med. Sch. OWL, Bielefeld University, Bielefeld, Germany, [3]Bielefeld Univ., Bielefeld, Germany

Abstract: The temporal pole has traditionally been considered a hub for the semantic network, but it is also involved in social and emotional processes. One challenge in studying the functional role of this structure is that conditions causing temporal pole lesions (e.g. semantic dementia) typically affects other brain structures as well. In this study we included rare patients with focal temporal pole lesions and pharmaco-resistant epilepsy, while measuring the function of the amygdala with intracranial recordings. The patients performed two memory tests, one verbal free recall task and one visual recognition task. In the verbal free recall task, the patients studied lists of 14 words of which one was an emotionally negative oddball and one was a perceptual oddball (presented in a different font) and subsequently performed a free recall test. A control group of 15 pharmaco-resistant epilepsy patients without temporal pole lesions showed the expected increase in memory for both emotional and perceptual oddballs compared to control words. Interestingly, patients with lesions in the left temporal pole showed a selective decrease in memory for emotional oddballs relative to control words. This reduction was present in all 5 patients with left temporal pole lesions, but not in one patient with right temporal pole lesion. In contrast, memory for emotional pictures was normal in the left temporal pole patients in the visual recognition task, suggesting that the emotional memory impairment was specific to the verbal domain. Analysis of intracranial EEG showed that a gamma response to emotional items was normal in the left TP patients in both tasks indicating that the emotional memory deficit cannot simply be explained by disruption of the amygdala as a consequence of the left temporal pole lesion. Taken together the results suggest that the left temporal pole is involved in episodic emotional memory in the verbal domain.

Disclosures: R. Hellerstedt: None. **M. Costa:** None. **R. Toledano:** None. **A. Gil-Nagel:** 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 Biocodex, GW Pharma, PTC Therapeutics, UCB Pharma and Zogenix. F. Consulting Fees (e.g., advisory boards); Arvelle/Angelini, Bial, Biocodex, Eisai, Esteve, GW Pharma, Jazz Pharmaceuticals, Pharvaris, PTC Therapeutics, Rapport Therapeutics, Stoke, UCB Pharma, Zogenix and Xenon. **C.G. Bien:** None. **P. Grewe:** None. **J. Kissler:** None. **B.A. Strange:** None.

Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR305.18/U34

Topic: H.07. Long-Term Memory

Support: James S. McDonnell Foundation
NSF Graduate Research Fellowship

Title: Hippocampal contributions to relational memory in human infants

Authors: *L. BEHM[1], T. S. YATES[2], J. E. TRACH[3], D. CHOI[3], N. B. TURK-BROWNE[3,4]; [1]Yale Univ., New Haven, CT; [2]Psychology, Yale Univ., New York, NY; [3]Psychology, [4]Wu Tsai Inst., Yale Univ., New Haven, CT

Abstract: Episodic memory requires integrating multiple elements of an experience into a unified representation. This encoding process is referred to as relational binding. In adults, relational binding depends critically upon the hippocampus, which is viewed as structurally and functionally immature in infancy. Despite this, infants can complete some behavioral tasks of relational binding, though how their brain supports this process is an open question. This question has remained unanswered because common methods for measuring infant brain activity (EEG, fNIRS) cannot resolve deep-brain structures such as the hippocampus. Here we leverage fMRI in awake and behaving infants (ages 3 - 24 months) to assess which regions of their brain support relational binding. Infants viewed a series of encoding trials in which a dynamic landscape scene video first appeared alone followed by a dynamic face video that was overlayed in the center of the scene. Test trials were interleaved among the encoding trials to probe relational memory for scene-face pairs. Each test trial began with one of the scene videos from encoding, after which two familiar faces were superimposed to create a visual paired comparison (VPC) between the target (face paired with the scene) and a foil (face paired with a different scene). We used infant gaze to the target vs. foil as a behavioral index of relational binding. We considered an infant to have successfully remembered a scene-face pair if they looked longer to the target than expected by chance. We then sorted the encoding trials according to these test labels and compared BOLD activity for subsequently remembered vs. forgotten relations. We included infants with a minimum of 12 usable pairs of encoding/test trials after exclusions for head motion and visual inattention. We used an infant-trained Automated Segmentation of Hippocampal Subfields (ASHS) atlas to extract regions of interest (ROIs) for the hippocampus and the medial temporal lobe cortex (entorhinal, perirhinal, and parahippocampal cortices). Data collection is ongoing, but preliminary analyses suggest that encoding activity in our ROIs was associated with VPC looking behavior. Overall, this work takes a step towards revealing how the developing brain supports early memory abilities, with implications for understanding infantile amnesia.

Disclosures: L. Behm: None. **T.S. Yates:** None. **J.E. Trach:** None. **D. Choi:** None. **N.B. Turk-Browne:** None.

Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR305.19/U35

Topic: H.07. Long-Term Memory

Support: European Research Council (ERC-2019-COG 866093)

Title: Uncovering the dynamics of human visual memory representations over time through convolutional neural networks

Authors: E. ZOHAR[1], ***S. KOZAK**[2], D. ABELES[2], M. SHAHAR[3], N. CENSOR[2,1];
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Abstract: Accurate retrieval of visual details from past events is fundamental to many cognitive functions relevant for daily life. While a visual experience contains an abundance of information, only some of it is later encoded into long term memory representations. However, an ongoing challenge has been to isolate memory representations which integrate various visual features and uncover their dynamics over time. To address this question, we leveraged a novel combination of empirical and computational frameworks based on the hierarchal structure of convolutional neural networks (CNNs), and their correspondence to human visual processing. This enabled to reveal the contribution of different levels of visual representations to memory strength, and their dynamics over time. Visual memory strength was measured with test distractors selected based on their shared similarity to the target memory along low or high layers of the CNN hierarchy. The results show that visual working memory relies similarly on low and high-level visual representations. However, already after a few minutes and on to the next day visual memory relies more strongly on high-level visual representations. These findings and empirical framework suggest that visual memory representations transform over time from a distributed representation to a stronger high-level conceptual representation, providing novel insights into the dynamics of visual memory and opening new avenues to study human memory representations.

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Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR305.20/V1

Topic: H.07. Long-Term Memory

Support: Max Planck Society, Max Planck Research Group awarded to RGB

Title: Successive suppression of aversive memories

Authors: ***F. BERGMANN**[1,2,3], H. STAUB[4,5], R. G. BENOIT[1,2,3];
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Neuroscience, University of Colorado at Boulder, Boulder, CO; [3]Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany; [4]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany; [5]University of Leipzig, Leipzig, Germany

Abstract: Many psychological disorders are marked by intrusive, negative memories. These often come to awareness when cued by a potent reminder. However, we can intentionally suppress unwanted retrieval. Repeated suppression attempts can gradually weaken a memory's neural representation, render it less intrusive, and eventually cause forgetting. Here, we employed fMRI (n = 39) and a novel version of the *Think/No-Think procedure* to examine and track the successive weakening of unwanted, aversive memories. Participants underwent six learning-suppression cycles. On each cycle, they first learned to associate each of a number of words with either a neutral or a negative scene. In the subsequent suppression phase, they were prompted with the words, paired with an instruction to either recall or suppress the associated scene. Consistent with previous findings, we observed a gradual reduction in the frequency of memory intrusions across cycles, indicating that participants become more efficient in deploying suppression throughout the experiment. Notably, within an individual suppression phase, too, the intrusiveness of a given memory declined, suggesting that it became successively weaker with each suppression attempt. Our fMRI data allows us to examine how a reduction in intrusiveness is associated with a reduction in a memory's neural reactivation. We also employed a recently developed regression model of negative affect to examine whether there are concordant changes in a neural signature of negative affect, too. Our preliminary analysis revealed that this neural marker of negative affect is more pronounced for deliberately recalled negative memories than neutral ones. Critically, suppressing the retrieval of these memories also resulted in less negative affect. So far, our results inform us that retrieval-induced negative affect can be quantified from brain responses and, importantly, that it can also be modulated by suppression.

Disclosures: F. Bergmann: None. **H. Staub:** None. **R.G. Benoit:** None.

Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR305.21/Web Only

Topic: H.07. Long-Term Memory

Support: R01MH133732

Title: Major event boundaries elicit the same cortical activity profiles during naturalistic movie-viewing and volitional web-browsing

Authors: H. LEE[1], ***Y. LEE**[2], J. CHEN[2];
[1]Dept. of Psychological Sci., Purdue Univ., West Lafayette, IN; [2]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Extant theories of how experience is segmented into events (Zacks et al., 2007) posit that event boundaries are perceived due to discrepancies between predicted and observed outcomes. However, internally generated (i.e., endogenous) event boundaries are challenging to incorporate into such a prediction error account (Wang & Egner, 2022). To better understand the neural correlates of event boundary perception, we conducted a study in which human subjects navigated a series of webpages, then verbally described their web-browsing experience from memory. During the web-browsing phase, subjects (N = 25) were allowed to browse and perform simple tasks on up to 12 different web pages in whatever order they wished. In typical studies of event segmentation, subjects passively view videos or listen to audio streams, and thus boundaries are exogenously generated; in contrast, in the current study, the subjects themselves initiated transitions between different webpages, and thus the boundaries were endogenously generated. We first investigated whether cortical signatures at webpage transitions resembled those observed at event boundaries during passive movie-viewing and movie recall. A prior study showed that posterior medial cortex (PMC), a key subregion of the default mode network (DMN), exhibits a stereotyped spatial activity pattern at boundaries between movies and between recall of different movies; we used the same dataset to obtain this PMC pattern (Lee & Chen, 2022). We calculated the similarity between 1) the stereotyped PMC movie-boundary pattern and 2) PMC activity at boundaries between websites, and observed positive correlations that peaked at 3 seconds after the offsets of webpages (r = 0.102). To assess whether web-browsing events were reactivated during spoken recall in a manner analogous to prior movie studies, we compared PMC activity patterns during browsing and recall of each webpage. Greater correlations were present for matching webpage-recall event pairs compared to non-matching pairs (r = 0.098 for matching pairs; r = -0.007 for non-matching pairs). Together, the results suggest that endogenous and exogenous boundaries have similar activity profiles in PMC, and that the web-browsing and recall paradigm shows promise as a volitional analogue for commonly used movie-viewing paradigms.

Disclosures: H. Lee: None. **Y. Lee:** None. **J. Chen:** None.

Poster

PSTR305: Human LTM: Encoding and Retrieval II

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Program #/Poster #: PSTR305.22/V2

Topic: H.07. Long-Term Memory

Support: NIH grant NS106611

Title: Predicting the Effects of Brain Stimulation from Observational Data

Authors: *R. DEHAAN[1], D. HALPERN[2], M. J. KAHANA[1];
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Abstract: Improving memory through neuroscientific interventions requires understanding of the neural activity that causes behavior. While decades of work link memory performance to neural activity, it remains unclear whether these conclusions from observational data reflect causes of successful memory encoding. Here we analyze intracranial electroencephalography recordings of 140 subjects performing a delayed free recall task. We compare models accounting for confounders of causal neural effects, including item and serial position effects, to unadjusted models. We find that accounting for these variables results in different conclusions about the relevant neural activity. Hypothesizing that this de-confounded model may better reflect an endogenous state of memory performance rather than the features of the presented stimuli, we predicted such a model should in turn better predict the effects of brain stimulation. We validate our model using a separate dataset of 20 subjects who received randomized electrical brain stimulation while performing the free recall task.

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Poster

PSTR305: Human LTM: Encoding and Retrieval II

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR305.23/V3

Topic: H.07. Long-Term Memory

Support: R01EY026701
P20GM103650
P30GM145646

Title: Brain networks involved in recognition memory are recruited more strongly, and more extensively, by real objects than by images of objects.

Authors: *G. T. FAIRCHILD[1], S. LEE[1], M. COMPTON[1], D. E. HOLLER[1], S. FABBRI[2], M. A. GOMEZ[1], C. P. NEMETH[1], L. STROTHER[1], J. C. SNOW[1];
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Abstract: Studies of human memory typically rely on two-dimensional (2-D) image stimuli. However, previous work has shown that images are less well-remembered than real objects,

which offer many more multisensory cues than images do. Here, we used fMRI to examine the neural basis of this difference. During an initial learning phase, participants were shown a large set of everyday items presented either as real objects or as images. During a subsequent recognition phase in the MRI scanner, participants viewed words that corresponded to items that either had been presented, or had not been presented ("foils"), during the learning phase. Participants' task was to judge whether they had seen each item, and if so, whether the item had been a real object or an image. Univariate analyses found that cortical networks commonly implicated in recognition memory were activated more strongly for real objects than for images; no regions showed the opposite pattern. Next, a multivariate searchlight classifier revealed successful decoding of recognition memory for both real objects and images versus foils, but this decoding was considerably more widespread for real objects. Moreover, an additional multivariate analysis revealed that several regions, including the hippocampus and parahippocampal cortex, represented the format in which the stimulus was presented during the learning phase. Together, our results show that brain networks implicated in recognition memory are activated more strongly, and more extensively, by multisensory real objects than by image displays, and that areas within this network represent the format in which a previously viewed item was seen.

Disclosures: G.T. Fairchild: None. **S. Lee:** None. **D.E. Holler:** None. **S. Fabbri:** None. **M.A. Gomez:** None. **L. Strother:** None. **J.C. Snow:** None.

Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR305.24/V4

Topic: H.07. Long-Term Memory

Support: NIMH P50 MH094271
NIH UG3MH123386
NIH U19 NS104653
NIH U24 NS109102
NIH UO1 EB026996
Stanley Center at Broad Institute
Alphabet Inc. (Google Research)

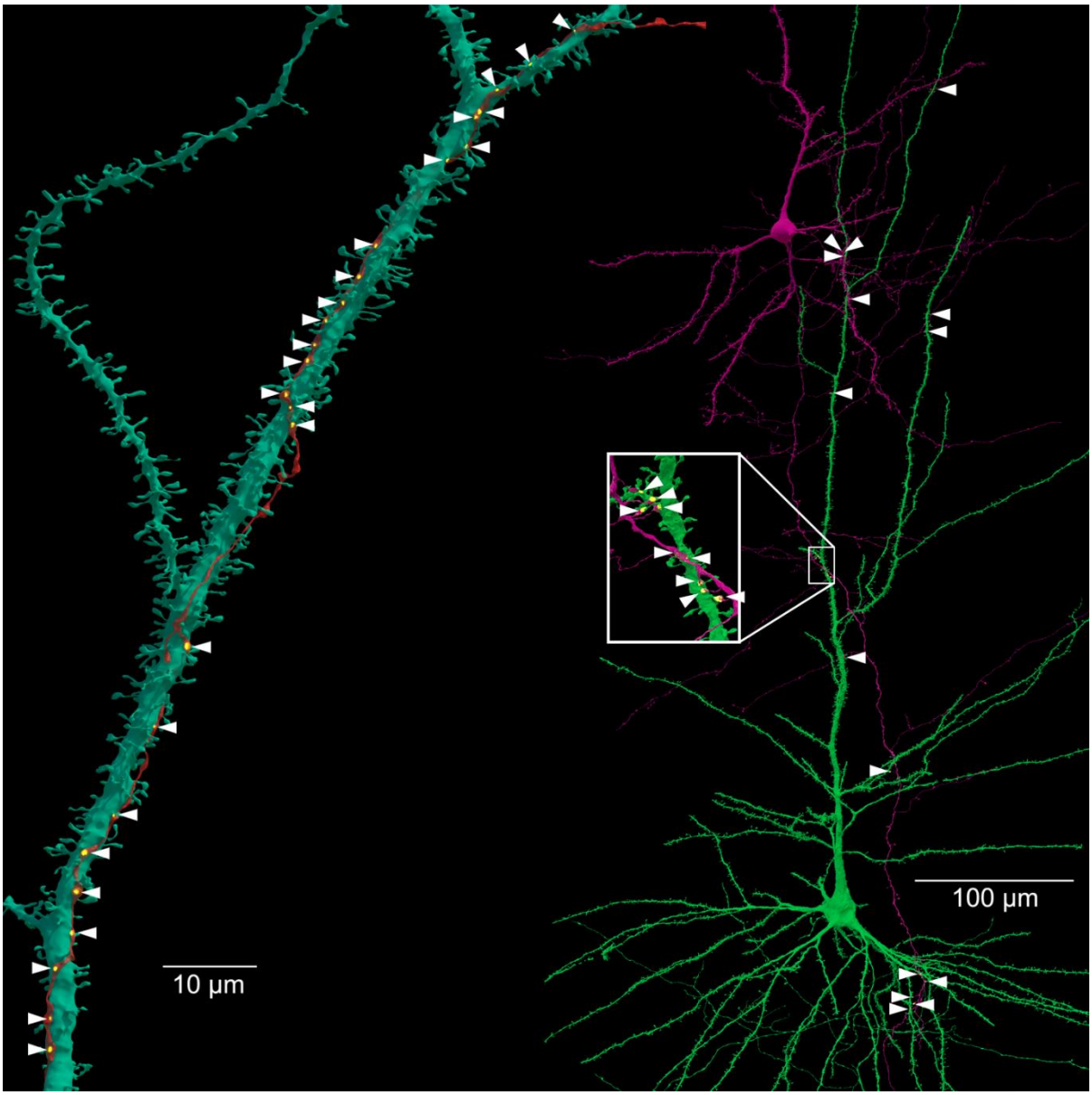
Title: Strong multi-synaptic connections in human cortex

Authors: *D. R. BERGER, J. W. LICHTMAN;
Harvard Univ., Cambridge, MA

Abstract: In collaboration with Google Research we recently generated a large dataset of human cortex (left anterior temporal lobe, 45 years old female; biopsy from surgery for hippocampal

epilepsy) at electron-microscopic resolution (Shapson-Coe et al., bioRxiv 2021, https://doi.org/10.1101/2021.05.29.446289) which allows for the reconstruction of synaptic connections between thousands of neurons, but requires manual proofreading. While most synaptic connections between neurons consist of 1-3 synapses, surprisingly, we also found rare, very strong connections, with dozens of synapses being established between specific pairs of neurons. In one such case a small layer 3 pyramidal neuron was found to establish a total of 53 synapses onto the dendrites of a nearby interneuron, and 52 synapses onto a second interneuron. We find such cases from both excitatory and inhibitory presynaptic to both excitatory and inhibitory postsynaptic partners. In addition, we find different styles of interaction. In some cases the axons appears to follow a dendrite through the tissue, establishing many en-passant synapses along the way (e.g. see figure, left; 25 synapses). In other cases the axon seems to traverse the tissue independently of a targeted dendrite, but makes synapses with the same target neuron whereever it happens to get close enough to one of its dendrites (less than ~5 micrometers), often employing several short axonal side branches with terminal boutons (e.g. see figure, right; 24 synapses). This suggests mechanisms of non-random, targeted enhancement of connectivity strength.

We plan to generate a larger set of proofread neurons to determine whether measurable characteristics like neuron type, size, or cortical layer correlate with connectivity style or the prevalence of establishing strong connections. We hope to shed more light on this phenomenon, ultimately to determine whether strong connections might provide a backbone scaffold for neural computations or could serve as a substrate for memory engrams.



Disclosures: D.R. Berger: None. **J.W. Lichtman:** None.

Poster

PSTR305: Human LTM: Encoding and Retrieval II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR305.25/V5

Topic: H.07. Long-Term Memory

Support: Natural Sciences and Engineering Research Council Discovery Grant RGPIN-2020-05747
James S. McDonnell Foundation Scholar Award
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University of Toronto Faculty of Arts and Science Top Doctoral Fellowship
Alexander Graham Bell Canada Graduate Scholarship – Doctoral for the Natural Sciences and Engineering Council of Canada

Title: Sleep is associated with preserved autobiographical memory richness, whereas dreaming impacts emotion

Authors: *N. MATORINA[1], J. SCOTT[1], A. AMADOR[1], M. D. BARENSE[1,2];
[1]Dept. of Psychology, Univ. of Toronto, Toronto, ON, Canada; [2]Rotman Res. Inst., Toronto, ON, Canada

Abstract: Sleep plays a role in the consolidation of episodic memories, yet little is known about how the very first night of sleep impacts autobiographical memories. In the current study, participants recorded one morning and one evening event from their everyday lives on a smartphone application over the course of two weeks. They also completed two memory tests per day (one in the morning and one in the evening) that assessed the memory from 12 hours prior (i.e., the evening memory test pertained to the morning event and the morning test pertained to the evening event from the night before). Preliminary data indicated that following a sleep delay, compared to a wake delay, memories were more vivid, $t(532.45) = -3.74$, $p < .001$, easier to recall, $t(534.05) = 4.82$, $p < .001$, and felt closer in time, $t(534.76) = 5.88$, $p < .001$. We also found that dreaming about a memory impacted memory richness and emotion, such that evening memories that were dreamt about felt closer in time, $t(208.60) = -2.91$, $p = .004$, were associated with more preserved emotion, $t(211.17) = 2.74$, $p = .007$, and an increase in negative emotion, $t(215.98) = -2.19$, $p = .03$. Emotional events were also more likely to be incorporated into dreams, $z = 3.34$, $p < .001$. Overall, our findings provide evidence that sleep preserves autobiographical memory richness, whereas dreaming plays a particular role in the emotional component of an autobiographical memory.

Disclosures: N. Matorina: None. **J. Scott:** None. **A. Amador:** None. **M.D. Barense:** None.

Poster

PSTR306: Circuit Mechanisms of Learning, Memory, and Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR306.01/V6

Topic: H.08. Learning and Memory

Support: R01 MH119102
CURE grant, Pennsylvania Department of Health
Dean's Fellowship, Graduate School of Biomedical Studies and Professional Studies, Drexel University

Title: Retrosplenial neuronal subpopulations in slow-wave sleep and memory

Authors: *A. N. OPALKA, K. J. DOUGHERTY, D. V. WANG;
Neurobio. and Anat., Drexel Univ., Philadelphia, PA

Abstract: Understanding the intricate mechanisms underlying slow-wave sleep (SWS) is crucial for deciphering the brain's role in memory consolidation and cognitive functions. It is well-established that cortical delta oscillations (1-4 Hz) coordinate communications among various cortical, hippocampal, and thalamic regions during SWS. These delta oscillations have periods of Up and Down states, with the latter previously thought to represent complete cortical silence; however, new evidence suggests that Down states serve important functions for information exchange during memory consolidation. Among the many brain regions involved in consolidation, the retrosplenial cortex (RSC) serves a pivotal role due to its extensive connectivity with memory-associated regions. Here, we employed multi-channel *in vivo* electrophysiology to study RSC neuronal activity in freely behaving mice during natural SWS. Using independent component analysis, we discovered that the RSC contains a discrete assembly of putative excitatory neurons (~20%) that initiated firing at the Down state and reached maximal firing at the Down-to-Up transition. Therefore, we termed these RSC neurons the Down state assembly (DSA), and the remaining RSC excitatory neurons as non-DSA. This DSA activity preceded the activity of non-DSA excitatory and inhibitory neurons. Moreover, the DSA had higher firing rate and burst activity compared to non-DSA excitatory neurons. Subsequently, we investigated RSC neuronal activity during a contextual fear conditioning paradigm and found that both DSA and non-DSA neurons exhibited increased firing activity during post-training sleep compared to pre-training sleep, which correlated with their increased activity during contextual fear recall. Lastly, we investigated whether memory-associated inputs, the anteroventral thalamus, claustrum, and dorsal hippocampus, selectively targeted RSC neuronal subpopulations utilizing optogenetics combined with *in vivo* electrophysiology. We found that the thalamus targeted a portion of RSC excitatory neurons, while all three regions similarly targeted most RSC inhibitory neurons. Collectively, these findings provide insight on distinct RSC neuronal subpopulation activity in sleep and memory consolidation.

Disclosures: A.N. Opalka: None. **K.J. Dougherty:** None. **D.V. Wang:** None.

Poster

PSTR306: Circuit Mechanisms of Learning, Memory, and Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR306.02/V7

Topic: H.08. Learning and Memory

Support: R310-2018-3611
R436-2023-471
R436-2023-855

Title: Selective attenuation of layer 1 inhibitory circuit by cholinergic receptor activation in retrosplenial cortex: implication to memory processing under stress

Authors: A. TANIMURA[1,2,3], H. LOGIN[1,2,3], S. ØSTERGAARD FELD-JAKOBSEN[1,2,3], T. OVERMARK[1], J. RADULOVIC[1,2,3,5,6], ***N. YAMAWAKI**[4,2,3];
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Abstract: In healthy brain, acetylcholine (Ach) concentration rapidly shifts depending on cognitive demands including memory processing. However, under severe stress, the Ach level is thought to be elevated for a prolonged time and this is predicted to cause maladaptive memory processing, as seen in memory symptoms of patients suffering from post-traumatic stress disorder and depression. Nevertheless, how persistently elevated Ach affects memory circuit function still remains elusive. Using mice, we examined the effect of tonic cholinergic receptor activation on the layer 1 circuit function in the retrosplenial cortex (RSC), which is densely innervated by cholinergic axons and uniquely integrates the afferents from the anterothalamic nuclei (ATN) and CA1 necessary for memory acquisition.
Optogenetic stimulation of ATN axons in RSC slice, when performing a whole-cell recording from layer 1 neurons with cesium internal solution, evoked excitatory and inhibitory postsynaptic current (EPSC and IPSC) at the -70 mV and +10 mV command potential, respectively. Bath application of carbachol (CCh, 1 μM), a cholinergic receptor agonist, selectively reduced the IPSC without affecting the EPSC. A long-range inhibitory input from CA1 was also attenuated by CCh. The reduction of ATN-evoked IPSC appears to be caused by reduced excitability of layer 1 neurons due to reduced input resistance. Data from the Allen brain RNAseq database and our RNAscope demonstrated that all layer 1 neurons in RSC expressed muscarinic 1 (M1) receptor and α7 nicotinic receptors, and blocking M1 receptors with pirenzepine (1 μM) countered the CCh's attenuative effect on IPSC evoked by ATN axons. Preliminary data from activity monitoring of subset of layer 1 population with fiber photometry during trace fear conditioning indicated significant correlation of their activity with freezing behavior.
These data suggest that layer1 neurons in RSC participate in memory processing, and tonic activation of M1 (and α7) receptors on these neurons attenuates their inhibitory impact on local circuit. The effect of stress on layer 1 neurons' activity and memory acquisition is being investigated.

Disclosures: A. Tanimura: None. **H. Login:** None. **S. Østergaard Feld-Jakobsen:** None. **T. Overmark:** None. **J. Radulovic:** None. **N. Yamawaki:** None.

Poster

PSTR306: Circuit Mechanisms of Learning, Memory, and Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR306.03/V8

Topic: H.08. Learning and Memory

Support: NIH MH083809

Title: The retrosplenial cortex is not needed for generating novel trajectories in a water maze barrier navigation task.

Authors: *S. LI[1], D. M. SMITH[2];
[1]Cornell Univ., Ithaca, NY; [2]Dept. of Psychology, Cornell Univ., Ithaca, NY

Abstract: The retrosplenial cortex (RSC) is a key component of the brain's memory and navigation systems. It is interconnected with many brain structures known to be involved in spatial navigation, including the hippocampus, anterior thalamus, and entorhinal cortex. Recent studies have found that RSC neurons encode the subject's spatial location, as well as navigational cues and goal locations (e.g. Vedder et al, 2017, Cerebral Cortex) and the RSC may play a key role in translating between allocentric and egocentric spatial reference frames (for review see Alexander et al, 2023, Neuron). In many studies, RSC firing patterns are closely tuned to the subject's trajectory through space. These considerations suggest that the RSC may play an important role in generating novel trajectories to goal locations in a changing environment. We tested this hypothesis by training control rats and rats with DREADD inactivation of the RSC in a series of water maze tasks. First, we tested rats' ability to learn the location of a hidden platform using standard training conditions, followed by reversal learning and then a series of three test sessions in which we introduced clear plexiglass barriers which blocked the route to the escape platform. Each barrier test session began with four trials from a fixed start position across from the escape platform with no barrier in place. Before the fifth trial, we placed one of three different barriers directly in the path between the start position and the escape platform, forcing the rats to take an alternative path around the barrier. We found a sex-dependent effect of RSC inactivation on the initial regular (non-barrier) water maze learning session. An ANOVA of the latency data found no main effect of inactivation condition ($F(1, 96) = 1.43$, $p = 0.24$), and no main effect of sex ($F(1,96) = 0.62$, $p = 0.43$), but did reveal a significant interaction of the sex and inactivation conditions ($F(1,96) = 6.36$, $p = 0.01$), with female DREADD rats being significantly slower than controls to find the escape platform. A similar impairment in female rats was seen during reversal learning. However, contrary to our expectations, we found no evidence of an inactivation-induced increase in escape latency during the barrier test sessions (e.g. main effect of inactivation condition for the first barrier test, $F(1, 72) = 1.03$, $p = 0.31$). No consistent sex-dependent effects of the inactivation were found during the barrier test sessions. Detailed analyses of the rats' trajectories to the escape platform are

ongoing, but our results thus far suggest that RSC inactivation does not cause a large-scale impairment in the capacity to generate novel trajectories during goal-directed navigation.

Disclosures: S. Li: None. **D.M. Smith:** None.

Poster

PSTR306: Circuit Mechanisms of Learning, Memory, and Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR306.04/V9

Topic: H.08. Learning and Memory

Support: NIH MH083809

Title: The anterior thalamus is required for flexible learning of goal locations.

Authors: *Y.-Y. YANG[1], C. JIANG[2], J. HO[1], I. WU[1], D. M. SMITH[1];
[1]Dept. of Psychology, Cornell Univ., Ithaca, NY; [2]Cornell Univ., Ithaca, NY

Abstract: The anterior thalamus (AT) has long been implicated in providing critical head directional input during spatial learning and memory processes. AT lesions produce deficits in both spatial learning and navigation, especially when the development of directional trajectories is required in the tasks (Clark and Harvey, 2016, *Neurobiol. Learn. Mem.*). However, the contribution of the AT to flexible route planning and spatial learning is not fully understood. In this study, we tested the ability of control rats and rats with AT inactivation to learn and remember a new array of goal locations each day using a cheeseboard maze task. Each day, rats learned three new goal locations, from among 121 reward wells arranged in a grid pattern on a circular arena. The goal locations were cued with an object next to the rewarded wells during the first five trials of each daily session, but the cues were removed for following 25 trials and the rats were required to navigate to the goals from memory. Under control conditions, the latency to retrieve rewards from all goal locations averaged ~ 23.5 seconds. Under AT-inactivation, rats spent significantly longer time retrieving the rewards, 54.1 seconds on average (DREADD inactivation: $F(1, 4.05) = 11.10$, $p = 0.029$; Muscimol inactivation: $F(1, 5.65) = 22.434$, $p = 0.004$). Ongoing trajectory analysis examined the extent to which rats deviated from the optimal path in the task under control and AT-inactivation conditions. Preliminary analyses of the rats' movement trajectories suggest that control rats developed highly efficient navigational strategies, adopting stereotyped trajectories that were close to the optimal path connecting all goal locations with the minimum possible distance. In contrast, AT-inactivated rats displayed less stereotyped trajectories that diverged widely from the optimal path and often involved missed rewards or backtracking to previously visited regions of the maze. The striking abnormalities in the trajectories to the goal locations is consistent with disruption of the AT head direction system. Moreover, the severe impairment in the ability to navigate efficiently to each day's goal

locations is consistent with spatial learning and memory impairments seen after AT damage. Overall, our results indicate that the AT is essential for the rapid acquisition of efficient navigational strategies during a flexible, unconstrained spatial learning task.

Disclosures: Y. Yang: None. **C. Jiang:** None. **J. Ho:** None. **I. Wu:** None. **D.M. Smith:** None.

Poster

PSTR306: Circuit Mechanisms of Learning, Memory, and Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR306.05/V10

Topic: H.08. Learning and Memory

Support: NSF Grant IOS-1656488
NIMH 1R01MH108729

Title: Chemogenetic disconnections between the orbitofrontal cortex and the parahippocampal region impair sensory preconditioning

Authors: *X. PENG[1,2], J. ZHOU[1], R. D. BURWELL[3];
[1]Chinese Inst. for Brain Res., Beijing, China; [2]Department of Cognitive, Linguistic, and Psychological Sciences, Brown University, Providence, RI; [3]Dept. of Cognitive, Linguistic, and Psychological Sci., Brown Univ., Providence, RI

Abstract: In the parahippocampal region of the meidal temporal lobe, the perirhinal (PER) and postrhinal cortex (POR, homologous to parahippocampal cortex in primates) have extensive connectivity with the entorhinal cortex and hippocampus, and they play crucial roles in recognition memory, spatial/context memory, and episodic-like memory. A previous study showed that both the PER and POR are necessary for retrieving latently-acquired associations in appetitive sensory preconditioning, likely because they collectively support a “gist-like” representation for associations between neutral events and context. The orbitofrontal cortex (OFC) is also capable of constructing associative structures and it is also critically involved in sensory preconditioning. Here, we used asymmetrical chemogenetic inhibition to examine whether disconnecting the OFC with the PER or POR impaired sensory preconditioning. In adult male rats, we injected an inhibitory DREADDs virus unilaterally into either the PER or POR, combined with either ipsilateral or contralateral injections into the OFC in the same rat. Before testing, all rats received a clozapine *n*-oxide injection that temporarily suppressed the areas with viral injections. Reduced sensory preconditioning effect was found in all groups except for the “POR - contralateral OFC” group, indicating that whereas bilateral interaction is necessary between the OFC and PER, only contralateral communication is required between the OFC and POR for associative value inference. These findings suggest that in addition to prefrontal-

hippocampal connections, cortico-cortical pathways between the prefrontal cortex and the parahippocampal region also serve important functions in associative learning and memory.

Disclosures: X. Peng: None. **J. Zhou:** None. **R.D. Burwell:** None.

Poster

PSTR306: Circuit Mechanisms of Learning, Memory, and Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR306.06/V11

Topic: H.03. Decision Making

Support: ARC Grant FT220100474

Title: Role of nucleus accumbens shell spiny projection neurons in mediating the influence of predictive learning on choice between actions

Authors: *O. SOEGYONO, B. CHIENG, B. K. LEUNG, B. W. BALLEINE, V. LAURENT; Univ. of New South Wales, Sydney, Australia

Abstract: Decision-making requires the capacity to extract predictive information from the environment to select the most optimal course of action. This capacity is commonly studied via the specific Pavlovian-Instrumental Transfer (PIT) paradigm, during which a stimulus predicting a particular outcome guides choice towards an action earning that same outcome but not a different outcome. Ample evidence indicates that the nucleus accumbens shell (NAc-S) as well as its projections to the ventral pallidum (VP) are critical for PIT. The NAc-S is predominantly composed of two populations of spiny projection neurons (SPNs), which differ in the particular dopamine receptors they express. One population expresses dopamine D1 receptors (D1-SPNs) and the other the dopamine D2 receptors (D2-SPNs). Here, we used optogenetic silencing and stimulation in freely moving rats (LE-Drd1$^{em1(iCre)Berke}$ and LE-*Adora2a*$^{em1(iCre)Berke}$) to uncover the role played by these two populations during PIT. Silencing D1-SPNs or D2-SPNs activity within the NAc-S at the time of choice abolished the ability of predictive stimuli to bias choice between actions. Conversely, stimulating either D1-SPNs or D2-SPNs within the NAc-S at the time of choice left PIT intact. We then investigated whether silencing NAc-S projections from D1-SPNs or D2-SPNs to the VP would also abolish the influence of predictive learning on choice between actions. The present findings demonstrate that activity in both D1- and D2-SPNs is necessary for the influence of predictive learning on choice between actions. These findings are also consistent with a recent model that describes the cellular mechanisms underlying PIT (Laurent & Balleine, 2021).

Disclosures: O. Soegyono: None. **B. Chieng:** None. **B.K. Leung:** None. **B.W. Balleine:** None. **V. Laurent:** None.

Poster

PSTR306: Circuit Mechanisms of Learning, Memory, and Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR306.07/V12

Topic: H.08. Learning and Memory

Support: Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant #400176

Title: Functional role of dopamine at D1 receptors within the perirhinal cortex in object memory malleability

Authors: *O. S. O'NEILL[1], B. D. WINTERS[2];
[1]Psychology, Univ. of Guelph, Paris, ON, Canada; [2]Psychology, Univ. of Guelph, Guelph, ON, Canada

Abstract: Consolidated long-term memories can be modified when presentation of a reminder cue triggers destabilization, the return of a memory trace to an active state before subsequent reconsolidation. Older or strongly encoded memories resist modification via reconsolidation due to biological boundary conditions. Destabilization of such memories is more likely with prediction error (PE) at reactivation. Accordingly, the neurotransmitter dopamine (DA), implicated in PE, has been linked to overriding boundary conditions using appetitive or aversive memory paradigms. However, more neutral memories also require modification to adapt to changing environments, and evidence suggests that a salient novel cue presented at reactivation can trigger destabilization of boundary condition-protected memories with less obvious valence. The present study investigates the functional role of dopamine at D1 receptors in object memory destabilization and overcoming boundary conditions for destabilization of object memories. Using a modified spontaneous object recognition task using male rats, we found that the D1 receptor antagonist SCH23390 administered systemically (0.1mg/kg, i.p.) blocked destabilization of recent and relatively remote object memories, preventing the impairing effects of a subsequent injection of NMDA receptor antagonist MK801 (0.1mg/kg, i.p.). SCH23390 administered intracranially (2μg/μL) to the perirhinal cortex (PRh), a brain region implicated in object memory consolidation and reconsolidation, also blocked recently encoded object memory destabilization, negating the amnesic effects of post-reactivation anisomycin (100μg/μL). Using the same paradigm, we next found that administration of systemic D1 receptor agonist SKF38393 (5mg/kg) was sufficient to induce destabilization of remote memories in the absence of a salient novel cue. Accordingly, using the post-reactivation object memory modification task as a model of contextual object memory updating, we found a consistent pattern whereby systemic administration of SCH23390 blocked both recently encoded and novelty-induced reactivation-dependant memory updating and SKF38393 permitted the updating of resistant

memory traces in the absence of salient novelty. Findings from previous literature and the present results implicating D1 receptors in destabilization of object memories and other memory types indicate that dopamine transmission is involved and potentially required for modification of newly encoded and relatively remote object memories and this effect is likely reliant on the PRh.

Disclosures: O.S. O'Neill: None. **B.D. Winters:** None.

Poster

PSTR306: Circuit Mechanisms of Learning, Memory, and Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR306.08/V13

Topic: H.08. Learning and Memory

Support: Einstein Foundation Research Grant

Title: Cortex-wide laminar differences during learning

Authors: *Y. E. POLLAK[1], A. GILAD[2];
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Abstract: The neocortex dynamically changes when we learn new tasks. One interesting characteristic of the cortex is its laminar profile comprising of 6 different layers, but the role of each layer during learning remains unclear. learning to discriminate between different stimuli, visual stimuli or texture touches leads to changes in the primary sensory areas, but what happens in other cortical areas? In this study, we performed wide field calcium (GCaMP6f) imaging of the whole dorsal cortex of layer-specific neuronal populations, either in L2/3 or L5 while mice learn a whisker-based texture discrimination task. Mice were trained to lick upon whisker-touch with a coarse texture surface. The respective other sandpaper type served as no-go stimulus with a white noise as a punishment when responded with a licking. As learning proceeds, a spatiotemporal activation sequence builds up spreading from auditory areas to rostro lateral immediately before texture touch and continuing into barrel cortex. In both layers as mice learn to discriminate between textures the barrel cortex activity intensifies. On the contrary, the activity in the rostro lateral across learning is enhanced in layer 2/3 but decreases in layer 5. Other areas such as the visual cortex and frontal area also display learning-related laminar differences. Overall, our results may aid in understanding the role of different layers during learning across the whole cortex.

Disclosures: Y.E. Pollak: None. **A. gilad:** None.

Poster

PSTR306: Circuit Mechanisms of Learning, Memory, and Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR306.09/V14

Topic: H.08. Learning and Memory

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NIH Grant R01 DC018545
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NSF Grant 2024776
Simons Collaboration on the Global Brain Pilot Award

Title: Learning-induced reorganization of thalamocortical interaction underlies the execution of learned movements

Authors: *A. RAMOT[1,2,3,4,5], F. H. TASCHBACH[6,2], Y. C. YANG[7,2,8,5], Y. HU[9,3,4,5], Q. CHEN[10,2,3,5], X. C. WANG[7,2,3,5], A. WU[9,4,3,5], K. M. TYE[11,12], M. K. BENNA[13], T. KOMIYAMA[14,2,4,5];
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Abstract: The primary motor cortex (M1) is central for the learning and execution of dexterous motor skills, and its superficial layer L2/3 is a key locus of learning-related plasticity. It remains unknown how motor learning shapes the way by which M1 circuits interact with their upstream inputs to execute learned movements. By longitudinal axonal imaging of main inputs to M1 L2/3, we identify the motor thalamus as the key input source that encodes learned movements in experts. We then used optogenetics to identify the subset of M1 L2/3 neurons strongly driven by thalamic inputs before and after learning, which showed that learning reorganizes thalamocortical interactions such that the thalamus selectively activates the neurons that uniquely encode upcoming learned movements in experts. Inactivation of the motor thalamus in experts impaired learned movements. Our study reveals that motor learning reshapes thalamocortical interactions for a reliable execution of the learned movement.

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Poster

PSTR306: Circuit Mechanisms of Learning, Memory, and Plasticity

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR306.10/V15

Topic: H.03. Decision Making

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NIH NS113110
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Simons Collaboration on the Global Brain

Title: Tracking the emergence and robustness of persistent activity in the premotor cortex across learning

Authors: *C. WANG[1,2], T. ABE[3], S. DRUCKMANN[4], N. LI[5,2];
[1]Baylor Col. of Med., Houston, TX; [2]Neurobiology, Duke University School of Medicine, Durham, NC; [3]Stanford Univ., Palo Alto, CA; [4]Stanford Univ., Stanford, CA; [5]Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Cognitive functions, such as short-term memory, are dependent on persistent neural activity that maintains information. Persistent activity is distributed across multiple brain regions, most prominently in frontal cortical areas. The functional couplings between brain regions must achieve a delicate balance: information must be shared across brain regions to coordinate coherent behavior; at the same time, the regions also need to be decoupled enough to limit the spread of noise in local parts of the network. It remains unclear how persistent representations and underlying network couplings change over learning.
In the mouse, neurons in anterior lateral motor cortex (ALM) exhibit preparatory activity during motor planning, a form of persistent activity. The two hemispheres of ALM coordinate to maintain robust preparatory activity (Li Daie et al Nature 2016; Chen Kang et al Cell 2021). We used longitudinal two-photon calcium imaging to track the same neuronal population in ALM in mice learning a motor planning task. Concurrently, we used optogenetics to silence ALM activity in the other hemisphere to probe functional coupling across hemispheres.
Mice learned to discriminate the position of a pole using their whiskers during a sample epoch and report choice using a right or left lick during a response epoch. The sample epoch and response epoch are separated in time by a delay epoch (3 s), in which mice must use short-term

memory to report the correct choice. Mice progressed from a naïve state to an expert state over weeks, as measured by the proportion of correct trials in a session (from 50% to above 70% correct). Learning was accompanied by a gradual increase of task selective neurons in all task epochs (sample, delay and response). This was further reflected in an emergence of stimulus and choice selectivity during the delay epoch. Functional coupling across hemispheres was altered across learning. The two hemispheres became progressively decoupled, or modular, following the emergence of persistent activity during the delay epoch. After a transient perturbation to one hemisphere, the unperturbed hemisphere was able to progressively better retain its selectivity during the delay epoch over learning. Behaviorally, mice also became progressively robust to optogenetic perturbations in the expert stage.
These results outline a learning process in which the frontal cortical networks first establish persistent neural representations that link past stimulus and future actions, followed by progressive refinement of network coupling that increases the robustness of the representations.

Disclosures: C. Wang: None. **T. Abe:** None. **S. Druckmann:** None. **N. Li:** None.

Poster

PSTR306: Circuit Mechanisms of Learning, Memory, and Plasticity

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR306.11/V16

Topic: H.03. Decision Making

Support: ERC Grant 2021

Title: Thalamo-cortical circuit dynamics underlying skilled performance of mice

Authors: *Y. LEVY[1], A. GILAD[2];
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Abstract: The brain continuously integrates sensory information from the external world with internal representations and states of experience, eventually leading to behavior. To achieve higher-order cognitive functions, the brain engages a brain-wide network spanning multiple cortical and subcortical areas. Two crucial areas that participate are the cortex and the thalamus. It is now thought that the cortex dynamically interacts with the thalamus via recurrent loops that encodes high-order processing of cognitive functions such as attention, working memory and learning. To date, we do not know what the interactions are between and within multiple thalamic and cortical areas during different sensory tasks, and weather there are any critical hubs that are especially related to higher-order functions such as choice. Our research goal is to simultaneously observe multiple thalamic nuclei and cortical areas as mice perform different sensory discrimination tasks. To achieve this, we first combine wide-field imaging and multi-

fiber photometry to simultaneously image the whole dorsal cortex and 13 different thalamic nuclei of mice expressing genetically encoded calcium indicators. Mice are imaged during skilled performance of two different behavioral go/no-go tasks; tactile (whisker sensation) and auditory (tone) discrimination. Tasks are constructed of repeating trials, each trial consisted of a uniform prior cue signal, tactile or auditory stimulus period and response period. Among our results we observed significant increase in cortical activity during tactile tasks in comparison to auditory tasks. Interestingly, this increase appeared on Hit trials (when mice acted appropriately) but not on correct rejection trials (CR, when mice avoided action appropriately). Thalamic activity differed significantly between tasks as well. Whereas cue periods characterized in thalamic inhibition across modalities, stimulus periods showed variation in responses to different stimuli; some nuclei responded in a similar inhibition or activation patterns across modalities, and some displayed significantly different responses. Interestingly, some higher-order thalamic nuclei and cortical areas encoded choice (i.e., Hit vs. CR) similarly in both sensory modalities. In summary, our unique experimental system may aid in understanding the wide complexity of thalamocortical loops.

Disclosures: Y. Levy: None. **A. gilad:** None.

Poster

PSTR306: Circuit Mechanisms of Learning, Memory, and Plasticity

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Topic: H.03. Decision Making

Support: ERC Grant 2021

Title: Interhemispheric Sensory Flow: Unraveling Sensory Information and Working Memory Transfer in Mouse Cortical Posterior Areas

Authors: *E. AVIDAN, A. GILAD;
Med. Neurobio., Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: The interhemispheric transfer of information in the cortex plays a significant role in the way we perceive the world. The cortex does not only integrate sensoryinformation but is also required to retain information in memory for several seconds. This function is defined as working memory (WM) and is thought to be encodedacross many brain areas. Whereas the process of sensory information and WM in one hemisphere has been broadly investigated in previous studies, interhemispherictransfer of sensory information and WM is poorly understood. We trained mice expressing calcium indicators across layer 2/3 of the cortex, to match between twotextures (smooth or rough sandpapaers) from both sides of their whiskers in a go/no-go task. Matching textures required mice to lick for a reward (Hit), and non-matching textures required

withholding licking (correct rejection; CR). Once the mouse has reached expert level, we introduced a delay period in-between texturespresentation. Thus, the WM of the first stimulus needs to be retained and transferred to the other hemisphere. Using dual-hemisphere wide-field calcium imaging, wecompared cortex-wide neuronal activity to study interhemispheric transfer of sensory information and WM. We found involvement of the Barrel cortex (BC) inprocessing choice-information, where 'Hit' displayed higher activity compared to 'CR'. Furthermore, BC did not encode the type of texture, as there was no discernibledifference in BC activity between 'Hit'-smooth and 'Hit'-rough during the sensation epoch. By introducing a delay period (1-3 sec) between texture presentations, weobserved sequential activity across hemispheres in posterior areas. Specifically, the left posterior area (P) initially held the sensory and type-information at thebeginning of the delay period and subsequently transferred it to the homotopic P area even before the arrival of the second texture. Moreover, we found additionalposterior areas such as the posterior-medial (VISpm) and the retrosplenial-dorsal (RD) involved in the transfer of WM. To further investigate the areas involved inchoice and/or texture information during the WM transition, we used a support vector machine (SVM) to classify choice or texture type based on neuronal signalsacross the entire cortex. This analysis enabled us to outline which areas contribute to the mouse's decision based on the sensory input. Consequently, our dualhemisphere behavioral task and imaging approach provide valuable insights into the processing and availability of sensory information for daily activities.

Disclosures: E. Avidan: None. **A. Gilad:** None.

Poster

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Program #/Poster #: PSTR306.13/V18

Topic: H.03. Decision Making

Support: ERC grant 2021

Title: Brain-wide dynamics underlying object identification and localization

Authors: *R. OZ ROKACH, A. GILAD;
Med. Neurobio., Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: The study of sensory integration in the brain cortex stands at the core of neuroscience research, contributing to our understanding of the neural mechanisms underlying cognition. To further this understanding, we turned our focus to the whisker system of mice, drawing inspiration from the two-stream hypothesis in primates' visual system. In our study, we hypothesized analogous pathways in mice, diverging from the Barrel Cortex (BC). Utilizing wide-field calcium imaging and body cameras, we assigned mice to perform texture

discrimination ('what') and location identification ('where') tasks. Our findings revealed varying activation patterns that did not completely align with the task type. We also observed alternating activity patterns in areas P and M2. To better understand these patterns, we employed various analysis methods, including linear modeling, ensemble learning, and regularization, elucidating the intricate interplay between sensory input and cognitive processing. The analysis revealed minimal impact of the task type variable, while factors such as the mouse's initial training task, the first task performed on a specific day, choice, and action planning had significant effects. Additionally, unsupervised learning and clustering methods were employed to detect possible brain pathways and relate them to behavioral and task-related variables. In summary, our study highlights unique processing streams that are influenced by both external task-related factors and, importantly, internal processes that may reflect internal states or strategies. This enhances our insight into the mechanisms governing brain function.

Disclosures: R. Oz Rokach: None. **A. gilad:** None.

Poster

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Topic: H.08. Learning and Memory

Support: STI2030-Major Projects

Title: Value information representation during stimulus reward-value associative learning in primate posterior parietal cortex

Authors: *Z. LIU;
Peking Univ., Beijing, China

Abstract: Value information representation during stimulus reward-value associative learning in primate posterior parietal cortexZiang Liu[1], Zhuangyi Jiang[1], Yang Zhou[1]*[1]School of Psychological and Cognitive Sciences, PKU-IDG/McGovern Institute for Brain Research, Peking-Tsinghua Center for Life Sciences, Peking University, Beijing, 100871, ChinaCorresponding author e-mail address: yangzhou1@pku.edu.cn***Abstract**Animals interact with environmental stimuli, attributing varying degrees of importance to them based on outcomes, often forming associations between stimuli and value through prior experiences in dynamic environments. Survival hinges heavily on animals' ability to swiftly adapt and continuously form new associations with valuable information. While previous research has identified brain regions like the orbitofrontal cortex involved in predicting rewards and representing stimulus value, the neuronal mechanisms underpinning the long-term establishment of these associations remain elusive. In this study, we employed two-photon calcium imaging to

observe over 3,000 neurons in monkey 7a, a subregion of the PPC (posterior parietal cortex) crucial for associative processing but not previously linked to associative learning. Monkeys engaged in a stimulus-reward association learning task, where various visual cues denoted different reward levels after a delay period. We discovered that a significant portion of 7a neurons encoded abstract stimulus-reward associations during the delay period preceding reward reception. Notably, this encoding was absent initially but became pronounced as monkeys learned the associations. Importantly, the encoding of stimulus value was distinct from that of stimulus category or reward prediction. Moreover, neuronal representations of stimulus-value associations reorganized as monkeys transitioned to learn new associations, evolving gradually over multiple learning days. These findings suggest that the PPC may play a crucial role in associative learning by maintaining memory traces of stimulus value to guide adaptive behavior.

Disclosures: Z. liu: None.

Poster

PSTR306: Circuit Mechanisms of Learning, Memory, and Plasticity

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Topic: H.08. Learning and Memory

Support: CIHR Grant 507489 (Junchul Kim)
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NSERC CGS M (Andrew Cheon)

Title: Neuronal dynamics of olfactory memory: in vivo calcium imaging of anterior olfactory nucleus circuits

Authors: J. BANNING[1], ***A. CHEON**[1], Y. CHOW[2], C. ZHANG[2], J. KIM[1,2];
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Abstract: The anterior olfactory nucleus (AON) is a central node in olfactory memory circuits. It shapes odor-guided behaviours by integrating bottom-up olfactory bulb (OB) inputs with top-down hippocampal (HPC) inputs. Our previous work demonstrated the necessity of the HPC-AON circuit for episodic odor memory and established the AON as an odor engram repository. Thus, we hypothesize that partial cues reminiscent of prior odor experiences reactivate hippocampal engrams, which then reactivate AON odor engrams. However, it remains unknown how odor memory is represented in spatiotemporal activity dynamics in AON circuits. We coupled *in vivo* fiber photometry with our novel context-dependent olfactory go/no-go paradigm and found that the temporal dynamics of AON activity transform as simple odor memories develop into odor-context memories. In particular, the AON preemptively activates in response to contextual information alone prior to receiving odor input during the retrieval phase of our

odor-context memory task (one-way ANOVA, n = 9, F(1.654, 13.23) = 10.29, p = 0.0029). This distinction between simple odor memory and odor-context memory processing in the AON supports our hypothesis that the AON functions as an odor-context memory repository. Notably, whole-brain c-Fos expression mapping revealed no significant differences in the spatial patterns of neural activity representing context-independent and context-dependent odor memories in olfactory memory circuits, highlighting the need for higher spatiotemporal resolution recordings to characterize olfactory memory circuits. We subsequently performed dual-site fiber photometry of the AON and the anterior piriform cortex (APCx), both primary olfactory cortical structures important for odor memory, to characterize the real-time statistical relationships between AON and APCx activity dynamics in odor-context memory. Overall, this study provides novel insights into the central role of AON circuits in processing odor-context memory. Our research on the temporal dynamics of AON circuit activity will deepen our understanding of how the brain processes sensory elements of episodic memory.

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Poster

PSTR306: Circuit Mechanisms of Learning, Memory, and Plasticity

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Program #/Poster #: PSTR306.16/V21

Topic: H.08. Learning and Memory

Title: Plasticity of amygdala interneuron subtypes in associative learning

Authors: *N. FAVILA[1], J. CAPECE MARSICO[1], B. ESCRIBANO[1], C. M. PACHECO[2,1], Y. BITTERMAN[3], J. GRUNDEMANN[1], A. LUTHI[4], S. KRABBE[1];
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Abstract: The basolateral amygdala (BLA) is a cortex-like structure known to be involved in simple forms of emotional learning such as fear conditioning. Local plasticity in the BLA is crucial for associative memory formation. While plastic changes of glutamatergic projection neurons (PNs) have been well characterized, little is known about the contribution of GABAergic interneurons. Although inhibitory interneurons only constitute around 20% of the neuronal population in the BLA, they can tightly control PN activity. Nonetheless, the behavioral relevance of different interneuron subtypes and their plasticity upon learning remain largely unexplored. The present study aimed to assess how the activity of different subtypes of interneurons in the BLA is modulated across fear conditioning and extinction. To address this, we performed deep-brain calcium imaging with miniature microscopes and a gradient-index (GRIN) lens implanted in the BLA of freely behaving mice. We targeted the overall inhibitory

interneuron population by injecting a cre-dependent GCaMP6 into the BLA of GAD2-Cre mice, and compared it to defined molecular interneuron subtypes using VIP-Cre and SST-Cre mice. Interneuron activity was recorded during a discriminative fear learning paradigm and subsequent extinction sessions, where a tone (CS+) was paired with a foot-shock (US), while another one served as a control cue (CS-). During fear learning, the overall inhibitory population of GAD2 interneurons exhibited synchronized activity in response to both CS+ and CS- presentations, as well as to the foot-shock, displaying a diversity of activity patterns. Moreover, interneurons persisted in encoding both CS+ and CS- during extinction sessions. Upon examining distinct interneuron subtypes, VIP interneurons exhibited heightened modulation in response to aversive stimuli and the predictive cue (CS+), whereas SST interneurons displayed a preference for the auditory tone associated with safety (CS-). However, overall, interneuron subtypes showed highly diverse activity patterns during both the conditioning paradigm and across days to both the CS+ and the CS-, with distinct plastic responses upon fear and extinction learning. In summary, our findings suggest that different subtypes of BLA inhibitory interneurons display preferential tuning to various aspects of learning, which is crucial for the acquisition and expression of fear memories. However, the precise role of these interneurons in associative learning remains complex and will likely require a fine-grained analysis of molecular and anatomical subtypes.

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Poster

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Topic: H.08. Learning and Memory

Support: HFSP Postdoctoral Fellowship LT0005/2024-L

Title: State-dependent dynamics and plasticity gating in inhibitory circuits

Authors: *C. MIEHL, B. DOIRON;
Univ. of Chicago, Chicago, IL

Abstract: Neuronal circuits in the brain that are characterized by a diversity of neuron types can appear dauntingly complex. The intricate operation of these circuits depends on the precise synaptic connectivity, neuronal nonlinearities, and external contextual signals. Experimental research has leveraged tools to probe neuronal circuit dynamics, but despite these advances, a comprehensive theoretical framework elucidating the interplay of connectivity, plasticity, nonlinearities, and contextual signals in shaping circuit dynamics is notably absent. In this work

we study an inhibitory circuit model that incorporates somatic and dendritic nonlinearities, allowing us to discern the critical parameters influencing circuit dynamics. We organize our work around examples of how contextual signals, which define the 'state' of parvalbumin (PV) and somatostatin (SST) interneurons, influence circuit activity. We demonstrate how the state-dependency of PV and SST determines the polarity of a rate change (exemplified through PV, SST, and VIP paradoxical effects), and circuit gain and stability in response to an input perturbation. Furthermore, we elucidate how the state of PV and SST neurons influences the gating of synaptic plasticity at the dendrite. By establishing a dendritic plasticity rule, we validate experimental findings from the olfactory cortex, where excitatory plasticity at the dendrite is induced by activating VIP and deactivating PV or SST neurons. Taken together, our study reveals how the state of PV and SST neurons exert control over inhibitory circuit dynamics and computations. Hence, we argue that experiments probing circuit dynamics need to be carefully interpreted in the context of the states of its components.

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Poster

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Topic: H.08. Learning and Memory

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Parkinson's and Movement Disorders Center (VCU PMDC) pilot grant

Title: Deep Brain Stimulation of Nucleus Basalis of Meynert improves learning in rat model of dementia: Role of different stimulation parameters.

Authors: J. P. WILSON, Jr[1], M. RAJAGOPAL[2], *J. TOMS[3], A. FREELIN[4], G. JADWIN[5], K. L. HOLLOWAY[2], D. KUMBHARE[6];
[1]Neurosurg., Louisiana State Univ. Hlth. Sci., Shreveport, Shreveport, LA; [2]Neurosurg., Virginia Commonwealth Univ., RICHMOND, VA; [3]Neurosurg., Louisiana State Univ. Hlth. Sci. Ctr. Shreveport, SHREVEPORT, LA; [4]UT Southwestern Med. Ctr., Dallas, TX; [5]Neurosurg., Louisiana State Univ. Hlth. Sci. Ctr., Shreveport, Shreveport, LA; [6]Dept. of Neurosurg., Louisiana State Univ. Hlth. Sci. Ctr. Shreveport, Shreveport, LA

Abstract: ***Background.*** Deep brain stimulation (DBS) of the nucleus basalis of Meynert (NBM) has been preliminarily investigated as a potential treatment for dementia. The degeneration of NBM cholinergic neurons is a pathological feature of many forms of dementia. Although

stimulation of the NBM has been demonstrated to improve learning, the ideal parameters for NBM stimulation have not been elucidated. This study assesses the differential effects of varying stimulation patterns, duration, and timing on learning and memory in a dementia rat model.
Methods. 192-IgG-saporin (or vehicle) was injected into the NBM to produce dementia in rats. Next, all rats underwent unilateral implantation of a DBS electrode in the NBM. The experimental groups consisted of i-normal, ii-untreated demented, and iii-demented rats receiving NBM DBS. The stimulation paradigms included testing different modes (tonic and burst) and durations (1-hr, 5-hrs, and 24-hrs/day) over ten daily sessions. Memory was assessed pre- and post-stimulation using two established learning paradigms: novel object recognition (NOR) and auditory operant chamber learning. Brain sections spanning through the NBM were processed for Choline Acetyltransferase (ChAT) immunostaining.
Results. Normal and stimulated rats demonstrated improved performance in NOR and auditory learning compared to the unstimulated demented group. The burst-stimulation groups performed better than the tonic-stimulated groups. Increasing the daily stimulation duration to 24-hr did not further improve cognitive performance in an auditory recognition task and degraded the results on a NOR task compared with 5-hr. The number of ChAT cells from different rat categories was correlated with their performance in auditory operant learning. There was variability in the ChAT count in all animals, including normal animals not treated with saporin. In both normal and demented rats, the ChAT count was linearly correlated (Correlation coefficient, $R = 0.83$, $p < 0.05$) with the rat's ability to improve in the auditory task. In contrast, no such relationship was seen in the stimulated rats, where significant improvement was seen in rats with low ChAT counts. Further work on evaluating the effects of timing of stimulation is underway.
Conclusion. The present findings suggest that naturalistic NBM burst DBS may offer an effective therapy for treating dementia and suggest potential strategies for reevaluating current human NBM stimulation paradigms.

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Poster

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Topic: H.08. Learning and Memory

Support: NIH Grant R03AG075637
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Parkinson's and Movement Disorders Center (VCU PMDC) pilot grant

Title: Long Term Behavioral and Histological Effects of Deep Brain Stimulation of Nucleus basalis of Meynert in a rat model of dementia

Authors: M. RAJAGOPAL[1], *D. KUMBHARE[3], K. L. HOLLOWAY[2];
[1]Neurosurg., Virginia Commonwealth Univ., RICHMOND, VA; [2]Neurosurg., Virginia Commonwealth Univ., Richmond, VA; [3]Neurosurg., Louisiana State Univ. Hlth. Sci. Ctr. Shreveport, Shreveport, LA

Abstract: Background. Deep brain stimulation (DBS) of the Nucleus Basalis of Meynert (NBM) is being explored as a treatment for dementia. Its role in cognition and memory has been well studied with brief durations of stimulation. A recent primate study reported persistent benefit even after the end of stimulation. Our laboratory has identified that NBM-DBS using delta-gamma burst parameters for 5-hrs/day can providing effective learning improvement in a rat model of dementia. This study assesses the long-term effects of this NBM DBS paradigm.
Methods. Eighteen Long Evans wild-type male rats were divided into three groups: healthy, demented, and demented with stimulation. A memory impaired rat model that mimics cholinergic denervation of dementia was created with bilateral 192-IgG-saporin injections into the NBM. A monopolar DBS electrode was implanted in the NBM, and the stimulated rats underwent a total of 50 hours of stimulation over a 2-week period. During the stimulation phase and weekly for 6 weeks afterwards, the rats were tested on an audio-cue based learning paradigm in an operant chamber. After sacrifice, brain slices were then multiplex stained with BRDU, ChAT, NeuN, Nestin, and doublecortin.
Results. Normal rats demonstrated improvement in accuracy over time (8.39%), whereas the demented rats showed significantly less improvement (1.82%). Stimulated rats demonstrated accuracy improvement that was significantly better than unstimulated demented rats and exceeded normal rats (10.5%, all $p<0.05$). The improved learning ability in the stimulated rats continued post-stimulation. The stimulated group had significantly increased staining compared to the demented and healthy groups for BRDU, BRDU + NeuN, and BRDU + Nestin in the NBM, hippocampus and SVZ regions.
Conclusion. NBM-DBS in a rat dementia model cause long-lasting restorative of learning capabilities in demented rats. One of the reasons for this persistent restoration could be the stimulation induced neurogenesis in the NBM, hippocampus and SVZ regions.

Disclosures: M. Rajagopal: None. **D. Kumbhare:** None. **K.L. Holloway:** None.

Poster

PSTR306: Circuit Mechanisms of Learning, Memory, and Plasticity

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Program #/Poster #: PSTR306.20/V25

Topic: H.10. Human Learning and Cognition

Support: NSF Grant 2245712

Title: Embodied Neuromorphic Robot System for Replicating Associative Learning of Rodents in Open-Field Maze

Authors: T. LIU[1], Y. ZHANG[2], ***H. AN**[3];
[1]Electrical and Computer Dept., [2]Dept. of Biol. Sci., [3]Michigan Technological Univ., Houghton, MI

Abstract: This research emulates associative learning in rodents using a neuromorphic robot within open-field maze environments. The neuromorphic robot is constructed by deploying computational models of spatial cells, such as place cells, grid cells, head direction cells, in a mobile robot for perception and navigation. Various coding schemes, including rate coding and population coding, are utilized for different perception signals. The simulations and experiments demonstrate that our neuromorphic robot successfully replicates the classic associative learning experiments of rodents by memorizing the causal relationship between visual cues and other favorable or unfavorable stimulus locations in an open-field maze. The learning process in our neuromorphic robot relies on synaptic plasticity. By incorporating excitatory and inhibitory synaptic plasticity mechanisms into our neuromorphic system, we emulate the dynamic process of synaptic modification observed in associative learning. In addition, our neuromorphic robot mirrors the training time of rodents for visual clue association tasks. The robot undergoes multiple training sessions over days acquire and consolidate associations. This highlights the fidelity of our approach in capturing the temporal dynamics of associative learning processes, offering valuable insights into neural mechanisms in learning and forgetting. Through repeated training sessions and intervals between trials, the robot will experience a decline in performance over time, mirroring the decay of memories as depicted by the forgetting curve. This work has two unique contributions. Firstly, the neuromorphic robot offers a new embodied simulation platform for studying memory and learning research that does not rely on animal models. By embodying computational models within a physical robotic platform, our neuromorphic robot enables the study of spatial memory and cognitive processes in a real-world context. Specifically, it offers a novel method that can monitor precise neural circuitry and activity changes over time during learning, memorizing, and forgetting processes during interactions with real-world. Secondly, this research provides a new approach to studying the pathological mechanisms underlying degenerative disease in memory and learning. This is accomplished by adjusting the controllable parameters in computational models of embodied neuromorphic robots in real-world scenarios. By replicating cognitive processes and neural circuits associated with these disorders, our embodied neuromorphic robot platform can assist in the development of therapeutic interventions and treatment strategies.

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Poster

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Topic: H.08. Learning and Memory

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Title: Multi-demand brain systems contribute to math academic performance during childhood and adolescence

Authors: *J. CUI[1], Y. ZHAO[3], H. ZHAO[1], S. QIN[2];
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Abstract: Mathematics is considered to be a crucial but challenging subject in primary education. It is associated with multiple cognitive functions, severing as a prominent predictor of children's general academic performance. However, few studies investigate how functional organization of large-scale brain networks involved in multi-demand operating systems account for the mutual influences between elementary cognitive abilities and math performance. Mathematics processing requires various executive functions and subject to affective factors. Hence, we investigate how the multiple-demand systems support math academic performance in school-aged children. We included 310 typically developing children aged 6 to 12, performing four cognitive tasks to assess their attention, working memory, decision-making and emotion matching. They also finished two academic tests in both math and reading. Based on task-related individual networks with 268 nodes, we utilized Partial Least Squares Regression (PLSR) and the Hidden Markov Model (HMM) to identify the multiple-demand system and further examine its dynamics across cognitive domains, respectively. Based on these task-specific features, we first identified a math-related multi-demand system with 121 common edges across 4 tasks, including 78 functional regions. They were summarized at a nodal level by the sum of their absolute loading. The top 10% nodes were the regions with the highest loading in the prediction overlapped with most multi-demand network regions, which were entered into further analysis. Note that six cerebellar areas, serving as the cerebellum's cognition function cores, were found to be related specifically with the math for the first time. Further dynamic analysis with HMM showed that four out of six states' occupancy time was associated with math performance and had more variant brain connectivity values. Moreover, the individual level transition probabilities between states were associated with math performance. No similar patterns were found in the validation analysis with reading performance as the contrast. Our findings uncovered the static and dynamic neural mechanisms underlying math performance and cognitive functions. Particularly noteworthy is the discovery of cerebellar regions previously believed to be solely involved in motor functions. These findings establish a robust foundation for future interventions in mathematics education.

Disclosures: J. Cui: None. **Y. Zhao:** None. **H. zhao:** None. **S. Qin:** None.

Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR307.01/V27

Topic: H.12. Aging and Development

Support: NWO cross-over grant 17611 (MOCIA)

Title: The relation between MIND diet adherence, low-grade systemic inflammation and neuroinflammation in Dutch older adults

Authors: *L. B. REMIE[1], G. WOORT[1], M. R. VAN LOENEN[1], M. P. H. VAN TRIJP[2], J. M. OOSTERMAN[1], E. AARTS[1];
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Abstract: The worldwide double ageing of the population causes a sharp increase in the prevalence of ageing-related cognitive decline. Chronic neuroinflammation is increasingly recognized as a potential mediator of cognitive decline. In ageing, an overall low-grade inflammatory state is commonly seen, which has also been linked to neuroinflammation. The Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet has been designed to reduce the risk of dementia and loss of brain function in ageing. The mechanisms mediating dietary effects on cognition are not completely clear, but inflammatory pathways are likely to be involved as the MIND diet includes various food components that have anti-inflammatory effects.

In this study we investigated the currently unexplored relation between MIND diet adherence, low-grade systemic inflammation and neuroinflammation in Dutch older adults. Using baseline data of the HELI study (NCT05777863), we included 94 older adults (60-75 year) at risk for cognitive decline based on modifiable lifestyle-related risk factors. MIND diet adherence was assessed using the MIND score, which was calculated via a food frequency questionnaire. Blood C-reactive protein levels, white blood cell counts and neutrophil-to-lymphocyte ratio were collected via a finger prick and converted into a composite systemic inflammation score. Magnetic resonance spectroscopy was used to measure myo-inositol, choline and creatine levels within the left dorsolateral prefrontal cortex, which were converted in a composite neuroinflammation score.

Multiple linear regression analysis showed that MIND diet adherence and systemic inflammation do not independently predict neuroinflammation ($F=1.59$, $p=0.210$). However, the interaction between MIND diet adherence and systemic inflammation significantly predicted neuroinflammation ($\beta=-0.12$, $p=0.020$), meaning that only in individuals with low adherence to the MIND diet, low-grade systemic inflammation positively correlated with neuroinflammation. Our findings suggest that MIND diet adherence might protect against the detrimental effect of systemic inflammation on neuroinflammation, which should be confirmed in randomised controlled trials.

Disclosures: L.B. Remie: None. **G. Woort:** None. **M.R. van Loenen:** None. **M.P.H. van Trijp:** None. **J.M. Oosterman:** None. **E. Aarts:** None.

Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

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Topic: H.12. Aging and Development

Support: NWO cross-over grant 17611 (MOCIA)

Title: The relation between physical activity, cerebral blood flow, blood pressure and episodic memory function in Dutch elderly at-risk of cognitive decline

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Abstract: The current rate of population ageing is unavoidably associated with an increased prevalence of age-related cognitive decline. This emphasizes the need for interventions to combat established modifiable risk factors. One of these risk factors is physical inactivity, which impacts facets of cardiovascular health such as blood pressure. Cerebral blood flow (CBF) - a measure closely related to brain health and thereby cognitive functioning - is strongly impacted by cardiovascular health, and previous studies have shown positive effects on CBF after short physical exercise training programs in elderly. However, the relation between current physical (in)activity and cognitive functioning in older adults remain to be further explored. With this study, we aim to investigate the link between current physical activity levels and episodic memory in a population of Dutch older adults (60 - 75 years old) at-risk of cognitive decline (n = 156), using CBF and mean arterial pressure (MAP) as potential mediating factors. We used the SQUASH and LASA Sedentary Behavior Questionnaire to measure physical activity and sedentary behavior respectively, and utilized arterial spin labelling MRI to non-invasively quantify CBF. Multiple outcome measures of the Rey Auditory Verbal Learning Test (RAVLT) were used as indices of episodic memory functioning, including learning and delayed recall. MAP was calculated using averaged readings from multiple electronic blood pressure measurements. Multiple linear regression analysis showed no predictive value of physical activity or sedentary behavior on learning (physical activity: t = -0.883, p = 0.379; sedentary behavior: t = 1.037, p = 0.302) or delayed recall (physical activity: t = -0.623, p = 0.535; sedentary behavior: t = -0.704, p = 0.483). CBF and MAP were not identified as mediating factors in this model, but predictably blood pressure (MAP) did significantly correlate with hippocampal perfusion (β = -0.221, p = <0.001) after correcting for age, sex and education (F(4, 135) = 7.898, p = <0.001, R^2 = 0.166). Although the findings from this observational study

indicate no apparent predictive value of subjective physical activity and sedentary behavior scores on episodic memory, we should further investigate the potential of lifestyle interventions (including physical activity) to delay cognitive ageing through potential underlying brain mechanisms such as CBF and its relation to blood pressure. We will discuss potential explanations for these null findings that contradict our initial hypothesis, such as the characteristics of this specific at-risk study population and methods of acquiring physical (in)activity scores.

Disclosures: M.R. van Loenen: None. **L. Wekking:** None. **L.B. Remie:** None. **J.M. Oosterman:** None. **E. Aarts:** None.

Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

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Topic: H.12. Aging and Development

Support: NIH grant 5R01AG063930

Title: Chronic stress is associated with alterations in large-scale functional brain network organization among middle-aged adult humans

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Abstract: Chronic stress negatively impacts brain anatomy and function (McEwen & Gianaros, 2010; Lenart-Bulga et al. 2022), and has been linked to impairments in cognition and behavior (Booth et al. 2015). While relationships between stress and the brain have primarily been examined in specific regions, the impacts of stress are presumed to alter distributed networks of the brain.
Previous work has demonstrated that increasing age is associated with alterations in functional brain network organization (Wig, 2017) and varies according to environmental exposures as indexed by measures of socioeconomic status (SES). For example, age-related decline in brain network segregation is more rapid in adults without a college degree (Chan et al. 2018; 2021). We hypothesized that these education-related brain network alterations may be due to the greater impacts of chronic stress on brain network organization among people of lower SES.
Data from a representative sample of healthy middle-aged adults (40-64y) in the Midlife Brain and Environment Study (N=148; 74F; 50% lower income [below 80% of median county

income]) were used to examine whether functional brain network organization varies with allostatic load (AL), a measure of cumulative stress. AL was calculated using 7 measures: systolic and diastolic blood pressure, waist-to-hip ratio, total over HDL cholesterol ratio, C-reactive protein, interleukin-6, & body-mass-index. Sex-specific quintile rank scores for each variable were calculated and summed to derive an Allostatic Load Score (ALS).
We further examined whether this link is moderated by educational attainment. Each participant's brain network was constructed from preprocessed and motion-corrected resting-state fMRI data. Brain network nodes were defined using resting-state functional correlation (RSFC) area parcellations (Wig et al. 2014) and labeled with a priori functional brain systems (Power et al. 2011). The RSFC matrix was used to calculate system segregation, a graph measure that estimates the degree to which functional systems are segregated from each other (Chan et al., 2014).
Our findings suggest that elevated ALS was associated with less segregated brain systems, particularly in adults lacking a college degree. Furthermore, this relationship was primarily observed in the association systems responsible for higher-order cognitive operations. These results indicate that elevated chronic stress degrades patterns of functional brain network organization. These stress-related alterations are particularly prominent in lower SES individuals who may have fewer resources to counteract the deleterious impacts of stress on brain aging.

Disclosures: M.Y. Chan: None. **C. Sullins:** None. **J. Reingle Gonzalez:** None. **D.C. Park:** None. **E. Brown:** None. **G.S. Wig:** None.

Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

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Topic: H.12. Aging and Development

Support: NIH R01-DC019394
NIH T32-DC00046

Title: Physiological Functional Connectivity Changes during Difficult Listening in Older and Younger Adults.

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Abstract: Listening in difficult, noisy conditions affects the cortical neural circuits that underlie speech comprehension. These directional circuits convey neural signals between cortical regions, encode information related to processing of the stimulus, and are characterized by their dominant frequency band, e.g., delta band or theta band. Here we elucidate how these circuits change as listening conditions become increasingly adverse, and we reveal differences in regional recruitment between older and younger individuals. We utilize the Network Localized Granger Causality (NLGC) framework applied to magnetoencephalography (MEG) data to simultaneously estimate neural currents in cortex and the graph network that connects current sources to one another. This directional connectivity is analyzed in multiple non-overlapping regions that span the entire cortex. Additionally, a Temporal Response Function (TRF) analysis is performed on the estimated current sources to probe hierarchical processing of speech features among network-connected current sources and to determine to what extent these circuits convey signals that temporally track the stimulus. Broadly, we estimate the connectivity of the cortical neural circuits in physiological frequency bands that are involved in processing speech, and we examine how the circuits change with age and listening difficulty. We also demonstrate how to combine these circuits with established TRF analysis to localize hierarchical processing of speech. We present results on a listening data set, but note that the methods are widely applicable to most MEG data sets. This work was supported by NIH grants R01-DC019394 and T32-DC00046.

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Poster

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Topic: H.12. Aging and Development

Support: R01AG057184

Title: Age-related differences in amygdala subregion functional connectivity with ventromedial prefrontal cortex and dorsolateral prefrontal cortex

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Abstract: Age-related differences in amygdala subregion functional connectivity with ventromedial prefrontal cortex and dorsolateral prefrontal cortexK. Alemu[1,2], K. Nashiro[1], H.J. Yoo[1], P. Nasseri[1], M. Mather[1,21]*Leonard Davis School of Gerontology, University of Southern*

*California; [2]Department of Psychology, University of Southern California*Despite physical and cognitive decline, emotional well-being is well maintained as people get older. There even is an age-related positivity effect in which older adults (OA) prioritize positive over negative information in attention and memory compared with young adults (YA), leading to age-by-valence interactions. Relatedly, younger participants show relatively more amygdala activity during viewing negative than positive stimuli compared with older adults who show relatively more amygdala activity during viewing positive than negative stimuli. The amygdala serves as a hub for neural networks involved in emotion processing but is not a single uniform structure. It is a complex structure comprising structurally and functionally distinct nuclei that have different functional networks. Both the ventromedial prefrontal cortex (vmPFC) and dorsolateral prefrontal cortex (dlPFC) are involved in emotion regulation networks that include the amygdala. In addition, the anterior (pregenual PFC) and posterior (subgenual PFC) portions of the vmPFC are differentially involved in response to positive and negative emotional stimuli, respectively. Most studies investigating the positivity effect and age-related differences in amygdala activity have predominantly employed task-based paradigms. Therefore, the present study investigated age-related differences in resting-state functional connectivity patterns between seven amygdala subregions and pregenual and subgenual PFC, as well as the dlPFC in YA (n = 113; ages 18-31) and OA (n = 60; ages 65-80). Mixed effects analysis of variance were used to examine age-related functional connectivity differences. Results revealed stronger functional connectivity between the amygdala and subgenual PFC in YA than in OA. Relative to the subgenual PFC, amygdala connectivity with the pregenual PFC is maintained in OA. We did not observe age-related differences in amygdala functional connectivity with the dlPFC. These findings lend additional support for task-based studies that have shown increased functional coupling between pregenual PFC and amygdala in old age is associated with the positivity effect.

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Poster

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MOE AcRF Tier 1 Award RS01/19

Title: Prefrontal circuit dynamics in cognitive aging

Authors: H. CHONG, Y. RANJBAR-SLAMLOO, M. HO, X. OUYANG, P. RAMESH, J. TAN, ***T. KAMIGAKI**;
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Abstract: The cognitive domain of executive function, encompassing higher-level mental processes and cognitive control, is particularly susceptible to the effects of aging. Among the facets of cognitive decline, working memory (WM) deterioration stands out for its early onset and integral role. Extensive research implicates the prefrontal cortex (PFC) in various executive functions, including WM, and suggests that its malfunction may be a critical contributor to cognitive aging. To elucidate the impact of aging on WM-related neural processing in the PFC, we employed a delayed two-alternative forced-choice (d2-AFC) task with mice from young adulthood to advanced age. During the task, mice were presented with sensory cues (auditory, tactile, or bimodal) and responded after a two-second delay. All age groups successfully acquired the task, albeit with an age-related decrement in learning rate. Calcium imaging of the medial PFC (mPFC) revealed intriguing age-related alterations: with advancing age, there was a decrease in the fraction of action-plan coding neurons and a reduction in the strength of the action-plan signal, leading to impaired decoding of action plans at the population level. Further analysis of the same mPFC neural population using distinct sensory modalities (auditory vs. tactile) in the d2-AFC task uncovered dynamic age-related changes. In young adult mice, population activity exhibited dissociable yet overlapping patterns between modalities, facilitating concurrent modality-general and modality-dependent coding. However, as mice transitioned to middle age, modality-general coding significantly diminished while modality-dependent coding persisted, and both forms of coding were disrupted in advanced age. Resting-state functional connectivity measurements indicated a decrease in connectivity among action-plan-coding neurons as early as middle age. Additionally, optogenetic experiments revealed heightened susceptibility to perturbation in the middle-aged mPFC. Our findings collectively demonstrate that functional alterations in the prefrontal circuit underlying memory-guided behavior emerge in middle age and progress with advancing age, providing valuable insights into the neural dynamics of cognitive aging.

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Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

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Title: Regulation of trophic factors in the choroid plexus of aged mice

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Abstract: The choroid plexus (CP) is a complex network of capillaries lined by specialized cuboidal epithelial cells, which are mainly involved in the production of cerebrospinal fluid (CSF). The CP also constitutes the blood-CSF barrier. Furthermore, the CP produces numerous neurotrophic factors (NTF), which circulate to different regions of the brain along with CSF. Regulation of NTF in the CP during natural aging is largely unknown. Here, we investigated the age and gender-specific transcription of NTFs along with the longevity factor klotho. We used male and female mice for the study. Age-related transcriptional changes were analyzed using quantitative PCR at three different time points: mature adult (5-6 months), middle-aged (11-12 months), and aged (18-24 months). Transcriptional changes during aging were further confirmed with the digital droplet PCR. Additionally, we used immunohistochemical (IHC) and western blot analysis for the evaluation of in vivo protein expression. We also determined the cellular phenotype of these NTF'S and klotho CP by co-labeling them with the classical vascular marker, Isolectin B4, and epithelial cell marker, plectin. Aging significantly altered the NTF's gene expression in the CP. Brain-derived neurotrophic factor (BDNF), Midkine, VGF, Insulin-like growth factor (IGF1), IGF2, Erythropoietin, and its receptor were reduced in the aged CP of males and females. Vascular endothelial growth factor (VEGF) transcription was gender-specific; in males, gene expression was unchanged in the aged CP, while females showed an age-dependent reduction. Age-dependent changes in VEGF localization were evident in vasculature and epithelial cells. IGF2 was localized in the basolateral membrane of the CP and showed an age-dependent reduction in epithelial cells. Longevity factor Klotho transcription in CP showed a decline in the aged CP of males and females. Klotho protein was localized in the basolateral membrane of epithelial cells, and a significant reduction in the aged CP was observed. Our study highlights gene and protein level changes in the CP during aging. The age-related transcriptional changes exhibit similarities as well as gene-specific differences in the CP of males and females. Importantly, reduction in the neurotrophic factors and longevity factor Klotho can play a role in regulating brain aging. Future directions involve the AAV-mediated knockdown of the Klotho gene in the CP of adult mice to study Klotho-mediated brain aging.

Disclosures: J. Sadanandan: None. **M. Sathyanesan:** None. **S.S. Newton:** None.

Poster

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Title: The effects of increasing neural noise in healthy younger adults simulates age-related resting state functional networks

Authors: *G. H. SHERARD[1], B. B. BISWAL[2], X. DI[2], B. P. RYPMA[3];
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Abstract: Neurocognitive aging studies assess the role of functional network changes in age-related cognitive decline. Using graph theory, these studies have shown that aging is associated with decreased *modularity* (a decrease in the efficient segregation between networks), decreased *nodal cluster coefficient* (a decrease in the connectivity within networks), and increased *path length* between *nodes* (selected brain regions from each functional network). One mechanism that may underlie such functional network changes is an increase in spontaneous baseline neural activity, also known as neural noise. These noise increases would be expected to disrupt neural communication and exert deleterious effects on cognitive performance. In this study, we sought to examine the effects of neural noise on age-related functional network changes. We collected fMRI data from 50 young adults (19-24 yr, M_{age}=22.34 yr), scanned at rest during a 3T fMRI gradient echo planar imaging sequence (TR/TE=2000 ms/30 ms; flip angle=90°; FOV=240×240 mm; matrix size=64×64; axial slice number=42 with slice thickness=3mm). Average time series BOLD signal was extracted from each of the 214 non-cerebellar functional network-based ROIs as defined in the Shen 268 Atlas. Based on previous literature, brain regions from six known networks (eg, the Visual and Fronto-Parietal networks) were selected for analysis. For each ROI time series, in each participant, the time series mean and standard deviation (SD) were estimated by fitting the time series to a Gaussian distribution. To simulate age-related reductions in signal-to-noise ratio, we added Gaussian random white noise with the same mean and varying SDs to the ROI time series for each participant. As hypothesized, the addition of this noise successfully simulated previously-observed age-related changes in all six functional networks (ie, decreased modularity and nodal cluster coefficients, and increased path length), with the Visual network showing the most rapid decline. The current study suggests the hypothesis that age-related increases in baseline neural activity (ie, neural noise) disrupts communication in functional networks, possibly slowing internodal and internetwork transmission, resulting in the processing speed declines that underlie cognitive aging.

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Poster

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Program #/Poster #: PSTR307.09/V34

Topic: H.12. Aging and Development

Title: Spatial and temporal scales of resting-state fMRI throughout lifespan

Authors: *J. BERO IV[1], Y. LI[2], A. KUMAR[3], C. J. HUMPHRIES[4], H. LEE[5], M. SHINN[6], J. D. MURRAY[7], T. J. VICKERY[8], D. LEE[9];
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Abstract: Temporal and spatial scales of blood-oxygen-level-dependent signals in functional magnetic resonance imaging (fMRI) data reliably and parsimoniously characterize the topological structure and temporal dynamics of cortical activity in the human brain. For example, timescales of resting-state fMRI signals can predict a significant amount of the individual variability in functional connectivity (FC) networks. Nevertheless, little is known about how the spatial and temporal scales of resting-state fMRI change across the lifespan. Therefore, in the present study, we quantified and compared temporal and spatial scales in resting-state fMRI data collected from 2,352 subjects between the ages of 5 and 100 in Developmental, Young Adult, and Aging Human Connectome Project (HCP) datasets. We found that the temporal and spatial scales varied similarly across different cortical areas throughout the lifespan, with the visual cortex and limbic network showing the largest and smallest correlations between them. In addition, these two scales displayed similar non-monotonic trajectories during adolescence in some cortical regions, peaking around the same time during adolescence, while decreasing throughout the rest of the lifespan. By contrast, cortical myelination increased monotonically throughout the lifespan, and its rate of change was significantly correlated with the changes in timescale across different cortical regions in adulthood. These findings suggest that temporal and spatial scales in fMRI signals as well as cortical myelination are closely coordinated during development and aging.

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Poster

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McDonnell Center for System Neuroscience Washington University St. Louis

Title: Negative association between neurovascular coupling and cortical gray matter volume during the lifespan

Authors: *P. CHRISTOVA[1,2], L. M. JAMES[3], A. P. GEORGOPOULOS[3];
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Abstract: Recent studies have established the moment-to-moment turnover of the blood-oxygen-level-dependent signal (TBOLD) at resting state functional Magnetic Resonance Imaging as a key measure of local cortical brain function. Specifically, TBOLD is heritable and varies across cortical brain areas, and corresponds with the distribution of acetylcholine projections (James at al. 2022, Christova et al. 2022). Separately, we have shown that gray matter volume changes during the lifespan (Christova and Georgopoulos 2023 a, b). Specifically, we found that, during both development and aging, cortical brain volumes decreased with age. Here we sought to bridge these lines of research and extend findings from prior TBOLD studies by evaluating TBOLD with respect to brain volume, age, and sex across the lifespan for 70 cortical areas. We analyzed data from 1,344 healthy participants including 633 from the Human Connectome Project (HCP)-Development cohort (HCP-D, 294 males and 339 females, age range 8-21 y) and 711 healthy participants from HCP-Aging cohort (HCP-A, 316 males and 395 females, 36-90 y old). In both development and aging groups we found that (a) TBOLD increased with age, (b) brain volume decreased with age, and (c) TBOLD and volume were highly significantly negatively correlated, independent of age. We evaluated partial correlation coefficients, when controlling for age, between TBOLD and normalized volume for the HCP-D and HCP-A groups. In the HCP-D group, all correlations, except one, were negative (98.6%), whereas in the HCP-A group 88.6% were negative. None of the positive correlations were statistically significant. Finally, the 1.5 times higher correlation between mean TBOLD and volume in the development cohort relative to aging cohort was documented. Partial correlations between TBOLD and volume, when controlling for age, were consistently more negative in males than in females in both HCP groups, a difference that attained statistical significance only in the HCP-A group. We

hypothesized that the strong correspondence between TBOLD and volume across age and sex suggest a common influence such as chronic neuroinflammation contributing to reduced cortical volume and increased TBOLD across the lifespan. We attribute this association to a possible chronic, low-grade neuroinflammation, probably induced by various neurotropic pathogens, including latent/reactivated human herpes viruses known to be dormant in the brain in a latent state and reactivated by stress, fever, and various environmental exposures.

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Poster

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Program #/Poster #: PSTR307.11/V36

Topic: H.12. Aging and Development

Support: Balchem, Inc

Title: Effects of Choline on Brain Functioning in Postmenopausal Women

Authors: *J. DUMAS[1], A. TESTO[2], A. SENFT MILLER[3], J. ZHANG[4], M. ABOUKHATWA[5], J. BOYD[6];
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Abstract: Choline is an essential nutrient that, in addition to its role in the brain, has a number of critical structural and physiologic roles throughout the body. Aside from dietary intake, the only source of choline in the body is de novo synthesis of phosphatidylcholine, catalyzed by phosphatdylethanolamine-*N*-methyltransferase (PEMP) that has several estrogen-responsive components in its promoter region and is induced by estrogen. This study used a randomized placebo-controlled trial to examine the ability of an acute dose of dietary choline to influence brain functioning in health postmenopausal women. This study was a cross-over, placebo-controlled trial of the effects of choline on brain functioning in 19 healthy postmenopausal women. Each woman participated in two study days with choline or placebo administered on each day three hours before a functional and structural MRI and MRS scans. Choline was administered at a dose of 1650 mg choline bitartrate. Placebos consisted of similar capsules filled with microcrystalline cellulose. Structural and BOLD task functional MRI scans of 19 cognitively healthy postmenopausal women were collected on each choline and placebo day. A ROI-to-ROI correlation (bivariate) analysis comparing connectivity on the choline vs placebo days during an Nback task was run among the 68 ROIs. Decreased activation was found for the

choline day compared to the placebo day during the working memory task in the right frontal pole ($p<.05$ cluster corrected). Increased activation was observed for the choline compared to placebo day during the working memory task in the central opercular cortex right, insular cortex left, and left postcentral gyrus ($p<.05$ cluster corrected). Decreased connectivity was observed for the choline compared to the placebo study during the working memory task in four clusters. The most significant cluster (p-FWE=0.034) showed a pattern of decreased functional connectivity between inferior parietal (R), and parahippocampus (R), the inferior parietal (R) and parahippocapus (L), and the rostral middle frontal (R) and rostral anterior cingulate left. These results illustrated the effect of choline on working memory related brain activation and functional connectivity. These measures indicate that choline decreased activation and connectivity. We propose that choline may increase brain functional efficiency with these results that show similar patterns to our prior work with three months of estradiol treatment in postmenopausal women. Future studies should examine different doses or length of choline administration to observe effects of choline on behavior in postmenopausal women.

Disclosures: J. Dumas: 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.; Balchem, Inc. **A. Testo:** None. **A. Senft Miller:** None. **J. Zhang:** None. **M. Aboukhatwa:** None. **J. Boyd:** None.

Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR307.12/V37

Topic: H.12. Aging and Development

Support: National Institutes for Aging Grant R01AG075440

Title: Intranasal Administration of Extracellular Vesicles from hiPSC-derived Neural Stem Cells in Early Middle Age Can Slow Down Brain Aging

Authors: *G. SHANKAR, S. ATTALURI, M. KODALI, V. RAO, S. RAO, B. SHUAI, Y. SOMAYAJI, P. PANDA, L. N. MADHU, A. K. SHETTY;
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Abstract: Aging is the primary risk factor for developing neurodegenerative diseases. Since moderate neuroinflammation, increased mechanistic target of rapamycin (mTOR) signaling, and reduced autophagy can contribute to age-related cognitive and mood impairments, strategies that can positively modulate these changes are of interest. Recent studies have shown that

extracellular vesicles (EVs) released by neural stem cells (NSCs) derived from human induced pluripotent stem cells (hiPSCs) carry therapeutic miRNAs and proteins capable of modulating neuroinflammation, mTOR signaling, and autophagy. Therefore, in this study, by employing C57BL6/J mice, we investigated whether intranasal (IN) administrations of hiPSC-NSC-EVs in early middle age (12 months) or at both early and late middle age (12 and 16 months) would maintain better cognitive and mood function in old age with modulation of neuroinflammation, mTOR signaling, and autophagy. A cohort of mice received IN administrations of either vehicle or hiPSC-NSC-EVs (10×10^9) at 12 months only (early middle age) or at 12 and 16 months (early and late middle age). These animals underwent multiple cognitive tests examining the integrity and function of brain regions such as the hippocampus, medial prefrontal cortex and the perirhinal cortex. The tasks included novel object recognition, object location memory, object-in-place, temporal pattern processing, pattern separation, and delayed alternation Y-maze tests. In all these tasks, animals receiving hiPSC-NSC-EVs at 12 months of age or both 12 and 16 months of age showed an improved cognitive ability than their counterparts receiving the vehicle. In addition, these animals displayed better mood function in the sucrose preference test for anhedonia, novelty-suppressed feeding, and elevated plus maze tests for anxiety. Notably, enhanced cognitive and mood function in animals receiving hiPSC-NSC-EVs was associated with decreased neuroinflammation, apparent from reductions in astrocyte hypertrophy, microglial clusters, and microglia exhibiting the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome complexes. Furthermore, animals receiving hiPSC-NSC-EVs exhibited diminished mTOR signaling and increased autophagy apparent from decreased pS6 and increased p62 expression in the medial prefrontal cortex, hippocampus, and entorhinal cortex. Additional biochemical analyses are underway to validate the above effects of hiPSC-NSC-EVs. Thus, IN administrations of hiPSC-NSC-EVs have the potential to be an effective treatment to prevent age-related neuroinflammation and cognitive and mood impairments.

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Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR307.13/V38

Topic: H.12. Aging and Development

Title: Prediction of individual cognitive test scores from imaging and non-imaging data in older males and females

Authors: *C. MENDL-HEINISCH[1,2], N. BITTNER[1,2], T. MILLER[1,2], P. R. DELLANI[2,1], S. CASPERS[1,2], C. JOCKWITZ[2,1];
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Abstract: Prior research has shown that cognitive performance profiles of males and females may not only differ, but may also be differentially predictable from brain structural data. However, as the overall predictive power for age-related cognitive decline based on brain structural data is limited for the two sexes, the aim of the current study was to optimize this predictive potential by adding non-brain health-related and demographic data. We systematically investigated predictability differences across males and females with respect to i) varying input modalities, i.e. imaging, health-related and demographic data, and ii) different cognitive functions assessed by sixteen standard cognitive tests in 494 older males (N = 247, 67 +/- 7 years) and females (N = 247, 66 +/- 7 years) from the 1000BRAINS study. Brain summary statistics (e.g. total grey and white matter (WM) volume, WM lesion load; 13 features), health-related (e.g. body-mass-index, diabetes diagnosis and cholesterol levels; 12 features) and demographic (i.e. age, sex, education; 3 features) data served as input to predict cognitive test scores (e.g. Trail Making Test) using a machine learning (ML) approach. ML performance, i.e. coefficient of determination (R-squared), was assessed using a repeated nested 10-fold cross-validation and four regression algorithms. Sex differences emerged across modalities and cognitive tests. Brain imaging data led to higher prediction performance in males (Mean R-squared: Males: -0.23 to 0.11; Females: -0.22 to 0.03), while demographic data was more successful in females (Mean R-squared: Males: -0.23 to 0.21; Females: -0.11 to 0.35). In both males and females, health-related data led to the lowest prediction performance (Mean R-squared: Health-related: -0.22 to -0.03; Imaging: -0.23 to 0.11; Demographics: -0.23 to 0.35). Analyses of individual cognitive functions revealed vocabulary test performance to be better predicted in females (Mean R-squared: Females: -0.09 to 0.35; Males: -0.09 to 0.07), while executive function and attention test scores (Mean R-squared: Males: -0.12 to 0.10; Females: -0.10 to 0.04) were better predicted in males. Despite rather low overall ML performance, we found sex-specific predictability differences across modalities and cognitive tests. While health-related data led to the lowest ML performance in males and females, sex-specific particularities emerged for the importance of brain and demographic data. Sex-specific predictability differences in cognitive tests, further, appear to mirror well-known sex differences in cognition. Results highlight the importance of considering sex differences in future prediction studies.

Disclosures: C. Mendl-Heinisch: None. **N. Bittner:** None. **T. Miller:** None. **P.R. Dellani:** None. **S. Caspers:** None. **C. Jockwitz:** None.

Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR307.14/W1

Topic: H.12. Aging and Development

Title: Prediction of individual cognitive performance from imaging and non-imaging data in decades of adulthood

Authors: *C. JOCKWITZ[1,2], C. KRÄMER[2,1], T. MILLER[2,1], P. R. DELLANI[1], N. BITTNER[2,1], S. CASPERS[2,1];
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Abstract: The identification of effective biomarkers for cognitive performance prediction is gaining much attention in light of demographic change. Results so far, however, remain controversial. While some studies found acceptable prediction results based on (non-)brain metrics, others, particularly in older adults, showed non-significant results. These differences might be due to the fact that a) age-related cognitive changes are partly non-linear and b) demographic and health-related factors might affect cognitive abilities differentially across adulthood. Therefore, the current study used a large sample from the German National Cohort (NAKO) to systematically examine the predictability of cognitive abilities based on brain, health-related and demographic data across the adult lifespan. First, the NAKO sample (N = 23,863; 48 +/- 12 y.) was stratified into decades: 25-35y.; 35-45y.; 45-55y.; 55-65y.; 65-75y. Brain summary statistics (e.g. total grey and white matter (WM) volume; 23 features), health-related (e.g. body-mass-index, cholesterol levels; 12 features) and demographic (i.e. age, sex, education; 3 features) data were used as input to predict four cognitive scores (episodic memory, interference, verbal fluency, verbal working memory) using a machine learning (ML) approach. ML performance, i.e. coefficient of determination (R^2), was assessed using a repeated nested cross-validation and four regression algorithms. Regarding the whole sample, results revealed adequate predictability for episodic memory (R2: < .21) and interference (R2: < .19) and low predictability for verbal fluency (R2: < .06) and working memory (R2: < .08). Moreover, prediction power notably decreases when examining each decade separately, with almost no differences between decades (R2: -.09 to .09). Regarding the input features, demographic factors seem to best predict cognitive performance across functions, with only small added value when integrating brain or health-related factors into the models. Current results accentuate demographics to outperform both, brain and health-related factors in predicting cognitive abilities in a large sample spanning the whole adulthood. Contrary to the hypothesis of a worse prediction at older ages, prediction appeared to be similarly low in each decade. Hence, sample size seems to matter even more than sample homogeneity. Including a wide age range for reaching large sample sizes, though, could come at the cost of predicting a hidden age effect. Given the differential predictability of distinct cognitive abilities even in the large sample calls for further investigation of the complex relation of various factors with cognition.

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Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR307.15/W2

Topic: H.12. Aging and Development

Title: Age associated alterations in the cortical representation of cardiac rhythms

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Abstract: Neural responses to the cardiac rhythms, known as heartbeat evoked responses (HER), provides a unique avenue for investigating the interaction between the brain and the heart, pivotal for various physiological and cognitive functions. Age is recognised as a significant factor influencing this interaction, given its impact on both the structural and functional aspects of the brain and the cardiovascular system. However, the precise nature of this interaction during the healthy aging process remains elusive. To gain a deeper understanding, our objective in this study is to examine how the cardiac rhythms influence spontaneous brain rhythms by utilizing HER as a measure, alongside exploring the underlying networks across a healthy aging (N=620; Female = 49.52%) cohort and to identify and characterize the workings of brain networks which prominently represents the HER. We report that (1) HER exhibit time-locked activity within the 180-320 ms post R peak of the electrocardiogram (ECG) waveform and the amplitude of this evoked activity significantly decreases across lifespan aging for both genders ($r= -0.1963$; $p<0.001$), (2) assessment of the mechanism behind HER shows the increasing inter-trial phase coherence (ITPC) values in the theta frequency band ($r = 0.2236$; $p<0.001$) than altering the spectral power across the age, (3) neural sources of HER were source localized to the right orbitofrontal cortex (OFC), right anterior prefrontal cortex (PFC) and left temporal pole (TP) (4) causal functional maps using granger causality (GC) revealed bidirectional interactions between OFC and PFC, OFC and TP and unidirectional interaction between PFC to TP. Additionally, GC values showed an increase from TP to OFC ($r = 0.1728$; $p<0.001$) and from PFC to TP ($r= 0.1530$; $p<0.001$) across age, indicating compensatory mechanisms in play to maintain homeostasis. Overall, these findings provide a comprehensive understanding of the interaction between heart and brain in healthy aging and present opportunities to identify non-invasive markers for characterizing pathological development in neurological and cardiovascular functions.

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Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

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Topic: H.12. Aging and Development

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Title: White Matter Lesion spatial distribution patterns follow arterial supply territories

Authors: *T. MILLER[1,2], N. BITTNER[1], P. R. DELLANI[2], J. QUABS[1], S. CASPERS[1,2]; [1]Inst. for Anat. I, Med. Fac. & Univ. Hosp. Düsseldorf, Heinrich Heine Univ., Düsseldorf, Germany; [2]Inst. of Neurosci. and Med. (INM-1), Forschungszentrum Jülich GmbH, Jülich, Germany

Abstract: Aiming at understanding the pathomechanism of white matter lesions (WML), decoding their spatial patterns might improve our understanding of their relation to the subject's cardiovascular health. While most studies focus on quantifying WML total burden or visually classifying superficial and deep WML, this study aims at identifying WML spatial distribution patterns and characterize them based on the arterial blood supply to the white matter. Data, 2 large population-based cohorts: 1000BRAINS (n=1,040, 18-87 years) & NAKO (n=27,559, 19-74 years). Total amount, and the center of WML mass were calculated - BIANCA. Risk factors for WML development and progression, i.e. age, sex, blood pressure, hypertension medication, smoking status, diabetes diagnosis and cholesterol levels were summarizes in a composite 'cardiovascular age' (CA) score. A clustering analysis was applied to all 3 features: WML total volume, WML center of mass, and the CA. To characterize each pattern, we assessed WML loads in 30 territories of the Digital 3D Brain MRI Arterial Territories Atlas and tested for each cluster the mean WML distribution in each arterial territory surpassing 95% bootstrap confidence. Analysis was conducted independently in both cohorts. 4 clusters were identified as common in terms of WML load, affected arterial territories and CA. In the 1st cluster, participants show the youngest CA, lowest overall WML load focused in the frontal lobe, while in this stage only the lenticulostriate (LS) territory was affected. With rising CA, patterns seem to evolve to wider distributions with heavier load and more affected territories described as the 2nd and 3rd common cluster: one with frontal WML distribution affecting the medial & lateral LS territory, and frontal part of the middle cerebral artery (MCA); and another with occipital WML distributions affecting the occipital part of the posterior cerebral artery (PCA). In the 4th cluster, participants with the oldest CA showed highest volume of lesions across all lobes affecting widespread arterial territories, comprising parts of the MCA and also PCA, and medial

and lateral LS territory. Specifically in NAKO subjects, one additional cluster was found characterizing a stage between clusters 3 and 4, affecting the parietal and temporal parts of the MCA and the PCA. We were able to automatically identify patterns that exhibit varying degrees of WML accumulation in specific arterial territories, providing valuable insights of the WML spatial distribution in relation to the subject's cardiovascular health. This could help explain associated cognitive alterations or used as new markers to characterize WML in cohort studies.

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Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

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Program #/Poster #: PSTR307.17/W4

Topic: H.12. Aging and Development

Support: NRF-2021R1C1C2012889

Title: Vesicular zinc mediates exercise-induced adult neurogenesis and reverses cognitive decline induced by aging

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Abstract: The neurogenesis-promoting effects of exercise have been extensively studied, yet the molecular mechanisms that underlie these responses remain unclear. Here, we propose that this is mediated by the exercise-induced vesicular Zn^{2+} release. Using knockout mouse models, we confirmed that zinc transporter 3 (ZnT3), which is a membrane Zn^{2+} transporter that is responsible for concentrating Zn^{2+} into neuronal presynaptic vesicles, is essential for the exercise-induced increase in adult hippocampal neurogenesis. In vivo zinc infusion increased hippocampal neuronal progenitor cell (NPC) proliferation and adult neurogenesis. The combination of dietary zinc supplementation with exercise restored neurogenesis and reversed age-related cognitive decline, suggesting potential therapeutic relevance. These results provide a molecular mechanism linking exercise-induced changes in the brain environment to the activation of quiescent hippocampal NPCs and their subsequent recruitment into the neurogenic pathway.

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Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

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Program #/Poster #: PSTR307.18/W5

Topic: H.12. Aging and Development

Support: NIA/NIH Grant 1U19AG076581-01A1

Title: Exploring the impact of COVID-19 on cognitive decline and Alzheimer's disease risk

Authors: C. G. FRANKLIN[1], W. J. ALLEN[3], I. ZWIR[4], M. HABES[2], *D. B. NEIDRE[5], G. A. DE ERAUSQUIN[6], P. T. FOX[7];
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Abstract: COVID-19 has affected over 160 million individuals globally and the long-term effects on older adults are still being elucidated, with potential insights into the connection between viral infections and Alzheimer's disease (AD) and related dementia awaiting exploration. A cohort of 529 older adult Amerindians from Argentina, including 453 individuals with confirmed COVID-19 and 76 controls, underwent comprehensive neuropsychiatric and cognitive assessments, neurological exams, and T1-weighted anatomical MRI scans. Volumetric segmentation of the MRI scans was conducted using MUSE. The neuroimaging volumetric data, along with demographic information, COVID-19-related variables such as vaccines, anosmia, age, PCR, variants, and risk of hospitalization, and cognitive assessments, were utilized for clustering techniques employing Ward’s method with half-square Euclidean distance, allowing for the categorization of subjects within each domain of knowledge. Subsequently, clusters were co-clustered across domains, and the coincidence rate between domains (co-clustering) was assessed using hypergeometric statistics (Fisher’s Exact Test) and regular regressions accompanied by T-tests. Four cognitive clusters were identified, assessing different aspects of cognitive function, including cognitive processing speed and executive functioning, visual and semantic memory, comprehensive attention, memory, and executive function, and semantic memory retrieval and category-specific knowledge. Additionally, three volumetric clusters indicated reduced brain volumes in regions related to memory, emotion regulation, and sensory processing, including the angular gyrus, corpus callosum, amygdala, hippocampus, thalamus,

and fornix. Significant correlations observed between imaging and cognitive data suggest an expected link between clusters, with anosmia analysis possibly indicating olfactory dysfunction with both imaging and cognitive clusters. Furthermore, analysis revealed significant associations between cognitive data and COVID-19 variables and COVID-19 and imaging data, implying potential relationships between different domains, as anticipated. However, the expected decline in cognitive functions is significantly influenced by the presence or risk conditions associated with COVD-19 variables, highlighting the importance of considering these factors. Our findings suggest that all SAR-CoV-2 variants, including those up to the omicron wave, are equally likely to contribute to cognitive impairment in older adults, with acute illness severity playing a modulatory role.

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Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

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Program #/Poster #: PSTR307.19/W6

Topic: H.12. Aging and Development

Support: NSF Career Grant
NIH UG3
University of Notre Dame Internal Science of Wellness Grant

Title: Neural and Behavioral Differences in Working Memory Updating Between Younger and Older Adults: An EEG Decoding Study

Authors: *J. FRAGETTA[1], C. XU[1], C.-M. CHAO[2], M. BENITEZ[1], Z. XIE[3], D. HENRECKSON[4], N. S. ROSE[5];
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Abstract: Working memory (WM) deficits play a significant role in age-related cognitive decline, but the underlying mechanisms remain unclear. The present study explores behavioral (n-back performance) and neural (EEG decoding) differences in WM updating between healthy younger adults (YA) and healthy older adults (OA). Specifically, this study investigates whether YA and OA rely on active or passive retention mechanisms during a WM updating task, and how these mechanisms relate to behavioral performance. To address these questions two age groups, (YA N=48, M_{age}=19.9 years; OA N=47,M_{age}=70.68 years) performed 1-back and 2-back WM tasks during EEG recording. Preliminary analyses of the 1-back task revealed significant

decoding of stimulus representations for both YA and OA during both the stimulus presentation and the early delay period, but not the late delay period. This lack of sustained decoding throughout the delay period is consistent with hypotheses on passive, “activity-silent” short-term retention mechanisms. Additionally, OA showed higher, more sustained delay-period decoding, consistent with a compensatory recruitment account of age differences in WM. Preliminary analyses of the 2-back task revealed further evidence for passive retention of stimulus representations, as both YA and OA failed to show significant decoding throughout the delay period of the task. In contrast to the 1-back task, there were no significant differences in decoding between age groups for the 2-back task. Interestingly, both age groups showed lingering decoding of the 1-back item, as well as “activity-silent”retention of the 2-back item, when participants should have been actively processing the 2-back item in preparation for the recognition probe. This suggests that participants were not appropriately switching attention to actively prioritize maintenance of the relevant item over the potentially interfering item during the task. Surprisingly, both younger and older adults appear to rely on passive retention and stimulus-driven recognition processes, in place of active maintenance and updating processes, to perform the n-back task. This study provides insight into age-related differences in WM updating, indicating potential compensatory mechanisms in older adults due to less efficient reliance on passive short-term retention and recognition memory processes.

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Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

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Program #/Poster #: PSTR307.20/W7

Topic: H.12. Aging and Development

Support: NIH Grant R01AG076082

Title: Healthy age alters source localized resting state alpha and beta power

Authors: *J. PARK[1], R. HO[1], W.-E. WANG[2], S. COOMBES[1];
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Abstract: Advanced aging is associated with robust changes in neural activity. In addition to the well-established age-related slowing of the peak alpha frequency, there is a growing body of evidence showing that older age is also associated with changes in alpha power and beta power. Despite the important progress that has been made, the interacting effects of age, brain region, and frequency band have not been directly tested in source space while controlling for aperiodic components. In the current study we address these limitations. We recruited 54 healthy young

and elderly adults and measured neural oscillations using a high-density EEG system during a resting-state with eyes closed. After preprocessing the EEG data and controlling for aperiodic components, we computed alpha and beta power in both sensor and source space. Permutation two-way ANOVAs between frequency band and age group were performed across all electrodes and across all dipoles. Our findings revealed significant interactions in sensorimotor, parietal, and occipital regions. The pattern driving the interaction varied across regions, with older age associated with a progressive decrease in alpha power and a progressive increase in beta power from posterior (parietal) to anterior (sensorimotor) regions. Our findings demonstrate that age-related changes in neural oscillations vary as a function of brain region and frequency band, and have implications for better understanding age-related changes in the neural circuits that subserve cognitive function and motor performance.

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Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

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Topic: H.12. Aging and Development

Support: CIHR-PJT162292
CIHR-PJT162274
CIHR-PJT173336

Title: Exploring the relationship between medial temporal lobe structure and visual discrimination in older adults with varying cognitive abilities

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Abstract: Tau pathology accumulates in the perirhinal cortex (PRC) of the medial temporal lobe (MTL) during the earliest stages of Alzheimer's disease (AD), often appearing decades before clinical diagnosis. It has been demonstrated in previous research that the PRC plays a critical role in the visual discrimination of novel objects as well as faces. To detect subtle cognitive impairment associated with volume changes in the PRC, we assessed visual discrimination of computer-generated novel objects (“greebles”) and faces in 70 community-dwelling older adults (age range = 60-87 years; mean age = 73.0; mean education = 16.7 years; range = 9-27; 39 female) who varied in cognitive status as determined by the Montreal Cognitive Assessment (MoCA). Furthermore, we examined the relationship between visual discrimination performance

and MTL subregion volumes, including the PRC, as established by high-resolution structural T2-weighted MRI scans. In order to determine MTL subregion volumes, we used the Automatic Segmentation of Hippocampal Subfields (ASHS) and an atlas that was trained based on the Olsen-Amaral-Palombo (OAP) manual segmentation protocol. Detailed quality control was carried out on the automated segmentations and manual corrections were made in order to obtain the final volumes. Results indicated that age was significantly negatively associated with visual discrimination performance on the greeble condition (R =-0.34) and face condition (R=-0.39), MoCA score (R=-0.33), and PRC volume (R=-0.26). As expected, we observed significant positive correlations between MoCA scores and PRC volumes (R = 0.29). We observed significant positive correlations between PRC volumes and visual discrimination for faces (R = 0.22) but not for greebles (R = 0.01). The significant relationship between PRC volumes and MoCA scores, as well as the relationship between MoCA scores and visual discrimination task performance, supports the potential use of visual discrimination tasks to detect subtle cognitive changes in mild cognitive impairment and AD.

Disclosures: L. Jiang: None. **X. Zhang:** None. **J.D. Ryan:** None. **M.D. Barense:** None. **R.K. Olsen:** None.

Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR307.22/W9

Topic: H.12. Aging and Development

Support: CIHR Grant

Title: Magnetic Resonance Imaging (MRI)-derived measures of intracortical myelin and cortical thickness correlated with measures of cognitive performance in healthy humans

Authors: *M. ELKHAYAT[1], S. HEO[1], M. B. KOVACHEFF[1], C. D. ROWLEY[2], N. GAZOR[3], R. MANSUR[6], R. MCINTYRE[7], R. MILEV[8], L. MINUZZI[4], V. H. TAYLOR[9], R. UHER[10], G. VAZQUEZ[11], L. YATHAM[12], B. FREY[5], N. A. BOCK[1];
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Abstract: Background: A key question in neuroscience is the extent to which brain composition and structure contribute to cognitive performance. Hence, we explored the influence of two features of the cerebral cortex on neuropsychological test performance: its myelination and cortical thickness. Myelin found within the cerebral cortex (intracortical myelin) is proposed to facilitate the synchrony of neural networks within the brain, contributing to cognition (Haroutunian et al., 2014). There is limited information on whether a thicker cortex is linked to better cognitive performance, however, cortical thinning has been implicated in cognitive decline. We investigated associations between neuropsychological test scores and cortical features measured with 3D whole-brain magnetic resonance imaging (MRI). These were: the longitudinal relaxation rate (R_1) (a proxy measure of intracortical myelin amounts), and cortical thickness. **Methods**: MRIs were obtained from 78 healthy individuals using 3T scanners across 5 sites, and corrected for inter-site variation. An inversion-recovery gradient-echo T_1-weighted anatomical image was collected at 1 mm isotropic resolution for registration and segmentation. R_1 maps were calculated from two inversion-recovery gradient echo images made at different inversion times. FSL's FLIRT tool was used to register the anatomical image and R_1 maps into the same space. Regions of interest (ROIs) were defined from the anatomical images using Freesurfer, Connectome Workbench, and the MMP atlas (Glasser et al., 2016). Participants were tested on: the Wechsler Abbreviated Scale of Intelligence-II (WASI-II) Vocabulary and Matrix Reasoning subtests, the Weschler Test of Adult Reading (WTAR), the Stroop Adult Colour and Word, Trail-Making Test (TMT) A and B, the Brief Assessment of Cognitive in Schizophrenia (BACS) Symbol Coding, the Weschler Memory Scale-III (WMS-III) Spatial Span, Letter-Number Span, and Category Fluency tasks from the MATRICS Consensus Cognitive Battery. Pearson's correlation coefficients were calculated between scores on each task and each of R_1 and cortical thickness. These were plotted across ROIs in surface space. **Results**: R_1 generally showed weak positive correlations (r^2 = 0-0.17) in most cortical regions for most tests administered, suggesting that intracortical myelin might support some aspects of cognition. For BACS Symbol Coding, Letter-Number Span, Category Fluency, Matrix Reasoning and Vocabulary tasks, the directionality of the correlations varied regionally. Cortical thickness showed more regional variation in directionality, and more negative correlations with test scores (r^2 = 0-0.12).

Disclosures: M. Elkhayat: None. **S. Heo:** None. **M.B. Kovacheff:** None. **C.D. Rowley:** None. **N. Gazor:** None. **R. Mansur:** None. **R. McIntyre:** None. **R. Milev:** None. **L. Minuzzi:** None. **V.H. Taylor:** None. **R. Uher:** None. **G. Vazquez:** None. **L. Yatham:** None. **B. Frey:** None. **N.A. Bock:** None.

Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

Location: MCP Hall A

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Program #/Poster #: PSTR307.23/W10

Topic: H.12. Aging and Development

Support: NIH Grant R01AG047972
NIH Grant R01AG029523
NIH Grant R01NS106711
NIH Grant R01NS106702

Title: Age-related carotid and vertebral blood flow changes underlie processing speed decline

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Abstract: Neurocognitive aging studies aim to identify the neural mechanisms underlying cognitive decline in aging. Previous studies, including some from our lab, have demonstrated that cerebral blood flow (CBF) is integral for effective processing speed (PS) in both younger and older adults. As older adults decline in CBF, there is an associated downturn in PS. CBF can be estimated as the summation of flow through the left and right internal carotid arteries (ICAs) and the left and right vertebral arteries (VAs), and our lab has previously shown that age-related decreases in total flux through the four arteries has similarly been associated with PS decline. However, it has yet to be investigated (1) whether variance in some arteries contributes more to PS variance than others, (2) whether there are hemispheric distinctions in these contributions, and (3) whether distinctive contributions are different between age groups. In this study, 74 younger adults (18-34 yr, M_{age} = 23.33 yr) and 65 older adults (50-81 yr, M_{age} = 61.68 yr) completed an outside-scanner neurocognitive battery including tasks of PS (i.e., the Digit-Symbol Substitution Task, the Number Comparison Task, and the Box Completion Task). Additionally, while at rest, participants completed four 3T phase contrast (PC) MRI scans. Each scan was localized at the level of the neck to left or right VAs and left or right ICAs (single slice, flip angle = 15°, TR = 20 ms, TE = 6.9 ms, voxel size = 0.45 × 0.45 × 5 mm, maximum velocity encoding (V_{ENC}) = 80 cm/s, 4 signal averages, scan duration = 0.5 min). PC MRI utilizes phase information to encode the flux, cross-sectional area, and velocity of the venous blood. For both age groups, blood flow flux in the left hemisphere (IA + VA) was associated with PS while no arterial characteristics were associated with PS in the right hemisphere. In particular, left VA was associated with PS in younger but not older adults, while left ICA was associated with PS in older but not younger adults. Results suggest the hypothesis of a plausible vascular basis for age-related PS declines that underlie cognitive aging.

Disclosures: G.H. Sherard: None. **D.H. Abdelkarim:** None. **B. Rypma:** None.

Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR307.24/W11

Topic: H.12. Aging and Development

Support: RF1AG067429
R01AG060977

Title: Age associated changes in brain phospholipids are mitigated by vagus nerve stimulation

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Abstract: Phospholipids play a crucial role in brain functions, including neuronal communication and synaptic plasticity. Brain phospholipid composition changes in aging and neurodegenerative diseases and has been implicated in cognitive decline. The goals of this study were to characterize exactly how aging alters the spatially-resolved brain lipidome and to determine the degree to which such age changes are modifiable with chronic vagus nerve stimulation (VNS) in different brain regions. Brains from young (4 months) and aged (24 months) Fischer 344 x Brown Norway F1 hybrid rats were processed for spatial lipidomics by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-IMS) imaging. Relative to young, aged rats had increased long chain highly saturated phospholipid species which are typically associated with inflammation and oxidative stress. In contrast, aged rats had reduced long chain unsaturated phospholipid species which are generally neuroprotective. We next determined the extent to which these age-related changes were modifiable by VNS. A separate cohort of aged (24 months) Fischer 344 x Brown Norway F1 hybrid rats were surgically implanted with a 4-channel VNS cuff electrode around the left vagus nerve. After recovery, rats received 30 days of VNS using stimulation parameters (100 stimulus trains over 1 hr; 30Hz, 120 µS pulse width, 700 µA, 0.8 s train duration) previously shown to enhance cortical plasticity. MALDI-IMS imaging compared the lipidome between VNS and aged rats receiving sham stimulation. Overall, VNS mitigated many of the age-associated changes in phospholipid composition, indicating both that lipid composition is highly modifiable even in the aged brain and that VNS has potential to rewire age-related changes in brain lipid metabolism. Additional spatial analysis is ongoing to characterize regionally specific effects of aging and VNS on brain lipidome.

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Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR307.25/W12

Topic: H.12. Aging and Development

Support: Tata Education Trusts , India

Title: Understanding the Link between Insulin Resistance and Cognition: Lessons from a South Indian Cohort

Authors: *T. G. ISSAC[1], L. DIWAKAR[2];
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Abstract: Understanding the Link between Insulin Resistance and Cognition: A cross-sectional study conducted in an Urban, South Indian cohort-Authors Thomas Gregor Issac, Latha Diwakar; Centre for Brain Research, Indian Institute of Science, Bengaluru, India**Disclosure**Thomas Gregor Issac: None, Latha Diwakar: None**Abstract**Recent research suggests that metabolic dysregulation caused by insulin resistance (IR) can have a negative impact on cognition. Therefore, the objective of this study is to explore the role of IR as an independent metabolic risk for decreased cognitive performance. The study included 1072 non-demented participants aged 45 years and above were recruited from the Tata Longitudinal Study of Aging (TLSA). Fasting insulin and blood glucose levels were collected during the baseline visit. Homeostatic Model Assessment of IR (HOMA-IR) formula was used to calculate IR. Cognition was assessed using Addenbrooke's Cognitive Examination III (ACE III) and COGNITO neuropsychological test battery. Generalized Linear Model (GLM) was performed to find the relationship between the IR category and cognition. The brain imaging was conducted using a 3 Tesla MRI system. The cortical volumes were acquired using Freesurfer software (v7.2.0)[12]. Further, GLM analysis was performed for MRI variables. The estimated general prevalence of IR among study participants is 56.3%. After adjusting for sociodemographic characteristics, affective disorders, metabolic risk factors and APOE4, GLM showed that participants with IR scored less in ACE attention (β=-0.396, $p<0.05$) and auditory attention (β = -0.392, $p<0.05$) tasks in comparison with healthy participants. GLM analysis for MRI indicated that participants with IR had a decrease in the left hemisphere brain volumes like amygdala ($p=0.0012$), inferior temporal lobe ($p=0.002$), lateral orbitofrontal cortex ($p=0.005$), superior temporal insula ($p=0.017$), middle temporal lobe ($p=0.002$), entorhinal ($p=0.049$), and right hemisphere brain volumes like precuneus ($p=0.025$), and insula ($p=0.002$). Our study findings conclude that participants with IR experienced a significantly poorer cognitive performance related to auditory attention. Furthermore, the study also revealed that IR is associated with decreased brain volumes in specific regions. With the changes in lifestyle and dietary pattern, there is an increased risk of developing metabolic syndrome that can further cause cognitive deficits. Therefore, if the metabolic risk factors are addressed at the earliest, progression to cognitive decline can be prevented or delayed.

Disclosures: T.G. Issac: None. **L. Diwakar:** None.

Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR307.26/W13

Topic: H.12. Aging and Development

Support: NIA Grant R56AG060052

Title: Age-related differences and practice effects on fMRI BOLD signals during multitasking.

Authors: M. ANDREO[1], P. SKOLASINSKA[2], ***C. BASAK**[3];
[1]Univ. of Texas at Dallas, Richardson, TX; [2]Behavioral and Brain Sci., The Univ. of Texas at Dallas, Addison, TX; [3]Univ. of Texas at Dallas, Dallas, TX

Abstract: In this study, we evaluated age-related differences in fMRI activations in cognitive control, sensory-motor processing, and practice effects during multi-tasking. This fMRI task is a slightly modified version of our previously developed embedded-cue, hybrid-block design, task-switch fMRI paradigm (Basak et al., 2018; Nashiro et al., 2018), but using a very fast TR (~500 ms). The task has two single task blocks (one for odd/even judgment and one for higher than/lower than 5 judgement) and three dual mixed-task blocks, where the two single tasks are randomly mixed. Our sample consisted of 24 younger adults (18-30 years) and 60 cognitively healthy older (65 years or more) adults. The age-contrasts of the block design, whole-brain analyses showed significant age-differences only in dual, but not single, tasks. Younger adults, compared to older, recruited lateralized prefrontal brain regions that serve cognitive control processes (left and right middle frontal gyrus; left frontal pole). However, older adults showed overactivations in motor processing areas of the brain, viz. left and right cerebellum. We further investigated the effects of practice on cognitive control aspects of task-switching by analyzing the three dual task blocks separately. For the first dual block, younger adults overactivated left frontal pole, right and left middle frontal gyrus, and right occipital gyrus, whereas older adults overrecruited cerebellum. In the later dual blocks, younger adults showed consistent overactivations of the lateral prefrontal cortices, but older adults showed overactivations not only in the cerebellum but also in the precuneus. We therefore conclude that younger adults, in contrast to old, show greater neural efficiency in sensory processing of visual information with practice, but continue to engage the lateral prefrontal cortex needed for cognitive control during task-switching. However, older adults, compared to the young, show greater and consistent reliance on motor processing brain regions as well as increased over recruitment of precuneus that may be signs of attempted compensation in aging brains. These findings suggest that there are age variations in compensatory recruitment, neural efficiency, and consistent over-recruitment of differential networks with task repetition, which has been overlooked in past studies of cognitive control.

Disclosures: M. Andreo: None. **P. Skolasinska:** None. **C. Basak:** None.

Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR307.27/W14

Topic: H.12. Aging and Development

Title: Age-related changes in Higuchi's fractal dimension is anti-correlated with 1/f slope and oscillatory power in human EEG

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Abstract: Brain signals, which reflect cognitive and motor functions, often exhibit complex, non-linear dynamics. Researchers utilize methods like Higuchi's fractal dimension (HFD) to probe these nonlinear dynamics. However, there are discrepancies in the literature regarding how HFD changes with healthy aging, potentially stemming from differences in computational techniques and frequency ranges employed across studies. Further, previous studies have shown that oscillatory power in alpha and gamma bands reduces with healthy aging, as well as the slope of the power spectral density (PSD) becomes shallower. These changes could also influence HFD, but the relationship between HFD, oscillatory power and PSD slopes is unclear. To resolve this disparity, we studied frequency-dependent changes of HFD with age and its correlations with the known age-related changes of PSD oscillations and slope. We analyzed 64-channel electroencephalogram (EEG) data obtained from a cohort of 217 elderly individuals aged 50-88 years, both under baseline conditions with eyes open and during the presentation of an achromatic grating stimulus that induced strong gamma oscillations. In the baseline eyes open state, with age, HFD increased at frequencies upto 150 Hz and showed opposite trend at higher frequencies. Interestingly, this change in HFD was opposite to the age-related change in the PSD slope. Furthermore, the stimulus-induced change in HFD exhibited an inverse relationship with alterations in oscillatory power within the alpha (8-12 Hz) and gamma (25 - 70 Hz) frequency ranges, indicating that the presence of oscillations reduced HFD. Therefore, HFD effectively captures changes in the oscillatory signatures such as alpha/gamma oscillations as well as the 1/f slope of the PSD, both recognized markers of excitation-inhibition (E-I) balance in neural circuits. Consequently, HFD holds promise as a potential biomarker for evaluating E-I balance alterations associated with healthy aging as well as various neurological disorders, offering invaluable insights into brain function across health and disease states.

Disclosures: S. Aggarwal: None. **S. Ray:** None.

Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

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Topic: H.12. Aging and Development

Support: NIH Grant 1K99AG078903-01A1

Title: Serotonin 2C receptor signaling regulates learning and reverses memory loss in model of Alzheimer's disease

Authors: *H. LIU[1], Y. HE[2], H. LIU[1], N. YIN[1], S. FAROOQI[3], Y. XU[1];
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Abstract: Memory loss is a hallmark of Alzheimer's disease (AD), which is increasing in prevalence due to aging and obesity. Serotoninergic neurons are involved in the regulation of memory and body weight in rodents. Here, we find that young adults with severe obesity carrying loss-of-function mutations affecting the Serotonin 2C receptor (5-HT_{2C}R) have significantly impaired memory. Furthermore, a mouse model of a human 5-HT_{2C}R mutation has impaired learning and memory. We demonstrate that midbrain serotonin neurons synapse with neurons expressing 5-HT_{2C}Rs in the ventral CA1 (vCA1) region of the hippocampus. Disruption of serotonin synthesis and of 5-HT_{2C}Rs on vCA1 neurons, markedly reduces long-term potentiation, a component of synaptic plasticity, essential for learning and memory. Moreover, a selective 5-HT_{2C}R agonist, lorcaserin, improves synaptic plasticity and corrects memory loss in an AD mouse model. We conclude that 5-HT_{2C}R signaling regulates cognitive function and body weight, findings which have implications for the treatment of obesity and AD.

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Poster

PSTR307: Natural Brain Aging: Neural Mechanisms

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR307.29/W16

Topic: H.12. Aging and Development

Title: The Role of the Microbiome on Cognitive Function in the Aged Dog

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Abstract: There are several biomarker and brain changes associated with canine aging that parallel neuropathological changes seen in human Alzheimer's Disease (AD); the canine microbiome is no exception. Many inflammatory diseases are linked to the microbiome, often with changes in microbial communities preceding the onset of such diseases. This study aimed to explore the relationship between cognitive performance of aged dogs and both gut and salivary microbiota. The cognitive domain specifically examined in this study was short-term visuospatial working memory assessed with a variable delay non-matching to position (varDNMP) task with 20 and 90-second delays. Sixteen dogs, aged 6 to 15 years, were tested on the varDNMP task. Subjects whose accuracy was >60% were classified as cognitively competent, whereas subjects with accuracy of 60% or less were classified as cognitively impaired. Fecal and saliva samples were collected from all subjects, prepped and fixed in a Zymo DNA/RNA shield, and frozen to preserve the bacterial loads. DNA was extracted from the samples using the Qiagen MagAttract PowerSoil DNA KF Kit, optimized for automated extractions on the ThermoFisher KingFisher robot. Fecal samples were analysed using the shallow shotgun sequencing method, which involved sequencing on the Illumina Next Seq (2X 150 BP), targeting an average of 0.5-2 million reads per sample. Saliva samples were analysed using the shotgun metagenomic sequencing method, which involved sequencing on the Illumina Next Seq High Output (2X 150 BP), targeting an average of 7 million reads per sample. We found that Firmicutes and Bacteroidetes were the most abundant taxa in all subjects regardless of cognitive status. Previous canine studies identified fewer Actinobacteria in subjects with increased performance on memory tasks, and in humans, Actinobacteria is increased with AD. Both Actinobacteria and Proteobacteria taxa were found in less abundance overall in our samples, however analyses of these taxa exhibited high individual variation. The relative abundance of both Actinobacteria and Proteobacteria in this study was inversely correlated with cognitive status. Tracking cognition and microbiome composition over time may be a valuable tool in identifying trends that will help further establish the relationship between cognitive performance and gut and salivary microbial communities. Human gut-brain axis studies suggest that throughout aging, Firmicutes and Bifidobacteria decrease, but Proteobacteria increases. A larger sample size across a wider age range will help verify if this is a consistent finding in dogs and how such changes relate to cognitive function.

Disclosures: C. de Rivera: A. Employment/Salary (full or part-time):; Transpharmation Canada Ltd.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); InterVivo Solutions Inc. **A. Cyr:** A. Employment/Salary (full or part-time):; Transpharmation Canada Ltd. **R. Percival:** A. Employment/Salary (full or part-time):; Transpharmation Canada Ltd. **J.A. Araujo:** A. Employment/Salary (full or part-time):; Transpharmation Canada Ltd.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); InterVivo Solutions Inc..

Poster

PSTR308: Genomic and Transcriptomic Techniques II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR308.01/W17

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Transcriptomic Innovations in the Evolution of Mammalian Motor Cortex

Authors: *M. E. WIRTHLIN[1], J. PUCKETT[1], N. JOHANSEN[1], M. SCHMITZ[1], A. A. DE SOUSA[1], I. KAPEN[1], N. L. JORSTAD[1,2], J. GOLDY[1], A. C. HALLEY[3], G. BALMUS[4], E. G. BARRETT[5], D. C. BOLSER[6], K. L. DREW[7], D. FITZPATRICK[8], S. M. FREEMAN[9], J. H. KAAS[10], L. A. KRUBITZER[3], J. J. PADBERG[11], C. SHERWOOD[12], G. K. WILKERSON[13], B. P. LEVI[1], Y. KOJIMA[14], G. D. HORWITZ[15], J. T. TING[1], K. SMITH[1], R. D. HODGE[1], E. LEIN[1], T. BAKKEN[1];
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Abstract: Mammalian evolution has produced a broad diversity of behavioral phenotypes, from species that dig and climb to those that sing and speak. Understanding the genomic basis of these traits depends on deepening our understanding of the cell types and transcriptomic signatures of motor cortex (M1), the primary cortical output for behavior. To address this need, we conducted 10X single nucleus RNA-seq of mammalian motor cortex (M1) across 25 species, encompassing 13 primates and 4 rodents, as well as diverse taxa including scandentians (northern treeshrew), lagomorphs (European rabbit), carnivorans (cat, coyote, and ferret), artiodactyls (pig), cingulates (nine-banded armadillo), and marsupials (short-tailed opossum). Analyzing over 1.5 million sequenced cells, we generated a consensus M1 cell-type taxonomy, providing a foundation for investigating the genomic evolution of mammalian brains. We identify shifts in the proportions of M1 cell types that vary across taxa, transcriptional specializations associated with specific lineages and phenotypes, and trace the evolutionary origins of human M1 innovations. We anticipate that this multi-species, multi-omic dataset will provide a valuable resource for further investigations into the molecular basis of trait evolution.

Disclosures: M.E. Wirthlin: None. **J. Puckett:** None. **N. Johansen:** None. **M. Schmitz:** None. **A.A. de Sousa:** None. **I. Kapen:** None. **N.L. Jorstad:** A. Employment/Salary (full or

part-time):; Genentech, Inc., South San Francisco, CA, USA. **J. Goldy:** None. **A.C. Halley:** None. **G. Balmus:** None. **E.G. Barrett:** None. **D.C. Bolser:** None. **K.L. Drew:** None. **D. Fitzpatrick:** None. **S.M. Freeman:** None. **J.H. Kaas:** None. **L.A. Krubitzer:** None. **J.J. Padberg:** None. **C. Sherwood:** None. **G.K. Wilkerson:** None. **B.P. Levi:** None. **Y. Kojima:** None. **G.D. Horwitz:** None. **J.T. Ting:** None. **K. Smith:** None. **R.D. Hodge:** None. **E. Lein:** None. **T. Bakken:** None.

Poster

PSTR308: Genomic and Transcriptomic Techniques II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR308.02/W18

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH BRAIN CONNECTS U01NS132267

Title: Multimodal characterization of spatial variation in neuronal types in the mouse basal ganglia

Authors: *A. BUDZILLO[1], M. MALLORY[3], C. LEE[5], R. DALLEY[1], R. MANN[1], N. JOHANSEN[2], J. A. MILLER[2], B. TASIC[6], H. ZENG[1], B. E. KALMBACH[7], Z. YAO[1], T. JARSKY[8], S. A. SORENSEN[3], B. R. LEE[4], N. W. GOUWENS[9];
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Abstract: Recently generated taxonomies of transcriptomically defined cell types (t-types) have revealed fine-grained distinctions between neurons in structures throughout the brain. Spatial transcriptomic experiments demonstrate that many of these t-types are found in specific locations within brain regions, and continuous transcriptomic variation across related t-types often follows spatial gradients. In the basal ganglia, topographic projections into and out of the striatum enable the processing of information from different sensory and motor modalities in parallel; in addition, there are approximately fifty t-types of medium spiny neurons (MSNs) in the mouse striatum that exhibit specific spatial distribution patterns. However, it remains unclear how spatial and transcriptomic variation relate to each other and to other intrinsic properties of the neurons, such as their electrophysiological characteristics and morphologies. To characterize these relationships, we performed Patch-seq experiments on these MSNs as well as other cell types of the basal ganglia. We measured their electrophysiological intrinsic properties by a standard whole-cell patch-clamp protocol, filled the cells with biocytin for morphological reconstruction, and recovered the cytosol and nucleus for single-cell transcriptomic profiling and mapping to t-types. We also identified the locations of recorded cells in the Allen Common Coordinate Framework to relate the measured properties to spatial location. We find that major

gradients of transcriptomic expression, electrophysiological properties (such as the shape of the action potential), and morphological properties (such as the extent of the dendritic arbor) co-vary systematically across MSNs in the striatum. Cholinergic interneurons of the striatum also exhibit coordinated variation in transcriptomic expression and electrophysiological properties along similar spatial patterns. We also characterize differences across types in transcriptomic expression and other properties that do not follow the same spatial gradients. Identification of the aspects of cell type variation that do and do not correspond with spatial distributions improves our understanding and description of the cell type landscape and its relationship to topographically organized circuits across the brain.

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Poster

PSTR308: Genomic and Transcriptomic Techniques II

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Program #/Poster #: PSTR308.03/W19

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH grant UM1MH130981

Title: Cross-species and multi-modal atlas of the basal ganglia

Authors: *N. JOHANSEN[1], Y. FU[2], M. WIRTHLIN[3], A. GARCIA[4], A. YANNY[2], S. BARLOW[5], D. BERTAGNOLLI[2], A. CHAKKA[2], R. CHAKRABARTY[2], S.-L. DING[1], J. GOLDY[6], N. GUILFORD[7], K. JAMES[2], D. L. JONES[8], M. LEYTZE[2], C. RIMORIN[9], S. C. SEEMAN[1], M. TIEU[1], B. P. LEVI[10], J. T. TING[10], K. SMITH[2], R. D. HODGE[11], E. LEIN[10], T. BAKKEN[1];
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Abstract: The basal ganglia plays an essential role in associational learning, planning and coordination of movement and whose dysfunction results in a myriad of neurological conditions. At the Allen Institute, we are building atlases of the human and non-human primate brain and have deeply characterized diverse cell types of the basal ganglia across human, macaque and

marmoset. Using single nucleus multi-omic profiling, we jointly profiled the transcriptomic and epigenetic signals from over a million cells from the primate basal ganglia. Leveraging these multi-omic profiles, we identified markers and gene regulatory elements that are highly cell type specific. We aligned primate and rodent basal ganglia taxonomies to identify homologous cell types across species based on conserved gene expression, open chromatin, spatial localization from MERSCOPE experiments. These homologous types include conserved regulatory DNA sequences that determine cell-type specific molecular properties underlying their unique cellular biology. Characterizing this DNA sequence logic will help drive development of cell type-specific viral genetic tools for functional testing and therapeutic applications.

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Poster

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Program #/Poster #: PSTR308.04/W20

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH UM1MH130981

Title: Morpho-electric properties of transcriptomically-defined neuronal types in the primate basal ganglia

Authors: *X.-P. LIU[1], N. JOHANSEN[2], R. DALLEY[1], J. A. MILLER[2], M. WIRTHLIN[3], T. BAKKEN[1], S. A. SORENSEN[4], T. JARSKY[1], J. T. TING[2], H. ZENG[1], E. LEIN[2], B. R. LEE[5], B. E. KALMBACH[2];
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Abstract: The basal ganglia are a set of subcortical nuclei critical for motor selection and reinforcement learning, and with relevance to a range of disruptive human disorders, such as Parkinson’s disease, Huntington’s disease, and drug addiction. Despite the ancient nature of this structure, studies have revealed differences between rodents and primates, suggesting exaptation of this ancient design. Recent transcriptomic analyses in primates have pointed to considerable diversity of medium spiny neurons (MSNs) in the striatum (He et al., 2021), and primate-specific interneuron types (Krienen et al., 2020). While there exists an extensive literature on neuronal

properties in the rodent basal ganglia, relatively less is known about the electrophysiological and morphological properties of primate basal ganglia neurons. In conjunction with efforts to create high-resolution basal ganglia taxonomies in non-human primates as well as humans and mice, we are can now develop a rich characterization these transcriptomically-defined neuronal types. We collected patch-seq data in ex vivo brain slices from Macaca nemestrina and Macaca mulatta obtained from the WA National Primate Research Center tissue distribution program to test for cell type specific differences in intrinsic membrane and morphological properties. Using enhancer based adeno-associated viral vectors, we are able to target rare cell types, such as cholinergic interneurons, which comprise only ~1% of the striatal neuronal population. The morpho-electric properties of striatal interneuron subclasses were highly diverse, differing in electrophysiological properties such as spike width, input resistance, afterhyperpolarization, and I_h-related sag. The primate-specific TAC3 interneuron class had unique properties with some similarities to fast spiking interneurons. In contrast, MSNs showed graded differences across compartments and along anatomical gradients related to the topographical projections from and to motor, sensory, associational, and limbic areas. We expect the use of a common circuitry for control of these different functional systems to require adaptation of the common elements. Intriguingly, D1/D2-hybrid MSNs and a novel group of MSNs not found in mouse both had relatively distinct electrophysiological properties from other MSN types. These results will contribute to a broad understanding of neurons in the primate basal ganglia and provide more accurate data for future modeling explorations. Finally, comparisons with the rodent basal ganglia will provide essential considerations for the development of therapeutics as well as for the understanding of primate evolution.

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Poster

PSTR308: Genomic and Transcriptomic Techniques II

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Program #/Poster #: PSTR308.05/W21

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant UM1MH130981

Title: Progress Towards a Spatial Transcriptomic Atlas of the Marmoset Basal Ganglia

Authors: M. A. TURNER[1], *B. LONG[4], S. C. SEEMAN[2], M. HEWITT[2], K. LEVANDOWSKI[7], A. RUIZ[8], N. VALERA CUEVAS[9], N. MARTIN[3], J. NAGRA[2], P. OLSEN[2], M. VANNESS[9], J. CAMPOS[8], N. JOHANSEN[3], T. BAKKEN[5], D. MCMILLEN[8], J. WATERS[4], F. M. KRIENEN[10], R. D. HODGE[5], H. ZENG[2], E. LEIN[6];

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Abstract: The basal ganglia are a set of subcortical structures critical for motor control, particularly in the context of action selection, motor learning and emotional state, whose coarse functional organization is well-described in the literature. Molecular studies have further revealed the extensive cellular heterogeneity within the basal ganglia, with recent single-nucleus RNA sequencing of the striatum emphasizing the extensive transcriptomic diversity present in these structures. However, it has been difficult to relate this transcriptomic diversity to the existing spatial parcellations - anatomical, neurochemical , and functional- of the basal ganglia. Here, we present our progress towards bridging this gap by building a cellular-resolution, spatial atlas of transcriptomic diversity across basal ganglia structures in the marmoset as part of the NIH Brain Initiative Cell Atlas Network (BICAN). We describe how different elements of our data generation and processing pipeline —the spatial transcriptomic platform (MERSCOPE or Xenium), cell segmentation algorithm, and the method used to map spatial transcriptomic cells to single-nucleus RNAseq reference taxonomies—can impact spatial measurements of cell type distributions. In addition to detailing proportions and distributions of RNAseq-defined cell types within and across basal ganglia structures, we also focus on data-driven parcellation to identify and characterize structures within the basal ganglia based on spatial gene expression data alone. Furthermore, the simultaneous generation of complementary spatial transcriptomic atlases in the macaque and human, as part of BICAN, has enabled us to explore shared and divergent cell type features across marmoset, macaque, and human. This work will provide a detailed framework for a wide range of investigations into basal ganglia circuit function with unprecedented molecular resolution and the potential for translation of circuit and functional features across species.

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Poster

PSTR308: Genomic and Transcriptomic Techniques II

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Program #/Poster #: PSTR308.06/W22

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant UM1MH130981

Title: Mapping the primate basal ganglia using spatial transcriptomics

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Abstract: The primate brain is highly complex and composed of many cell types organized into distinct regions. Although single-cell RNA-Seq studies have greatly enhanced our understanding of cellular diversity in the brain, the exact spatial distribution of cell types within the brain has been difficult to determine. Using MERFISH, a method that enables spatial profiling of hundreds of genes at subcellular resolution, we assayed gene expression in the macaque basal ganglia. Our dataset consisted of approximately 100 coronal sections spaced 1 mm apart, sampling the caudate, putamen, nucleus accumbens, globus pallidus, and substantia nigra. To process and analyze this large dataset, we created a pipeline consisting of 1) cell segmentation, 2) quality control, and 3) assigning cell-type identities. For cell segmentation, we fine-tuned a CellPose model on our data, using DAPI as a nuclear stain and total mRNA density to approximate the cytoplasm. We successfully applied this model in 3D and across species, which captured more realistic cell shapes and substantially improved accuracy compared to 2D methods. We performed several quality control steps to filter low-quality cells and doublets. We then mapped the remaining high-quality cells onto a reference single-nuclei RNA-Seq taxonomy to identify their transcriptomic cell type. The result is a high-quality spatial atlas of cells and their transcriptomic identities within the macaque basal ganglia. Our findings recapitulate several known patterns of gene expression and cellular organization in the basal ganglia and also introduce new ones. In the striatum, we observe clear boundaries between medium spiny neuron (MSN) subclasses in matrix and striosome, as expected. At the cluster level, we observe additional spatial patterns that suggest more fine-grained organization of cell types within the basal ganglia. For example, D1 and D2 striosome clusters exhibit medial-to-lateral gradients. In the matrix, D1 and D2 cells exhibit a variety of spatial patterns, with some forming opposing spatial gradients. We have also generated similar datasets for marmoset and human basal ganglia to enable cross-species comparisons of spatial gene expression and cell-type distributions. Together, these high quality spatial transcriptomic datasets will serve as a foundation for future whole-brain atlasing efforts and will ultimately shed light on the organization and evolution of the primate brain.

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Poster

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Program #/Poster #: PSTR308.07/W23

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant UM1MH130981

Title: Comprehensive Cell Type Taxonomy of the Human Basal Ganglia via Single-nucleus Multi-omics Sequencing

Authors: *Y. FU[1], N. JOHANSEN[2], M. WIRTHLIN[3], A. GARCIA[4], I. KAPEN[1], A. YANNY[5], S. BARLOW[6], D. BERTAGNOLLI[1], A. CHAKKA[1], R. CHAKRABARTY[1], S.-L. DING[5], J. GOLDY[7], N. GUILFORD[8], K. JAMES[1], M. LEYTZE[1], B. NGUY[1], C. RIMORIN[9], S. C. SEEMAN[5], N. TASKIN[1], M. TIEU[5], B. P. LEVI[2], J. T. TING[2], K. SMITH[1], R. D. HODGE[10], E. LEIN[2], T. BAKKEN[5];
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Abstract: The basal ganglia (BG) are a group of subcortical nuclei extensively studied for their roles in motor control, cognition, reward, and emotion. Neurons in the BG have been characterized at the molecular, cellular, physiological, and circuitry levels. However, a comprehensive, high-resolution taxonomy of cell types has not yet been fully established for the human BG, nor is there a consensus taxonomy for primate BG. Here, we conducted 10X single-nucleus multi-omics sequencing on over half a million nuclei after sorting NeuN+, NeuN-OLIG2+, NeuN-OLIG2- nuclei isolated from adult human BG. This included nuclei from the caudate, putamen, internal and external segments of the globus pallidus (GPi/GPe), nucleus accumbens (NAC), subthalamic nucleus (STN), and substantia nigra (SN). More than 400,000 nuclei passed through our strict quality control, and over 300 clusters were grouped using hierarchical, iterative clustering algorithm. Leveraging hierarchical single-cell annotation tools and relevant accessible single-cell datasets, we identified each cluster and build the human BG

taxonomy across class, neighborhood, subclass, and cluster levels. Many clusters exhibited pronounced regional specificity, particularly in areas such as the GPi and NAC. Notably, both astrocytes and oligodendrocyte progenitor cells were categorized into several distinct subpopulations, each displaying unique molecular characteristics and regional preferences. We are aiming to integrate our human BG taxonomy with those from macaques, marmosets, and mice, creating a consensus cross-species cell type taxonomy. This integration will help us identify both conserved and species-specific cell types. Furthermore, by leveraging RNA and ATAC modalities, we can precisely identify enhancer elements associated with these cell types, facilitating the development of novel genetic tools for studying BG functions and disorders.

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Poster

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: HMBA Grant # 5UM1MH130981-02

Title: Transcriptomic and epigenetic profiling of 14 mammals provides insight on the evolution of the entorhinal cortex

Authors: *J. PUCKETT[1], M. WIRTHLIN[1], N. JOHANSEN[1], M. SCHMITZ[1], A. GARCIA[1], K. J. TRAVAGLINI[1], S. BARLOW[2], D. BERTAGNOLLI[1], A. CHAKKA[1], R. CHAKRABARTY[1], S.-L. DING[1], J. GOLDY[1], N. GUILFORD[1], A. C. HALLEY[3], C. RIMORIN[1], S. C. SEEMAN[1], M. TIEU[1], G. BALMUS[4], E. G. BARRETT[5], D. FITZPATRICK[6], L. A. KRUBITZER[7], J. J. PADBERG[8], C. SHERWOOD[9], G. K. WILKERSON[10], B. P. LEVI[11], J. A. MILLER[1], K. SMITH[1], R. D. HODGE[11], E. LEIN[11], T. BAKKEN[11];
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Abstract: The entorhinal cortex is a crucial structure in the brain known for its role in spatial navigation, memory, and the processing and relaying of sensory information. Understanding how this structure has evolved over 200 years of mammalian evolution provides key information on how these traits have changed to fit a species' niche, lifestyle, and functional needs. The entorhinal cortex is also highly associated with cognitive impairments including Alzheimer's disease, schizophrenia, and epilepsy. Using cross-species comparisons of the entorhinal cortex provides a new understanding of how disease-associated traits, cell types, and gene expression have changed throughout evolution and provides insight on potential model organisms that better reflect human disease phenotypes.
To generate this dataset, we used 10x multiomic snRNA-seq and snATAC-seq to sample the entorhinal cortex (EC) of 14 mammals, comprising six primates, three rodents, and a variety of other taxa including scandentians (northern treeshrew), carnivorans (ferret), artiodactyls (pig), cingulates (nine-banded armadillo), and marsupials (short-tailed opossum). Using the RNA-seq data we created a cross-species EC taxonomy showing conservation of cell types between species. We then utilized this taxonomy to observe lineage-specific differences in cell type proportions and abundance. We conducted differential gene expression analysis to investigate conserved and divergent patterns of gene expression, including primate lineage-, rodent lineage-, and human-specific features. This included identifying subclass-level marker genes across species, as well as transcription factors driving changes in gene regulatory networks within EC cell types. Finally, we provide a comparative analysis across mammals of transcriptional regulatory networks disrupted in human Alzheimer's disease, putting these critical gene networks in the broader context of their evolutionary origins.

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Poster

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: UM1MH130981
Paul G Allen Family Foundation

Title: Spatial transcriptomics of macaque brain and relationship to anatomical and functional organization

Authors: *S. SEEMAN[1], B. Q. ROSEN[2], R. D. HODGE[1], M. HEWITT[1], J. QUON[1], M. KUNST[1], J. WATERS[1], J. ARIZA TORRES[1], M. HUANG[1], L. NG[1], L. KRUSE[1], C. M. PAGAN[1], T. JARSKY[1], T. HAYASHI[3], M. F. GLASSER[2], F. YAZDANIBANAFSHEDARAGH[4], D. HU[4], D. C. VAN ESSEN[2], W. FREIWALD[4], E. LEIN[1], H. ZENG[1];
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Abstract: Understanding the spatial organization of cell types in the brain is crucial for unraveling its complex functional and anatomical architecture. Here we present a spatial transcriptomic atlas of a macaque hemisphere using the Stereo-seq platform combined with structural and functional magnetic resonance imaging (MRI) and histological staining. Together, these data offer insight into the spatial distribution of diverse cell types and their relationship to anatomical and functional boundaries. Stereo-seq is a commercial spatial transcriptomic platform that captures the whole transcriptome at single-cell spatial resolution. Through this high-resolution spatial transcriptomic profiling, we mapped the expression of thousands of genes across distinct anatomical regions of the macaque brain. Using spatial domain detection analysis, we were able to identify and align regional boundaries with spatial gene expression. Additionally, we used combinatorial gene expression in segmented cells to identify unique cell types. This analysis revealed areas of spatially restricted cell types along with broad, heterogenous spatial distributions of cell types. Furthermore, spatial patterns of cell types were also sufficient to identify regional boundaries. For example, cell types identified as layer 2/3 intertelencephalic projecting, along with layer 6b corticothalamic cells mark the transition from primary visual areas to higher visual areas. Overall, these data provide valuable insights into the spatial organization of gene expression and cell types in the macaque brain and offer a foundation for understanding how this organization contributes to its complex functional and anatomical architecture.

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Poster

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Program #/Poster #: PSTR308.10/W26

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH grant UM1MH130981

Title: Probabilistic inference of anatomical region underlying whole brain tiling

Authors: *I. KAPEN, M. SCHMITZ, A. GARCIA, R. D. HODGE, T. BAKKEN, N. JOHANSEN;
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Abstract: Building molecular atlases of the human and non-human primate brain requires dissecting complex anatomical regions in tiles that contain diverse functional areas and profiling with single nucleus multiomic sequencing. This strategy enables comprehensive coverage of brain regions but obscures the regional identities of cells that are dissected. Also, targeted dissection of brain regions can inadvertently include cells from neighboring anatomical regions which can complicate building regional cell type taxonomies. Here, we present a probabilistic framework for inferring the anatomical region from which a cell was isolated. Using Bayesian statistics paired with a mouse whole brain reference atlas of spatially localized cell types, we can infer the likely brain region of origin for cells sampled from other mammalian brains, including human and non-human primates. Such a strategy enables the categorization of cells into the most likely captured anatomic regions and expected coverage of cellular diversity.

Disclosures: I. Kapen: None. **M. Schmitz:** None. **A. Garcia:** None. **R.D. Hodge:** None. **T. Bakken:** None. **N. Johansen:** None.

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.01/W27

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Semi-automated hand annotation (saha) for mouse brain single cell and spatial datasets

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Abstract: Single cell and spatial omic technologies continue to inundate the field of neurobiology with increasingly complex data investigating cellular states and responses. Each dataset produced provides more context into how a particular cell reacts to genetic manipulation, the presence of disease-relevant stimuli, and the influence of environmental factors. For example, investigation into microglia in neurodegenerative diseases has allowed the discovery of several disease-associated cell states that appear to contribute to both protective and detrimental effects of the innate immune system. However, the field lacks a singular consensus methodology for cell classification that is robust enough to withstand the large batch-, laboratory-, and platform-specific noise present in single cell and spatial data. Although many computationally intensive pipelines are under development, there is a need to streamline iterative gene-by-cluster annotation strategies. We propose a visualization-forward approach to cell classification that we call semi-automated hand annotation (SAHA). By creating an easily accessible package in R, we have lowered the barrier for entry-level and skilled bioinformaticians alike to 1) compare the transcriptional state of clusters within their own data, 2) identify overlap between their own clusters and any publicly available database, and 3) implement marker-free cell similarity analysis to overcome challenges in targeted or low-sensitivity experiments. SAHA was specially designed to be able to run on a desktop or laptop computer, where only summary files of a dataset may be accessible. SAHA provides the user with a classification, summaries of how that classification was reached, and the flexibility to choose a marker database that is most appropriate for their study. The default databases for SAHA are PangloaDB and the Allen Brain Atlas; however, any custom annotation databases may be specified given a list of cell states and respective markers. In contrast to machine-learning or integration-based annotation approaches that are fully automatic, SAHA's semi-automatic pipeline allows researchers several checkpoints to fine-tune their annotations. Instructions for download and a vignette for implementation are freely available at www.github.com/neurogenetics/saha.

Disclosures: D.J. Acri: None. **R. Mustaklem:** None. **L. Horan-Portelance:** None. **L.C. Dabin:** None. **J. Park:** None. **K. Hartigan:** None. **H. Kersey:** None. **M. Mesecar:** None. **J. Gibbs:** None. **M.R. Cookson:** None. **J. Kim:** None.

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.02/W28

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH R01NS119813
NIH R01AG075114
NIH R21MH125107

NIH NIA K00AG084261
AFOSR FA9550-22-1-0337

Title: Whole brain, cell type specific transcriptomic profiling of in vivo neurochemical perturbation

Authors: *C. SLATER[1,2], M. VAIKUNTHAN[1], T. LANTIN[1], R. GIGLIO[3], S. WELLMAN[1], L. JIA[1], J. L. MCFALINE-FIGUEROA[1], Q. WANG[1];
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Abstract: The brain is composed of functionally distinct cell types that store and transmit information in a context-dependent manner. This interplay dynamically regulates both local and global neural activity. Relatively homogenous neuromodulatory networks, such as the locus coeruleus-norepinephrine (LC-NE) system, play an outsized role in dynamically regulating region- and cell-specific neural states through a host of receptor subtypes. Conventional methods lack the resolution or scalability to simultaneously measure receptor gene expression across multiple brain regions. Such methods are also unable to multiplex cell state readouts and perturbations. To this end, it is now possible to perform brain wide profiling of the transcriptomic response driven by selective release or inhibition of an active neurochemical such as NE. Until recently, the cost of single cell sequencing prohibited neuroscientists from routinely performing large-scale transcriptomic profiling. With the advent of efficient single-nuclei combinatorial indexing strategies, researchers can affordably profile millions of cells across the brain of intact animals undergoing controlled neural modulation. Here, we show the feasibility of this approach. We refine existing protocols for use in typical neuroscience experimental workflows and directly compare the impact of tissue dissociation methods and fixatives on transcript recovery, finding that the method performs similarly well across a range of conditions. To drive in vivo noradrenergic activity, we express excitatory designer receptors bilaterally in the LC of mice and administer clozapine-N-oxide for 10 days. Following tissue processing we recover the paired-end transcriptomic profiling of nearly 30,000 cells across the whole mouse brain. Using references such as the Allen Brain Cell atlas, we can predict individual cell type identity and confirm receptor gene expression distribution across cell types. In the cortex, adrenergic receptor gene expression is widespread, with a relative over-expression of the Adra1a subtype in inhibitory cells and Adra1d in some cortical intratelencephalic cell types. The Adrb1 gene is most abundant in the cortex and is distributed across numerous cell types. We additionally confirm several cell-specific marker genes (including *Slc17a7*, *Gad1*, *Aldh1l1*, *Mbp*, and *Pdgfrb*) that are preserved in our data as compared to the reference atlas. This approach can be used to describe broad transcriptomic changes that occur in response to prolonged noradrenergic modulation, resulting in the affordable, flexible, and scalable ability to understand the complex role of neuromodulators across the brain.

Disclosures: C. Slater: None. **M. Vaikunthan:** None. **T. Lantin:** None. **R. Giglio:** None. **S. Wellman:** None. **L. Jia:** None. **J.L. McFaline-Figueroa:** None. **Q. Wang:** E. Ownership

Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cofounder of Sharper Sense.

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.03/W29

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Multi-omic profiling of healthy and diseased brains with high-plex single-cell spatial molecular imaging

Authors: *C. WILLIAMS, A. HECK, L. WU, K. YOUNG, M. WALTER, R. LIU, A. WARDHANI, A. ROSENBLOOM, P. DANAHER, M. HOANG, J. BEECHEM; NanoString(R) Technologies (a Bruker company), Seattle, WA

Abstract: Single-cell transcriptomics and proteomics can provide complementary information about the form and function of neurons and glia throughout the brain. However, most high-plex spatial analyses to date have primarily utilized one of these two modalities to interrogate cell activity and cell-to-cell communication. Here, we simultaneously leveraged the detection of 68 proteins and over 6,000 RNA targets on the same formalin-fixed paraffin-embedded (FFPE) human brain sections to perform extended segmentation of neural processes and integrated analyses of protein and RNA expression.

Using a mult-iomic approach with the CosMx™ Spatial Molecular Imager (SMI), first high-plex protein panel targets were imaged via cyclic *in situ* hybridization chemistry. Next RNA targets on the same tissue section were exposed then hybridized, and finally RNAs were imaged using the same chemistry. The human neuro protein panel targets are particularly well-suited for dissecting neurodegenerative disease pathology, including various phospho-tau species and amyloid beta variants. Moreover, the protein panel includes markers for diverse neural cell types and enables robust cell typing, especially alongside the over 4,900 neuroscience-related genes covered by the Human 6K Discovery Panel. RNA targets focus on over 80 pathways, cell typing, and key ligand-receptor interactions. To demonstrate the capability of the single-cell high-plex multi-omic technique, we collected data from sections of FFPE male human brains, with samples derived from frontal, parietal, and occipital lobes, as well as the precentral/ postcentral gyri and cerebellum, of healthy individuals and Alzheimer's Disease patients.

Drawing on both the protein and RNA data, we achieved unparalleled segmentation of neurons and glia and increased transcript counts per cell. We also annotated cells with neuronal, glial, and vascular subtypes. By comparing RNA and protein expression, we identified genes and proteins with correlated and divergent patterns across our tissue space, highlighting the advantage of including the functional readout, protein, in understanding cell activity. Using open-source tools,

we assigned cells into niches based on protein patterns and then applied differential expression models to identify genes and gene sets which varied based on niche for individual cell types. Overall, by applying the SMI multi-omic platform to human brain samples, we were able to simultaneously probe cell shapes, cell types, cell neighborhoods, and cell activity in one experiment on a single slide.

Disclosures: C. Williams: A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **A. Heck:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **L. Wu:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **K. Young:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **M. Walter:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **R. Liu:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **A. Wardhani:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **A. Rosenbloom:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **P. Danaher:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **M. Hoang:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **J. Beechem:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies.

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.04/W30

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Enhanced, high-throughput single-cell DNA methylation analysis using massively paralleled indexing

Authors: M. NAKAMOTO[1], S. KHARE[1], D. SKINNER[1], *P. BOYD[2], D. POKHOLOK[1], J. THOMAS[1], F. SCHLESINGER[1];
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Abstract: The epigenetic landscape of the human brain shows changes in DNA methylation during malignant transformation. This epigenetic modification has been widely studied using traditional techniques like bisulfite sequencing and enzymatic methyl sequencing (EM-seq). However, these methods analyze bulk cell populations and lack the granularity of single-cell analysis, and although single-cell methylation analysis remains costly and laborious.To address these challenges, ScaleBio utilizes the cell itself as a compartment to perform 2-3 rounds of sequential barcoding in a plate-based workflow, eliminating the need for complex instrumentation. This technology has been successfully adapted to assess DNA methylation at the single-cell level offering a robust, affordable, high-throughput protocol that enhances yield, diversity, and coverage.In this study we used ScaleBio's single-cell RNA-seq and methylation kits to investigate gene expression and DNA methylation patterns during oncogenesis using human isocitrate dehydrogenase (IDH) mutant glioma cells. *IDH1/2* mutations are present in over 80% of low-grade gliomas while IDH-mutant gliomas constitute about 1/5th of all adult diffuse gliomas, thus making them one of the most common brain tumor subtypes. As such, IDH mutations have emerged as attractive therapeutic targets for glioma treatment. By uncovering DNA methylation patterns at the single cell level, we provide here an epigenetic map to better understand this complex disease and better inform clinical discoveries.We achieved high cell recovery and robust cytosine coverage throughout our analysis of single cell methylomes isolated from human glioma tumor tissue. Using this data we generated a ranked list of the top hypo- and hypermethylated genomic regions and identified cell type specific clusters seen in different pathological states by looking at Differentially Methylated Regions (DMR) uncovering unique single-cell methylation profiles that may be obscured by bulk or pseudo-bulk analysis. RNA analysis of these cells revealed unique populations that could be identified using transcriptome analysis, and comparison of the transcriptional and epigenetic diversity of these samples was compared.Together these data show that ScaleBio workflows can be used to profile complex samples, offering increased sensitivity, specificity, and accuracy in identifying DNA methylation sites and transcriptional profiles and providing insights into cellular heterogeneity and trajectories.

Disclosures: M. Nakamoto: A. Employment/Salary (full or part-time):; Scale Biosciences. **S. Khare:** A. Employment/Salary (full or part-time):; Scale Biosciences. **D. Skinner:** A. Employment/Salary (full or part-time):; Scale Biosciences. **P. Boyd:** A. Employment/Salary (full or part-time):; Scale Biosciences. **D. Pokholok:** A. Employment/Salary (full or part-time):; Scale Biosciences. **J. Thomas:** A. Employment/Salary (full or part-time):; Scale Biosciences. **F. Schlesinger:** A. Employment/Salary (full or part-time):; Scale Biosciences.

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.05/W31

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Simons Foundation for Autism Research Award 947591

Title: Cellular resolution multi-omics of white matter tracts in autism spectrum disorder

Authors: *Y. ZHANG, A. A. KULKARNI, G. KONOPKA;
Neurosci., UT Southwestern Med. Ctr., Dallas, TX

Abstract: The convergent molecular mechanisms that underlie autism spectrum disorder (ASD) remain mostly unknown. White matter abnormalities have been observed in brain imaging studies of individuals with ASD as well as mouse models of genetic forms of ASD. These results suggest that alterations in white matter may contribute to ASD-relevant phenotypes. White matter subregions represent distinct anatomical pathways and functional networks within the brain, and whether different subregions have distinict molecular features is unknown. Since the brain is composed of highly heterogeneous cell types, we hyptothesize that it is necessary to analyze human white matter subregions at cellular resolution to identify potential disease-related alterations in molecular pathways underlying ASD. Here, we performed single-nucleus multiome sequencing of fresh-frozen tissues from 6 white matter subregions in 10 ASD patients and 10 controls. Therefore, with these data, for the first time, we will: 1) Analyze regional variation of white matter cellular diversity in human brains and 2) Compare this variation between individuals with ASD and controls to reveal disease-relevant patterns specific to different cell types that may have laid foundations for ASD-relevant features. We identify cell proportional and differential gene and chromatin accessibility data in each cell type in each subregions of cases compared to controls. These data will provide a valuable public resource, enabling new discoveries and insights into human white matter cellular features and relevance to ASD.

Disclosures: Y. Zhang: None. **A.A. Kulkarni:** None. **G. Konopka:** None.

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.06/W32

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Ministry of Science and ICT, Republic of Korea, RS-2023-00266110

Title: Infrared laser-based single cell isolation: Paving way for spatially-resolved single cell transcriptomics in neuroscience

Authors: *E. H. KIM, S. LEE, A. C. LEE;
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Abstract: The locations and spatial contexts of individual cells undergoing single-cell RNA-sequencing (scRNA-seq) critically inform interpretations of their transcriptomic data. However, the fragility and complexity of neural tissues has posed challenges to implementing spatial transcriptomics into neuroscience. Neural cells are often too delicate to withstand the harshness of UV lasers used in laser capture microdissection techniques, while the most conventional method of single-cell isolation in neuroscience—flow cytometry—requires a homogenization step that makes it impossible to acquire spatial information about cells' spatial origins. Although newer imaging-based single cell methods such as Xenium and Visium from 10X Genomics are powerful, their high costs can make access infeasible. Here, we present Spatially-resolved Laser Activated Cell Sorting (SLACS) as an affordable and highly effective means of addressing these hurdles. SLACS isolates individual cells using an infrared (IR) laser-based punching mechanism. The IR laser is both gentle and extremely precise, befitting the technology for the sorting of delicate, rare, and difficult-to-capture neural cells. The preservation of transcriptome information in SLACS-isolated samples has been, and is continuing to be, demonstrated consistently. A mixed cell line sample consisting of 30,000 non-isolated HL60 control cells and 10,000 SLACS-isolated MCF7 cells, after scRNA-seq and analysis using the Seurat R package for single cell analysis, produced clustering that visibly differentiated between the two cell lines. A distinctly outlying cluster was confirmed to represent MCF7 cells by its high expression of the estrogen receptor alpha (ESR1) gene and negligible expression of the estrogen receptor beta (ESR2) gene. This demonstrates the capture and preservation of transcriptomic material in SLACS-isolated samples at the single-cell level. The follow-up sequencing of a mixed-species sample containing 30,000 HL60 control human cells and 10,000 SLACS-isolated spleen cells from mouse tissue has evidenced the technology's high compatibility with both cell line and tissue samples. Current confirmatory research involves isolation and sequencing of single-cell samples from a diverse range of tissue section types and brain organoids. Broader employment of SLACS can better establish avenues of spatial transcriptomics in neuroscience, bringing insights into cellular heterogeneity within and across microenvironments; cell-cell interactions and the biological structures containing them; and examination of localized diseases and targeted therapeutic approaches.

Disclosures: E.H. Kim: A. Employment/Salary (full or part-time):; Meteor Biotech, Co. Ltd. **S. Lee:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Meteor Biotech. Co. Ltd. **A.C. Lee:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Meteor Biotech. Co. Ltd..

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.07/W33

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Advancements in Spatial Transcriptomics: Unlocking Insights from Low-Quality Brain Tissue Samples

Authors: *R. CHEN, J. HE, B. WANG, B. YANG, T. WIGGIN, L. MAZIASHVILI, P. REINHOLD, M. RAY, S. TATTIKOTA, J. HE, G. EMANUEL;
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Abstract: The emergence of tools enabling researchers to perform high-plex spatial transcriptomics with single-cell resolution has revolutionized our understanding of neuronal function. However, tissue samples with degraded RNA or extensive crosslinking present challenges for gene expression measurements. The majority of human brain samples are archived using fresh frozen methodologies, yet many samples cannot be used for deep transcriptome analyses due to low RNA quality stemming from variable sample collection conditions.
Here we introduce the new MERFISH 2.0 chemistry and updated sample preparation workflow powered by the MERSCOPE® Platform, which facilitates direct RNA profiling of up to 1000 genes. The enhanced MERFISH 2.0 chemistry has been developed to maintain MERFISH sensitivity in lower quality tissue samples. We showcased its effectiveness in analyzing frozen archival human brain tissue previously deemed unusable due to degraded RNA, as well as frozen mouse brain tissue.
For each sample, cells were captured using the updated sample preparation workflow, and their spatial information was profiled using a 1000-gene panel containing markers for both cell typing and neurodegeneration pathways. We observed a significant increase in gene counts per 100 micron2 of tissue of the MERFISH 2.0 protocol over the previous version, in some cases exceeding 4x. Additionally, we demonstrated increased reproducibility between replicates with the streamlined workflow and enhanced MERFISH 2.0 chemistry. Finally, we constructed a spatially resolved single-cell atlas across low-quality healthy and diseased human brain tissue, mapped and cataloging different neuronal cell types, and performing ligand-receptor analysis to identify cell-cell interactions.
Spatially resolved transcriptomic profiling of low-quality samples at single-cell level offers significant opportunities for understanding how cells connect in the brain. These improvements will enable new genomic inquiries into previously intractable tissues like frozen brain tissue, leading to new biological insights.

Disclosures: R. Chen: A. Employment/Salary (full or part-time):; Vizgen. **J. He:** A. Employment/Salary (full or part-time):; Vizgen. **B. Wang:** A. Employment/Salary (full or part-time):; Vizgen. **B. Yang:** A. Employment/Salary (full or part-time):; Vizgen. **T. Wiggin:** A. Employment/Salary (full or part-time):; Vizgen. **L. Maziashvili:** A. Employment/Salary (full or part-time):; Vizgen. **P. Reinhold:** A. Employment/Salary (full or part-time):; Vizgen. **M. Ray:** A. Employment/Salary (full or part-time):; Vizgen. **S. Tattikota:** A. Employment/Salary (full or part-time):; Vizgen. **J. He:** A. Employment/Salary (full or part-time):; Vizgen. **G. Emanuel:** A. Employment/Salary (full or part-time):; Vizgen.

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.08/W34

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant R01

Title: Age-associated activation of cGAS-STING pathway underlies cognitive decline and hippocampal dysregulation in a mouse model of Alzheimer's disease

Authors: *S. LEE[1], N. R. ZEMKE[1,2], X. LIN[3], B. YANG[4], S. GRIECO[3,5], E. VELAZQUEZ[3], Z. TAN[6], N. C. BERCHTOLD[6], Q. YANG[4], H. INDRALINGAM[1], S. THAMILARASAN[7], Y. E. LI[1], A. WANG[4], S. PREISSL[4], X. XU[3,5], B. REN[1,2];
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Abstract: Age and sex contribute to the risk of Alzheimer’s disease (AD), but the underlying mechanisms remain elusive. Here, we describe the results of single-cell multiomic analysis of a mouse model of AD, focusing on the dorsal hippocampi from male and female mice at young (3-month old), mid- (9-month old), and old ages (18-month old). Our comparative analysis showed that microglia and oligodendrocytes exhibited AD and age-related changes in gene expression and chromatin accessibility in the AD mouse model. A degree of these alterations were notably more pronounced in female AD mice. In microglia, we observed a progression of gene program shifts from tumor necrosis factor production-related programs in young mice to interferon-associated programs in mid-aged mice and ultimately to gene programs linked to lipid metabolic processes in old AD mice. Interestingly, an upregulation of the cGAS-STING pathway was observed in the microglia from old AD mice. Inhibition of the STING pathway partially restored cognitive function in aged AD mice. Our findings therefore suggest cell types/states-specific AD

and age-associated changes in gene regulatory programs underlie AD pathogenesis and aging-associated cognitive decline.

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Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

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Program #/Poster #: PSTR309.09/W35

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: 5U19MH114830-05
1U01MH130962-01

Title: A spatial transcriptomics atlas of the adult mouse brain

Authors: *M. KUNST[1], R. MATHIEU[2], L. CHING[3], J. QUON[3], D. MCMILLEN[3], J. CAMPOS[3], M. CHEN[4], J. GEE[5], S. DANIEL[3], A. RUIZ[6], M. HEWITT[2], S. C. SEEMAN[2], C. M. PAGAN[7], J. ARIZA TORRES[8], S. M. SUNKIN[2], L. A. ESPOSITO[9], L. NG[2], C. VAN VELTHOVEN[3], Z. YAO[2], J. WATERS[2], H. ZENG[2];
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Abstract: Understanding the spatial organization of cell types in the brain is crucial for unraveling its complex functional and anatomical architecture. Spatial transcriptomics provides a method to relate transcriptomic cell types to their anatomical location. To further our understanding of cell type distribution within the brain we generated two sex-matched spatial transcriptomics dataset covering the entire mouse brain using the commercial MERFISH platform MERSCOPE. We created an automatic processing pipeline that consists of a) cell segmentation with a custom made Cellpose model, b) filtering of low quality cells, and c) mapping of the cell to our most recent version of the whole-mouse-brain RNAseq taxonomy.These data offer insight into the spatial distribution of diverse cell types and their relationship to anatomical and functional boundaries. One example we present is the habenula. Using cell-type maps at different level of taxonomic hierarchy (subclass, supertype and cluster) we were able to differentiate different subdomains of the habenula. This approach could recapitulate previously known domains as well as identify additional, more fine-grained

division.We also performed spatial domain detection, which allowed us to identify anatomical regions defined by shared gene expression. We are able to integrate the spatial clusters across the male and female brain allowing us to investigate sex differences in a region-specific manner.

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Poster

PSTR309: Single-Cell Techniques

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant 7R37DA054370-03

Title: A characterization of the transcriptional heterogeneity and spatial distribution of GABA neurons in the ventral tegmental area and rostromedial tegmental nucleus

Authors: *Z. J. HOUGH[1], N. SCHAFFER[1], B. W. HUGHES[2], C. W. COWAN[3], E. J. NESTLER[4], T. C. JHOU[5];
[1]Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Friedman Brain Institute, Dept. of Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]Neurosci., Med. Univ. of South Carolina, Charleston, SC; [4]Icahn Sch. of Med. At Mount Sinai, New York, NY; [5]Neurosci., Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD

Abstract: Recent studies have highlighted considerable diversity in the function and gene expression of ventral midbrain GABA neurons, which are pivotal in modulating motivated behaviors. Thus, a deeper understanding of the molecular distinctions among various GABA neuron populations and their spatial distribution is warranted. Our research primarily focused on two key regions in the midbrain implicated in the regulation of motivated behavior: the ventral tegmental area (VTA) and the rostromedial tegmental nucleus (RMTg, also known as the tail of the VTA). Notably, about one-third of VTA neurons are GABAergic, which are increasingly recognized as heterogeneous. In contrast, RMTg neurons are predominantly GABAergic, yet little is known about their potential diversity or overlap with VTA GABA neuron subpopulations. To address this, we conducted single-nucleus RNA sequencing on VTA and RMTg tissue from four 12-week-old male Sprague Dawley (SD) rat brains. Analysis of these datasets supported previous findings, indicating substantial diversity among VTA GABA neurons. Furthermore, we confirmed that *Foxp1* expression, present in over 90% of RMTg

GABA neurons, distinguishes RMTg from VTA GABA neurons, consistent with prior immunohistochemical (IHC) and fluorescence *in situ* hybridization (FISH) observations. Additionally, we identified several novel markers, including *Tll1*, *Sulf1*, *Cbln4*, and *Trpc4*, which were significantly upregulated in RMTg GABA neurons relative to GABA neurons in the VTA and surrounding brain regions. These marker genes were further assessed for their spatial specificity to RMTg GABA neurons in both mouse and rat brain slices via FISH. Consistent with the sequencing data, *Tll1, Sulf1, Cbln4, and Trpc4* exhibited elevated expression levels relative to adjacent cells and VTA GABA neurons, however, none of them were entirely restricted to the RMTg. *Sulf1* exhibited robust expression in both RMTg and VTA GABA neurons. *Sox14* expression was also found to be elevated in the RMTg but was also detected at lower expression levels in many adjacent brain regions in the rat brain. Overall, *Tll1* expression was the most specific to RMTg GABA neurons, however, it is also expressed robustly in dopamine neurons. Ongoing analysis, coupled with spatial characterization via a combination of IHC, FISH, and emerging spatial transcriptomics methods will further enhance our understanding of distinct GABA neuron subtypes within these brain regions. In summary, our current data indicates that RMTg and VTA GABA neurons exhibit distinct transcriptional profiles, suggesting divergent roles in the regulation of motivated behavior.

Disclosures: Z.J. Hough: None. **N. Schaffer:** None. **B.W. Hughes:** None. **C.W. Cowan:** None. **E.J. Nestler:** None. **T.C. Jhou:** None.

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.11/W37

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: The evolution of primate forebrain revealed by STARmap in situ transcriptomics

Authors: *Y. ZHOU[1], H. SHI[2], G. FENG[3], X. WANG[1];
[2]Xiao Wang Lab., [1]Broad Inst., Cambridge, MA; [3]Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: The brain structures of rodents and primates exhibit numerous conserved features, yet advanced cognitive functions typically depend on primate-specific characteristics. Previous comparisons between rodents and primates have relied on anatomical analogies or single-cell RNA sequencing. With the development of advanced 3D in situ sequencing methods (STARmap), brain regions defined by spatial transcriptomics features offer a more comprehensive and accurate framework for comparative studies of brain evolution. In our study, we employed STARmap to analyze the forebrains of mice and marmosets, enabling integrative molecular cell type and tissue region identification. We profiled 461 genes in 196,803 high-

quality cells in the marmoset forebrain, and 1,022 genes in 140,779 high-quality cells in the mouse forebrain. Our analysis identified a conservative cytoarchitecture of the cortex and septum between marmosets and mice, while the striatum, white matter, and ependyma exhibited greater divergence. When examining molecular cell types, we found that septum inhibitory neurons and cortical excitatory neurons exhibit conserved spatial layout across both mouse and marmoset forebrains. However, the marmoset striatum contains unique Tac3-MSN cells, which are absent in the mouse striatum. Additionally, we observed that two distinct types of ependymal cells are in the outer and inner layers of the ependyma in marmosets, respectively. Comparative analysis of molecular tissue regions revealed that the upper layers of the marmoset cortex harbor a greater density of interneurons, while in the mouse cortex, it is the deeper layers that exhibit a higher concentration of interneurons. The marmoset white matter microenvironment is more complex and composed of white matter-specific cells. Among homologous molecular tissue regions of mouse and marmoset forebrains, many genes exhibit differential spatial patterns between the two species. In summary, our study identifies primate-specific spatial gene expression, cell types, and tissue regions, shedding light on the molecular foundations underlying the evolution of the primate forebrain.

Disclosures: Y. Zhou: None. **H. Shi:** None. **G. Feng:** None. **X. Wang:** None.

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.12/W38

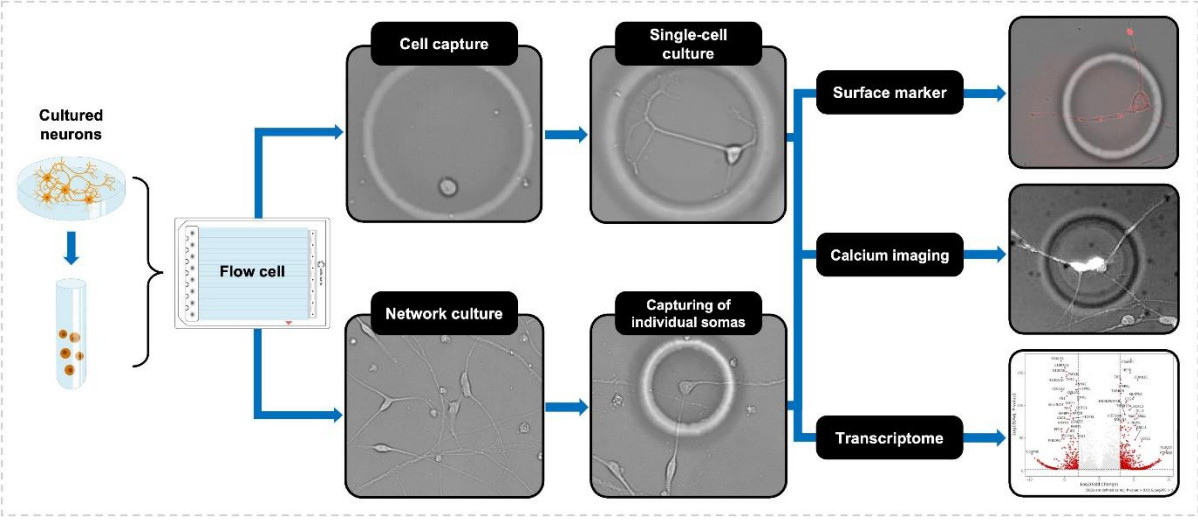
Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Concurrent live-imaging and single-cell transcriptome analysis of intact functional neuronal networks on a highly parallel scale

Authors: *O. VILA[1], N. ROUZBEH[1], S. FARAHVASHI[1], J. JONES[2], B.-A. WANG[2], S. SABRI[1], S. DESHMUKH[1], J. LAMSTEIN[1], E. FARJAMI[1], M. TAING[1], P. GHERARDINI[1], S. MOEINZADEH[1], T. KHURANA[1], F. H. GAGE[2], J. R. ECKER[2], M. RONAGHI[1], G. SCHROTH[1];
[1]Cellanome, Palo Alto, CA; [2]Salk Inst., La Jolla, CA

Abstract: Single-cell technologies have provided unprecedented insights into cellular functions and heterogeneity, holding great promise for precise mapping of neuron diversity. However, current single-cell technologies require cell dissociation, encapsulation into droplets, or handling with flow-based microfluidics. These procedures can be detrimental for neurons, perturb their transcriptomes, and therefore, reduce confidence in the biological relevance of findings from surviving cells. Furthermore, existing technologies do not allow the study of live neurons in functional networks, and due to their destructive nature, hinder the ability to conduct the multi-

modal assays needed to correlate physiological properties with molecular profiles.
To overcome these limitations, we have developed a platform to comprehensively study adherent neurons at single-cell resolution in isolation, or from within their functional networks. We leveraged a novel technology that can gently capture tens of thousands of individual, live neurons without requiring enzymatic cell dissociation or nuclei extraction. Further, this platform enables comprehensive multi-modal analysis at the single-cell level, encompassing morphology, function, and transcriptome, enabled by live imaging and mRNA capture capabilities, from the same cell.
Using our platform, we successfully demonstrate: 1) long-term culture (>3 weeks) of human neurons in adherent state, 2) their ability to form functional neuronal networks validated by calcium imaging, and 3) the capability to isolate either whole neurons or somas from interconnected networks for single cell multi-modal analysis. Our findings reveal differential gene expression between adherent neurons and dissociated neurons in suspension, demonstrating that physical context influences neuronal transcriptomic states (Fig 1). In preserving cell integrity and function, this technology will unveil previously unattainable insights and become an essential tool for exploring the interplay between the transcriptome and cellular behavior.



Disclosures: O. Vila: A. Employment/Salary (full or part-time):; Cellanome. **N. Rouzbeh:** A. Employment/Salary (full or part-time):; Cellanome. **S. Farahvashi:** A. Employment/Salary (full or part-time):; Cellanome. **J. Jones:** None. **B. Wang:** None. **S. Sabri:** A. Employment/Salary (full or part-time):; Cellanome. **S. Deshmukh:** A. Employment/Salary (full or part-time):; Cellanome. **J. Lamstein:** A. Employment/Salary (full or part-time):; Cellanome. **E. Farjami:** A. Employment/Salary (full or part-time):; Cellanome. **M. Taing:** A. Employment/Salary (full or part-time):; Cellanome. **P. Gherardini:** A. Employment/Salary (full or part-time):; Cellanome. **S. Moeinzadeh:** A. Employment/Salary (full or part-time):; Cellanome. **T. Khurana:** A. Employment/Salary (full or part-time):; Cellanome. **F.H. Gage:** None. **J.R. Ecker:** None. **M. Ronaghi:** A. Employment/Salary (full or part-time):; Cellanome. **G. Schroth:** A. Employment/Salary (full or part-time):; Cellanome.

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.13/X1

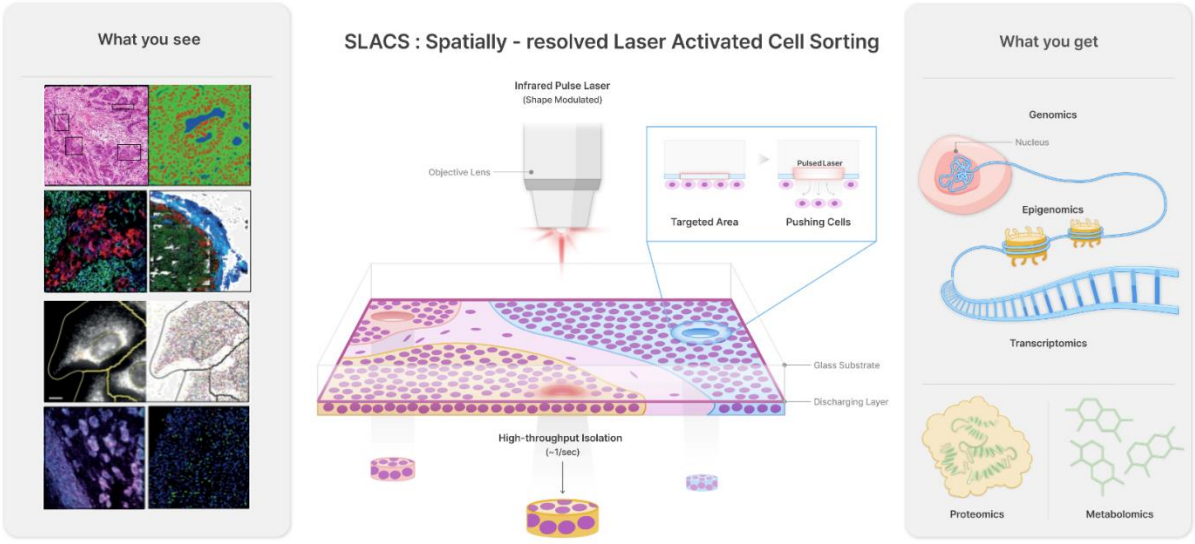
Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: RS-2023-00266110

Title: Next Generation Spatial Cell Sorter - A tool for neural cell sorting in neuroscience research

Authors: *S. LEE, A. LEE;
Meteor Biotech, Co. Ltd., Seoul, Korea, Republic of

Abstract: In neuroscience, understanding the brain's intricate complexity demands techniques that capture neural diversity while preserving spatial integrity. Despite advances in imaging and molecular biology, traditional staining techniques and next-generation sequencing (NGS) often struggle to integrate spatial and molecular data. This gap hinders our ability to fully elucidate cellular behavior in native environments. Addressing this challenge, Spatially Resolved Laser-Activated Cell Sorting (SLACS) emerges as a transformative methodology. SLACS preserves spatial information while integrating spatial assays with molecular assays like DNA sequencing, RNA sequencing, and protein profiling. Using a laser on a metal oxide-coated slide, SLACS propels isolated cells into PCR tubes for comprehensive analysis. Its dual functionality offers automated and manual control, enabling researchers to target cells with high precision based on fluorescence characteristics. SLACS's high throughput allows it to sort multiple targets rapidly, enhancing scalability. This technique isolates entities such as bacteria, chromosomes, and neural cells from complex biological matrices, providing researchers with a powerful tool for scientific investigations. SLACS offers unprecedented integration of molecular and spatial data by physically isolating cells within tissue landscapes. This enables mapping molecular profiles to anatomical locations, advancing our understanding of neural circuits and neurodevelopment. SLACS has already yielded significant insights into neurodegenerative diseases like Alzheimer's, where researchers isolated glial cells crucial for homeostasis and signal transmission. By analyzing glial cells in their native environment, SLACS unveiled potential treatment strategies targeting the broader cellular networks involved in disease. SLACS offers a paradigm shift in neuroscience research, revealing the brain's architecture and alterations in disease states to guide more effective diagnostics and therapeutics.



Disclosures: S. Lee: A. Employment/Salary (full or part-time):; Meteor Biotech, Co. Ltd.. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Meteor Biotech, Co. Ltd., Ministry of Science and ICT, Korea. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Meteor Biotech, Co. Ltd. **A. Lee:** A. Employment/Salary (full or part-time):; Meteor Biotech, Co. Ltd.. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Meteor Biotech, Co. Ltd., Ministry of Science and ICT, Korea. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Meteor Biotech, Co. Ltd..

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.14/X2

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Ministry of Science and ICT, Republic of Kroea RS-2023-00266110

Title: Host-virus spatial epitranscriptomics in Zika virus-infected brain organoids

Authors: *A. LEE, S. LEE;
Meteor Biotech, Co. Ltd., Seoul, Korea, Republic of

Abstract: Zika virus (ZIKV) poses a significant neurodevelopmental threat, evidenced by its association with microcephaly and other congenital abnormalities. Conventional models have failed to elucidate the complexity of ZIKV pathogenesis due to the lack of human pathophysiological context and spatial resolution. Recent advances in spatial epitranscriptomics hold promise for bridging this knowledge gap. This study aims to dissect the spatial epitranscriptomic landscape of ZIKV-infected brain organoids, focusing on the role of Adenosine-to-Inosine (A-to-I) RNA editing in modulating host-virus interactions and their implications for viral pathogenesis and potential therapeutic interventions. Utilizing iPSC technology, we differentiated brain organoids containing distinct neural progenitors and mature neuron populations. Upon ZIKV infection, we applied Select-seq to isolate and analyze discrete micro-niches. Our approach enabled full-length sequencing of host and viral transcriptomes, along with precise RNA editing profiling. Our findings reveal a distinct spatial distribution of A-to-I RNA editing events within ZIKV-infected organoids, correlating with unique gene expression signatures and cellular phenotypes. Notably, A-to-I editing was found to be differentially regulated in ZIKV envelope protein-expressing cells, suggesting a potential mechanism of viral evasion or adaptation. Furthermore, we identified patterns of host-virus interaction that may contribute to the neuropathological outcomes associated with ZIKV. The integration of spatial epitranscriptomics into the study of ZIKV infection provides insight into the virus-host interplay at a cellular level. Our approach underpins the significance of spatial context in understanding viral pathogenesis and opens avenues for the development of antiviral therapies. The application of advanced technology in virology enables the dissection of complex pathogenetic processes and fostering the development of personalized therapeutic strategies against ZIKV and other neuropathogenic viruses.

Disclosures: A. Lee: A. Employment/Salary (full or part-time):; Meteor Biotech, Co. Ltd.. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Ministry of Science and ICT, Republic of Korea. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); I hold share in Meteor Biotech. **S. Lee:** A. Employment/Salary (full or part-time):; Meteor Biotech, Co. Ltd.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sumin holds share in Meteor Biotech.

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.15/X3

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH grant 1T32GM14429
Duke-Coulter Translational Partnership

Title: Development of an electropolymer that can genetically link defined neurons to electrodes

Authors: *U. GHOSH[1], I. WEAVER[2], L. A. LIGONS[1], S. S.-X. LIM[2], B. C. SHIELDS[1], M. R. TADROSS[1];
[1]Biomed. Engin., Duke Univ., Durham, NC; [2]Duke Univ., Durham, NC.

Abstract: Traditional implantable neural technologies have the ability to record from thousands of neurons with millisecond temporal resolution. However, they face significant limitations: difficulty in achieving cell-type-specific interfacing due to the absence of a genetically encoded element, inability to record and stimulate the same neuron chronically due to neural drift, and neuronal degradation and gliosis often exacerbated by the high electrical stimulation required to bypass the resulting scar tissue. To address these challenges, we developed ElectroGLuE (Electropolymer that Genetically Links Neurons to Electrodes), an electropolymer that establishes covalent connections between neurons of interest and the electrode surface, creating a stable, cell-type specific electrical interface. We harnessed the abilities of the HaloTag technology by synthesizing a novel 3,4-ethylenedioxythiophene (EDOT) conjugated with the HaloTag ligand (HTL), a small chemical covalently captured by the HaloTag protein (HTP). Recordings with first generation PEDOT^{HTL}-coated multi-electrode arrays (MEAs) demonstrated a 10-fold increase in spiking amplitude in cultured neurons expressing a functional HTP as compared to neurons expressing a control protein. Nevertheless, significant polymer delamination and low (10%) covalent capture due to inefficient polymer packing was observed. We aimed to improve functionality in the MEA design through surface chemistry optimization of the polymer and incorporation of iridium oxide (IrOx), a material shown to form an ultra-stable bond with PEDOT. We developed and optimized procedures, including cyclic voltammetry (CV), to electropolymerize the EDOT^{HTL} monomer into a conductive PEDOT^{HTL} polymer to coat the surface of IrOx electrodes. We then used an enzymatic assay to determine optimal polymerization parameters, assessed by the covalent capture efficiency of PEDOT^{HTL}. Our results show an enhanced ability of the PEDOT^{HTL} layer to form stable covalent bonds with the HTP as a variable of polymerization parameters (concentrations of monomers, voltage ranges, duration, frequency). The enhanced polymer nanoarchitecture was able to withstand repetitive CV stress scans without delamination and loss of binding. Future work includes neuronal culture experiments with custom IrOx-MEAs to further validate ElectroGLuE, and ultimately adapting the technology to implantable electrodes for closed-loop recording and stimulation of individual neurons.

Disclosures: U. Ghosh: None. **L.A. Ligons:** None. **B.C. Shields:** None. **M.R. Tadross:** None.

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.16/X4

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant 1RF1MH117055-01
NIH Grant DP2-MH119425
AHA Predoctoral Fellowship

Title: Appraising receptor function by membrane location with subcellular pharmacology

Authors: *S. S. X. LIM, H. YAN, B. C. SHIELDS, S. C. BURWELL, S. SINGH, A. CHOUDHURY, M. R. TADROSS;
Duke Univ., Durham, NC

Abstract: Neurons are highly polarized cells with distinct anatomical compartments (axon, soma, dendrites, primary cilia). These subcellular compartments are thought to serve distinct functions. However, it remains unknown how the same receptor in different subcellular compartments impacts circuit dynamics and animal behavior. A prototypical example is in regard to the main inhibitory neurotransmitter, GABA, whose receptor (the $GABA_AR$) is present throughout all locales of neurons. Each compartment is biophysically positioned to perform distinct functions, and receives distinct streams of information. For instance, $GABA_ARs$ on distal dendrites are thought to counterbalance to local excitatory *inputs*, whereas those on the soma are thought to have veto power over action potential *output*. $GABA_ARs$ in the axon may decouple transmitter release from action potential firing, with potential to individually tune collaterals. Beyond these traditional compartments, the primary cilia has recently been shown to be a distinct postsynaptic target, raising the tantalizing prospect for specialized $GABA_AR$ regulation of signaling to the nucleus. A second transmitter implicated in subcellular processing is dopamine, for which receptors exists in all these anatomical compartments. Dopamine receptors may have particular significance to the primary cilium, where dopamine receptors are enriched. DART (Drug Acutely Restricted by Tethering) offers a groundbreaking new way to study native receptors, including dopamine and $GABA_ARs$, by making it possible to deliver pharmaceuticals to genetically defined cells. The cells of interest are made to express a protein that can covalently capture and concentrate drugs to levels ~1,000-fold higher than the ambient concentration, yielding a localized cell-specific pharmaceutical effect. Here, we describe three novel subcellular refinements of the technology. First, we describe a method to deliver DART pharmaceuticals to axon projections without diffusion into somatodendritic compartments, enabling axon-specific pharmacology. Second, we present novel soma-targeted versions of DART. Finally, we describe cilia-targeted versions of DART. Additionally, we develop functional assays to map target engagement with subcellular resolution, allowing us to validate the specificity of our novel subcellular DART variants. Altogether, these tools are compatible with use in freely behaving mice, and should provide a modular foundation for subcellular targeting of virtually any drug.

Disclosures: S.S.X. Lim: None. **H. Yan:** None. **B.C. Shields:** None. **S.C. Burwell:** None. **S. Singh:** None. **A. Choudhury:** None. **M.R. Tadross:** None.

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.17/X5

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant RF1 MH117055
NIH Grant DP2 MH1194025
MJFF/ASAP grant ASAP-020607

Title: Reward perseveration is shaped by $GABA_A$-mediated dopamine pauses

Authors: S. C. BURWELL[1], H. YAN[2], S. S. LIM[3], *B. C. SHIELDS[3], **M. R. TADROSS**[3]; [1]Dept. of Neurobio., Duke Univ., Raleigh, NC; [2]Dept. of Neurobio., Duke Univ. , Dept. of Biomed. Engin., Durham, NC; [3]Biomed. Engin., Duke Univ., Durham, NC

Abstract: Extinction learning is an essential form of cognitive flexibility, which enables obsolete reward associations to be discarded. Its downregulation can lead to perseveration, a symptom seen in several neuropsychiatric disorders. This balance is regulated by dopamine from VTA_{DA} (ventral tegmental area dopamine) neurons, which in turn are largely controlled by GABA (gamma amino-butyric acid) synapses. However, the causal relationship of these circuit elements to extinction and perseveration remain incompletely understood. Here, we employ an innovative drug-targeting technology, DART (drug acutely restricted by tethering), to selectively block $GABA_A$ receptors on VTA_{DA} neurons as mice engage in Pavlovian learning. DART eliminated $GABA_A$-mediated pauses—brief decrements in VTA_{DA} activity canonically thought to drive extinction learning. However, contrary to the hypothesis that blocking VTA_{DA} pauses should eliminate extinction learning, we observed the opposite—accelerated extinction learning. Specifically, DART eliminated the naturally occurring perseveration seen in half of control mice. We saw no impact on Pavlovian conditioning, nor other aspects of VTA_{DA} neural firing. These findings challenge canonical theories, recasting $GABA_A$-mediated VTA_{DA} pauses from presumed facilitators of extinction to drivers of perseveration. More broadly, this study showcases the merits of targeted synaptic pharmacology, while hinting at circuit interventions for pathological perseveration.

Disclosures: S.C. Burwell: None. **H. Yan:** None. **S.S. Lim:** None. **B.C. Shields:** None. **M.R. Tadross:** None.

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.18/X6

Topic: I.08. Methods to Modulate Neural Activity

Support: RO1-NS107472
RF1-MH117055
DP2-MH1194025

Title: Cell-specific modulation of voltage-gated sodium channels using DART.2

Authors: *H. YAN[1], Y. OH[2], B. C. SHIELDS[1], P. JEONG[2], J. HONG[2], M. R. TADROSS[1,3]; [1]Biomed. Engin., Duke Univ., Durham, NC; [2]Chem., Duke Univ., Durham, NC; [3]Neurobiology, Duke University, Durham, NC

Abstract: The ability to manipulate neural circuits with molecular and cellular specificity allows for causal interrogation of the connectome. Recently, we demonstrated that our DART.2 technology (Drug Acutely Restricted by Tethering) is able to antagonize and allosterically potentiate excitatory or inhibitory post-synaptic function with thousand fold cellular specificity. The DART method works by expressing the HaloTag Protein (HTP) on genetically defined cells of interest, enabling these cells to capture and locally accumulate drugs linked to the HaloTag Ligand (HTL), which can then act on endogenous neuronal receptors on the membrane. Ideally, this unique technology can be extended to pharmaceutical tools to modulate different voltage-gated ion channels. Specifically, voltage-gated sodium channels are the main mechanism supporting action potential (AP) firing in the central nervous system. We developed new JHNaV$^{DART.2}$ variants based on established sodium channel blockers and investigated the cell-specific effects of these DART variants on voltage-gated sodium channels. Using whole-cell patch recordings, we recorded voltage-gated sodium currents and AP firing in cultured hippocampal neurons expressing the HTP construct. We found that several new JHNaV$^{DART.2}$ variants successfully reduced voltage-gated sodium currents with a broad range of voltage. As a result, the APs firings were markedly diminished by these reagents. In contrast, a control DART (blank$^{DART.2}$) with no tethered drug resulted in no impact of voltage-gated sodium current and APs firings. In summary, our newly created JHNaV$^{DART.2}$ reagents offer cellular specific modulation of voltage-gated sodium channels and neuronal firing, providing a unique approach to cell-type and ion channel specificity in future studies on cellular excitability, animal behavior, and neuronal disease.

Disclosures: H. Yan: None. **Y. Oh:** None. **B.C. Shields:** None. **P. Jeong:** None. **J. Hong:** None. **M.R. Tadross:** None.

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.19/X7

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant DP2-MH119425
Duke University RECA 2022/0059

Title: Engineering the Reversibility of HaloTag Protein for Versatile Neuro-applications

Authors: *L. LI, V. GOLDENSHTEIN, B. C. SHIELDS, M. R. TADROSS;
Biomed. Engin., Duke Univ., Durham, NC

Abstract: In neuropharmacology, the Drug Acutely Restricted by Tethering (DART) system enables rapid and cell type-specific neuronal manipulation by integrating traditional pharmacology and genetically encoded tools. Based on the HaloTag system, DART facilitates the covalent capture and accumulation of drugs tethered to the HaloTag ligand on the cell surface where the HaloTag Protein (HTP) is expressed. The covalent capture creates a stable local concentration difference between HTP positive and HTP negative cells, however, covalent capture prevents sequential drug delivery for the same cell or the same animal over an acute time frame. Therefore, reversible DART is required for diverse neuro-applications. To achieve reversibility, we aim to leverage the inherent ligand hydrolysis from the original HTP enzyme. Via N272H mutation, HTP is able to hydrolyze the covalent bond and release the ligand. Although reversibility is achieved by the single mutation, this variant only holds the ligand for ten minutes, which is too short for some in-vivo applications. To slow down the hydrolysis rate of HTP, we used directed evolution to engineer the HTP and select for variants with a longer protein-ligand intermediate half-life. We targeted six residues close to the His272 which have direct influence on the hydrolysis rate. The protein library was created using site saturated mutagenesis on those residue locations. After performing iterative rounds of evolution, the selected variants were screened using a bacterial surface capture assay and further characterized by Surface Plasmon Resonance. By altering the hydrolysis rate of the HTP, we will be able to provide a versatile platform for temporally controlled manipulation of neuronal activity using reversible DART, paving the way for innovative therapeutic and neuroscientific applications.

Disclosures: L. Li: None. **V. Goldenshtein:** None. **B.C. Shields:** None. **M.R. Tadross:** None.

Poster

PSTR309: Single-Cell Techniques

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR309.20/X8

Topic: I.08. Methods to Modulate Neural Activity

Support: Duke-Coulter Translational Partnership
NIH Grant R33-DA051530

Title: Covalent attachment of neurons to electrodes for improved selectivity, sensitivity, and stability for in-vivo electrophysiology

Authors: *L. A. LIGONS[1], U. GHOSH[1], B. C. SHIELDS[2], M. R. TADROSS[2];
[2]Biomed. Engin., [1]Duke Univ., Durham, NC

Abstract: Recent decades have seen remarkable strides in neural activity recording, driven primarily by advancements in tools such as genetically encoded voltage/calcium indicators (GEVIs/GECIs). However, the integration of these optical methods faces tradeoffs between temporal resolution and spatial scale. Multi-electrode arrays (MEAs) have been introduced to address these challenges, but conventional MEAs encounter limitations in cell and cell-type specificity, as well as stable long-term cell registration. To overcome these obstacles, we introduce Electropolymer that Genetically Links Neurons to Electrodes (ElectroGLuE), a genetically encoded coating designed to enhance electrodes for long-term, cell-type-specific electrophysiology recordings and single-cell closed-loop stimulation. ElectroGLuE operates by establishing a covalent linkage between neurons expressing the HaloTag Protein and implanted electrodes coated with novel chemically synthesized HaloTag Ligand (HTL) and EDOT (3,4-Ethylenedioxythiophene). In this study, we present a systematic investigation, including ex-vivo experiments validating the polymerization of ElectroGLuE onto NeuralThread ultra-flexible nanoelectronic thread (NET) probes through scanning electron microscopy (SEM) imaging, as well as assessing its stability and biocompatibility through impedance spectroscopy and other innovative techniques. Additionally, we conducted pilot experiments in the mouse visual cortex to confirm the stability of ElectroGLuE in vivo. By addressing the limitations of existing methods, ElectroGLuE aims to significantly contribute to the advancement of neural recording and manipulation techniques, providing cell specific registration with increased sensitivity and long-term stability.

Disclosures: L.A. Ligons: None. **U. Ghosh:** None. **B.C. Shields:** None. **M.R. Tadross:** None.

Poster

PSTR310: Electrical Tools for Neuronal Probing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR310.01/X9

Topic: I.04. Physiological Methods

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NIH 1RF1NS12866701
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Title: Subcellular carbon fiber electrodes for intracortical neural recording: towards large animal models

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Abstract: Brain machine interfaces show promise in restoring neurological function, but current neural-recording electrodes are limited by low density, neuronal death, glial scarring, and material lifespan (Patel 2023, Sponheim 2022). 8.4 μm diameter carbon fibers (CFs) can be used as biocompatible and chronically stable electrodes (Welle JNE 2020), but their subcellular scale poses a challenge for mechanical insertion due to fiber buckling and brain dimpling. Our group has successfully implanted arrays of up to 16 CFs in rats (Patel JNE 2015), but large mammal validation is desired to transition CFs into humans. First, to test if CFs could record in large mammal brain, 3 High Density Carbon Fiber (HCDF) arrays of 8 or 16 CFs (8.4 μm diameter, blowtorch-sharpened, parylene C-coated, PEDOT:pTS-coated tips, spaced 80 or 160 μm apart) were inserted into the right parietal cortex of an anesthetized rhesus macaque. Compared to the 8-CF array, dimpling was increased in the 16-CF array and decreased after pial dissection. At peak-yield insertion depth, 29/35 functional electrodes recorded a minimum of 10 bipolar waveforms of 100 $\mu V_{peak\text{-}peak}$ amplitude, and under 1.5 ms duration. Second, to test if a larger array could penetrate large mammal brain through intact pia, we designed a 10 x 10 array (1.5 mm, 10 μm diameter, at 400 x 500 μm spacing, with 92 CFs after 8 fiber breakages before a silicone layer at the base was added for strain relief). CFs were inserted into 0.6% agarose (N=9 insertions), polyvinyl chloride (PVC, N=8) and ex-vivo sheep cortex (N=14) to an approximate depth of 1 mm at 0.1, 1 and 5 m/s insertion rates. Insertion of a 100-shank Utah electrode array (UEA) was tested for comparison. The CF array successfully inserted into agarose, PVC, and ex-vivo sheep with a force (mean ± SD) of 2.9 ± 0.3, 6.4 ± 1.6, and 10.1 ± 1.2 mN at 0.1 m/s and 4.8 ± 0.2, 6.0 ± 1.8, and 9.4 ± 2.4 mN at 1 m/s respectively with minimal dimpling. The UEA inserted into agarose with larger forces of 32.8 ± 4.0, and 43.6 ± 5.6 mN at 0.1 and 1 m/s respectively, and did not fully insert into PVC or sheep brain without forces exceeding the 10 g load cell capacity and causing significant dimpling. After 101 insertions, the CF array showed breakage of 2/92 CFs. These results suggest that CF arrays can penetrate large mammal leptomeninges without pneumatic impaction despite their small scale, and validate the recording capabilities of CFs. Together, these have implications for a clinically viable, high-channel-count,

intracortical implant. Future work involves chronic in vivo testing in large mammals, and validation of insertion and recording in humans.

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Poster

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Topic: I.04. Physiological Methods

Support: NIH Grant NS126046

Title: Development of surgical insertion methods for flexible neural probe arrays to reach deep brain targets

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Abstract: Penetrating polymer-based microelectrode arrays (MEAs) have gained popularity for long-term high-quality recordings of dynamic neural activity. Unlike metal wire and silicon MEAs, penetrating polymer-based MEAs exhibit improved device-tissue interface stability. However, implanting them in deeper brain regions is challenging as they lack axial stiffness. This study investigates and compares three insertion methods to achieve accurate implantation of polymer MEAs > 5 mm deep (dip coating, lamination, and insertion shuttles). Agarose gels (0.6%) were used as brain phantoms. To simplify the study, polymer MEA cutouts (not containing electrodes) were used for insertion testing. These shams were attached to a motorized frame and lowered into load cell mounted agarose gel blocks, enabling simultaneous force measurements. Shams were fabricated using Parylene C and polyimide thin films. Single shank and shank arrays (2, 4, 8 shanks) having different designs (5.5 – 20 mm long) were evaluated. The dip coating method entailed applying a thin layer of water-soluble polyethylene glycol (16 – 29 µm and 9 – 23 µm thick on Parylene and Polyimide probes) to shams then removing the coating at the tip, leaving up to 2 mm exposed length. Molten polyethylene glycol (PEG; 3350 MW) was applied with different thickness by controlling the retraction speed (0.1 – 1.5 mm/s). This method shortens the exposed length to achieve an increase in buckling force of the shanks according to Euler's equation. The lamination method aligns identical Parylene probes to double the overall thickness and increase the buckling force by 8×. Buckling force is further increased

by dip coating with PEG (3350/8000MW) with a controlled retraction speed (1.5 – 2.6 mm/s) to achieve uniform coatings (17 – 156 μm thick). Finally, tungsten wire (81 μm diameter) was used as an insertion shuttle and shams were attached using the surface tension of a PEG aqueous droplet (10%w/v; 35000 MW). The insertion shuttle was removed after placing the sham (retraction speed of 1 mm/s) and the tip displacement caused by shuttle retraction was measured (57 – 65 μm). All shams were successfully inserted into gels to their full depth though three different methods. Dip coating, lamination, and insertion shuttle methods can each achieve accurate placement up to 20 mm deep.

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Program #/Poster #: PSTR310.03/X11

Topic: I.04. Physiological Methods

Support: NIH Grant GR1061143

Title: A microfabricated Parylene cuff electrode for branched nerve stimulation

Authors: *A. ESTEBAN-LINARES[1], B. THIELEN[1], Q. REZARD[1], A. PETROSSIANS[2], J. WELLS[3], J. COATES[3], S. ELYAHOODAYAN[1], D. SONG[1], V. PIKOV[4], E. MENG[1]; [1]Biomed. Engin., USC, Los Angeles, CA; [2]Platinum Group Coatings, Pasadena, CA; [3]Med-Ally, Goose Creek, SC; [4]Medipace Inc, Pasadena, CA

Abstract: Peripheral nerve interfaces provide a therapeutic avenue for bioelectronic medicine applications and pain management, typically employing small electrodes on a target nerve. Minimally invasive interfaces, notably cuff electrodes, wrap around the nerve circumference to avoid nerve trauma and extend implant longevity compared to more invasive nerve interfaces that breach the epineurium and/or perineurium. However, existing designs produced using thick polymer substrates are not suitable for abdominal vagal nerve branches, which are desirable therapeutic targets in bioelectronic medicine due to their proximity to and greater selectivity in controlling internal organs.
We developed a thin-film cuff electrode suitable for stimulating sub-millimeter diameter nerves. This thin film cuff comprises a metal layer sandwiched between two insulating layers of Parylene C polymer and is enabled by advanced microfabrication methods. The structured and cut-out thin films are fixtured and thermoformed into a soft-closing and self-sizing cuff

configuration. We systematically assessed the electrochemical performance of the electrode sites with and without PtIr coatings to meet charge injection capacity requirements.
Concurrently, we developed a novel interconnect component that permits the cuff electrodes be mechanically and electrically connected to standard clinical-grade leads for interfacing with implantable pulse generators. The interconnect is a rigid substrate consisting of metal pads patterned on an adhered patterned platinum foil that allows reliable attachment of the lead wires (using welding) and thin film electrodes (using low temperature bonding).
We designed several cuff placement tools for cuff deployment using different surgical techniques (e.g. open surgery and laparoscopic surgery). Placement and stimulation will be evaluated in acute experiments using the rat sciatic nerve. Additionally, benchtop accelerated lifetime testing will be conducted to evaluate device longevity prior to chronic animal experiments. We report current results on cuff development towards the realization of an open-source implantable cuff electrode capable of targeting sub-millimeter abdominal vagal nerve branches.

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PSTR310: Electrical Tools for Neuronal Probing

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Program #/Poster #: PSTR310.04/X12

Topic: I.04. Physiological Methods

Support: Paul G. Allen Foundation

Title: Tools for mouse behavior and neurophysiology at the Allen Institute for Neural Dynamics

Authors: *A. CAHOON[1], J. JUNG[1], N. MARS[1], S. VASQUEZ[1], H. F. RODRIGUES[1], H. SMITH[1], A. GUTHRIE[1], C. BENNETT[1], A. A. LAKUNINA[1], T. OÑA JODAR[1], C. POO[1], J. Y. COHEN[1], C. FARRELL[2], K. SVOBODA[1], S. TURKYILMAZ[1];
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Abstract: The Scientific Instrumentation and Process Engineering (SIPE) team closely collaborates with and supports scientists at the Allen Institute. Here, we share four hardware advancements that we developed for the Allen Institute for Neural Dynamics and are currently being used in head-fixed mouse foraging decision-making tasks with *in vivo* neural recordings. The standardized mouse head-fixation system, initially designed for the Allen Institute Brain Observatory, has been adapted to accommodate projects in the Allen Institute for Neural Dynamics. We present the design process, criteria, and templates for a suite of new headframe designs. We also describe how we applied this method to develop new headframes that are used

for brain-wide electrophysiology and optical physiology in areas outside of the visual cortex. These headframes retain key components of the original design for the Allen Institute Brain Observatory and utilize new manufacturing techniques that facilitate increased design complexity, rapid prototyping, and low-volume manufacturing.
Closely related to the newly developed brain-wide electrophysiology headframes is a new variation of the standard cranial window that consists of a 3D-printed implant, “SHIELD”. We extend the SHIELD and manufacture a dual-hemisphere version which allows researchers to make simultaneous Neuropixels recordings from multiple regions distributed across the brain. We detail how we overcame challenges spanning the sagittal suture with an implant, in addition to design and manufacturing guidance for placing holes for probe insertion.
The new headframes and implants are complemented by a redesigned 6-inch running wheel equipped with an integrated encoder, variable resistance brake, and torque transducer that is controllable with a new HARP board for data acquisition. We present the apparatus, performance characteristics, and design files.
Lastly, the new foraging tasks required an updated lickspout stage that added a fourth axis for accommodating a second lickspout or an odor delivery nozzle. The dual lickspout configuration enables real-time and rapid movement (up to 100 m/s), while the odor delivery configuration enables the independent adjustment of the lickspout from the nozzle. We present on the stage's uses and applications along with performance characteristics and design files.

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Topic: I.04. Physiological Methods

Support: NSF CAREER #1943716
MSU Discretionary Funding Initiative

Title: Changes in gene expression at the single-cell level following the implantation of subcellular, ultra-flexible devices in the rat motor cortex

Authors: *L. C. KLEYN[1], C. H. THOMPSON[2], M. REIMERS[3], E. K. PURCELL[4];
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Abstract: Implantable electrodes with recording and stimulating capabilities have proven useful in both research and clinical settings. Specifically, differences between normal and pathological states can be investigated to determine their utility in therapeutic interventions for various neurological disorders and injuries. Following implantation, biological responses (i.e., 'foreign body response', glial encapsulation of the electrode, neuronal loss) have been observed through traditional immunohistochemistry techniques that are thought to be associated with the long-term instability of such electrodes. Various aspects of device design (i.e., Young's modulus, bending stiffness, materials, device dimension, architecture) contribute to these responses, but it is unclear as to which aspects of device design are specifically associated with which biological response(s). Analyzing these biological responses following implantation through immunohistochemistry has provided a foundational understanding of how the body responds to such devices, but these analyses are limited to a few pre-selected markers in each experiment. To build upon these traditional analyses, we propose using single-cell spatial transcriptomics alongside immunohistochemistry to characterize the changes in gene expression at a single-cell level following the implantation of devices with varying design properties. Specifically, we compared the biological responses observed between devices of different material compositions (silicon vs. polymer-based electrodes, including devices with state-of-the-art ultraflexibility) with different time points of recovery (1 week vs. 6 weeks). It is hypothesized that subcellular designs (≤10 µm) with ultra-flexibility (i.e., high bending stiffness and a low Young's modulus) will alter the expression of genes associated with more traditional device designs. Through the combined use of traditional immunohistochemistry techniques and single-cell spatial transcriptomics, we aim to build upon the foundational knowledge regarding the biological response following device implantation with the ultimate goals of (1) improving the chronic biocompatibility of such devices and (2) informing the development of effective therapeutic interventions.

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Topic: I.04. Physiological Methods

Support: NIH Grant R01NS116080

Title: Enhanced Neurotransmitter Detection Using Multichannel Boron Diamond Microelectrodes and Fast-Scan Cyclic Voltammetry

Authors: *J. SIEGENTHALER[1,2], V. ÖRNBRATT[1], B. KEPROS[1], B. GUPTA[3], M. PERILLO[4], G. BANNA[2], R. RECHENBERG[1], M. F. BECKER[1], E. K. PURCELL[4,3], W. LI[4,2,1]; [1]Fraunhofer USA, Inc., Ctr. Midwest, East Lansing, MI; [2]Electrical and Computer Engin., [3]Neurosci. program, [4]Biomed. Engin., Michigan State Univ., East Lansing, MI

Abstract: Neurodegenerative diseases such as Parkinson's and Alzheimer's affect millions of people globally. Carbon fiber microelectrodes have been extensively utilized using fast-scan cyclic voltammetry (FSCV) to measure and quantify both dopamine (DA) and serotonin (5-HT) release in real-time. One challenging aspect for microelectrodes for neurotransmitter measurements is that they are hand-fabricated individually, a tedious and highly variable process. Here, we report on our efforts to use boron-doped diamond (BDD) electrodes for neurochemical measurements as an alternative material for long-term chemical measurement. BDD is a versatile material that has been shown to have excellent biocompatibility, a wide working potential window in aqueous solutions, and has been shown to be an excellent material to study electrochemical systems. Through wafer processing, electrode variability is avoided, and batches of identical electrodes can be produced reproducibly. We fabricated freestanding BDD microelectrodes insulated with polycrystalline insulating diamond, both as single and multichannel (4 and 8 channels) single-shank Michigan-style microelectrodes. We characterized the impedance and electrochemical response of the electrodes using slow cyclic voltammetry and studied the effect of surface termination on electrode response. We studied how etching the BDD surface can increase the electroactive area and how the response changes for neurotransmitter measurement. We then characterized the electrodes' response to several common neurotransmitters, including dopamine, serotonin, and hydrogen peroxide, using FSCV, showcasing the linear dynamic range, detection limit, and noise of these electrodes. We also report on the usage of these electrodes for physiological measurements as well as circuit modifications necessary for FSCV headstages to enable multichannel, individually addressable waveforms. Using all-diamond electrodes for neurotransmitter analysis is advantageous as it is the gateway towards customized wafer batch fabrication of microelectrodes, thus decreasing both errors generated in the traditional hand fabrication methods and building towards a scalable batch method for electrode array technologies.

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Topic: I.04. Physiological Methods

Support: NIH Grant 5R44MH119870-04

Title: Enzyme-modified carbon fiber electrodes for the measurement of glucose and lactate in the brain using fast-scan cyclic voltammetry

Authors: K. TURNER[1], K. LINDER[1], D. AILLON[2], S. KAPLAN[2], *D. JOHNSON[2];
[1]North Carolina State Univ., Raleigh, NC; [2]Pinnacle Technology, DE LLC, Lawrence, KS

Abstract: This work describes 7 µm enzyme-modified carbon fiber electrodes (EM-CFEs) for sensing glucose and lactate *in vivo*. This technology was developed by Dr. Leslie Sombers at North Carolina State University and is being commercialized by Pinnacle Technology DE LLC (Lawrence, KS). The new electrode is formed on a 7 µm carbon fiber (100 µm length) in a pulled glass capillary. Enzyme (glucose oxidase or lactate oxidase) is electrochemically deposited as a hydrogel on the electrode surface to functionalize the carbon fiber for measurement of glucose or lactate. Enzymatically produced hydrogen peroxide is detected using Fast Scan Cyclic Voltammetry (FSCV, -0.2 to 1.4 V 400 V/S, 10 Hz). Glucose and lactate EM-CFEs demonstrate a linear response to analyte to over 2 mM glucose and 500 µm lactate (respectively). Glucose and lactate sensitivities are 16.7 ± 4.3 nA/mM and 6.6 ± 1.7 nA/100 µM respectively. Both new EM-CFEs have been tested to survive shipping and are stable for over 3 weeks. EM-CFEs were characterized *in vitro* using a flow cell and *in vivo* in anesthetized sprague-dawley rats. EM-CFEs were implanted targeting the dorsal striatum. A bipolar stimulating electrode was implanted in the ventral midbrain. Lactate and glucose (and dopamine) were evoked by electrical stimulation (60 Hz, 100 to 400 µA, 1ms pulse width) for 1 to 2 seconds. Voltammetric recording and electrode stimulation were controlled using Sirenia FSCV software (Pinnacle Technology).

Disclosures: D. Aillon: A. Employment/Salary (full or part-time):; Pinnacle Technology DE LLC. **S. Kaplan:** A. Employment/Salary (full or part-time):; Pinnacle Technology DE LLC. **D. Johnson:** A. Employment/Salary (full or part-time):; Pinnacle Technology DE LLC.

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Program #/Poster #: PSTR310.08/X16

Topic: I.04. Physiological Methods

Support: Paul G. Allen Foundation
Simons Collaboration on the Global Brain Transition to Independence Award (Poo: 863738)

Title: Lickety-split: a high-speed, noninvasive, capacitive lick detection system for behavior and electrophysiology

Authors: *S. VASQUEZ[1,2], B. CRUZ[3], T. OÑA JODAR[3], Y. I. BROWNING[3], X. YIN[3], K. JUNG[3], Z. SU[3], H. D. NGUYEN[3], G. F. LYNCH[3], H. F. RODRIGUES[3], J. Y. COHEN[3], K. SVOBODA[3], C. POO[3], J. H. SIEGLE[3];
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Abstract: Lick detection is a widely used method for measuring decisions and actions in head-fixed mouse behavior experiments. Concurrently, high-density electrode arrays such as Neuropixels offer an unprecedented ability to read out neural activity on a brain-wide scale. However, most lick detection methods either create unwanted artifacts in electrophysiological signals or make measurement compromises to eliminate these artifacts. Current lick detector designs are either (1) spatially cumbersome, requiring substantial real estate for cameras, mirrors, or beam-breaking devices, (2) electrically noisy, introducing transient voltages that interfere with electrophysiology, or (3) low-speed, requiring integration over cycles of mechanical vibrations to trigger a response. To overcome these limitations, we developed a novel electronic lick detection system, dubbed “Lickety-Split,” capable of detecting licks within 1 millisecond while remaining invisible to electrophysiology recordings. We do so by measuring changes in capacitance from a 100 kHz, 200 nA excitation signal using a self-contained embedded system. Our system requires only that a single wire be attached to a conductive water-dispensing lick tube. Each lick triggers a TTL signal sent via a BNC connector. Additionally, timestamped messages are made available via the Harp (USB serial) protocol. We present device performance metrics collected from behavioral experiments both with and without simultaneous electrophysiology. Finally, our system hardware and firmware are open-source (CERN-OHL-P and MIT, respectively) and can be readily manufactured in single units or at scale for less than $500. Lickety-Split is easy to adopt and makes it possible for any lab to combine lick detection and large-scale electrophysiology with minimal latencies and zero impact on the quality of recorded signals.

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Poster

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Topic: I.08. Methods to Modulate Neural Activity

Support: Bonsai Foundation CIC
Paul G. Allen Funds
Champalimaud Foundation

Title: Harp: A standard for self-synchronizing and event-driven hardware for neuroscience research

Authors: *B. F. CRUZ[1], F. CARVALHO[2], J. FRAZAO[3], G. LOPES[3], A. SILVA[4], S. VASQUEZ[5];
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Abstract: Observation, measurement, and control of discrete neural and behavioral events are a foundational tenet for understanding brain function. Nonetheless, efforts to promote the adoption of a common data acquisition framework have been largely unsuccessful. This is due, in part, to a lack of standardization across devices, acquisition software, and data formats, which creates a substantial overhead for scaling experiments, quality control, and sharing of experimental data. Here, we present Harp, an open-source standard for real-time data acquisition and experimental control in neuroscience. It addresses key limitations of previous approaches by defining a lightweight yet flexible binary protocol for communication between devices, clear conventions for hardware-level synchronization, and hardware implementation templates for creating new devices. A key advantage of Harp, in comparison to other systems for behavioral control, is the ability to synchronize multiple devices to a distributed clock. Harp devices are synchronized at the hardware level, allowing temporally precise logging of events with < 64 μs precision. Commands and events are hardware timestamped by each device's microcontroller and relayed back to the computer over USB with an average round-trip time of < 2 milliseconds. Thus, all data streams are temporally aligned before reaching software, even if they are acquired by different Harp devices connected to different computers. Furthermore, this allows Harp devices to leverage asynchronous hardware interrupts for efficient control flow, communication, and logging. We showcase the power and flexibility of the Harp ecosystem via integration with the Bonsai programming language to enable rapid prototyping of multi-modal, closed-loop experiments while maintaining precise temporal control and efficient logging of several parallel data streams. Because data collected across all devices are automatically aligned at acquisition time, and logging formats are standardized, this further eliminates the need for complex post-hoc data synchronization and thus greatly accelerates the analysis and exploration of behavioral and neural data. Altogether, Harp represents a significant advancement in experimental neuroscience instrumentation by providing researchers with a standard and general solution for monitoring and controlling behavior apparatuses. Adoption of the ecosystem will facilitate the sharing of resources and reproducibility across both experimental and analysis pipelines.

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Poster

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Topic: I.04. Physiological Methods

Support: NSF (Award # 2024270)
NIH (Award # R01NS116080)

Title: Quantitative Study of Insertion Mechanical Properties of Implantable Neural Probes

Authors: *Z. JIANG[1,2], X. LIU[3,2], Y. GONG[1,2], M. KAFI KANGI[1,2], A. CREECH[4,2], G. BANNA[1,2], W. LI[1,2];
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Abstract: Implantable neural probes are essential tools in modern neuroscience for effectively stimulating and recording neural activity. However, acute tissue damage occurring during penetration could trigger foreign body reactions in brain tissue. This tissue damage is attributed to the insertion forces experienced during the penetration process. To minimize acute tissue damage and enhance compatibility between neural probes and brain tissue, potential strategies involve optimizing the insertion parameters (e.g., speed, vibration, etc.) as well as material and device designs of implantable neural probes. Herein, the force exerted on brain tissue during insertion is utilized as an objective metric for assessing induced tissue damage. This study reports a fourth-degree polynomial correlation between insertion speed and insertion force of neural probes within a large range of insertion speeds (from 0.01 mm/s to 2 mm/s), with inflection points observed around speeds of 0.5 mm/s, 1.2 mm/s, and 1.8 mm/s. Additionally, the impact of different materials with varied Young's modulus (from 1500 kPa to 1200 GPa), including boron-doped diamond (BDD), on insertion force of neural probes was investigated. COMSOL finite element simulation results also indicate potential influences of different brain micromotion on foreign body reactions. This study addresses the deficiency in prior research by supplementing quantitative investigations into acute tissue damage due to a broad range of insertion speeds and probe material properties.

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Topic: I.04. Physiological Methods

Support: NIH Grant (R00MH120279)
NSF Career (CAREER, 2239030)
Brain&Behavior Research Foundation Young Investigator Grant (29878)

Title: Soft and Multifunctional Hydrogel Probes for Neuromodulation and Recording

Authors: *S. HUANG[1], S. RAO[2], Q. WANG[3];
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Abstract: Soft and Multi-functional Hydrogel Probes for Neuromodulation and RecordingAuthors Sizhe Huang[1], Qianbin Wang[1, *], Siyuan Rao[1, *].[1, *]Department of Biomedical Engineering, State University of New York at Binghamton,Binghamton, NY 13902, United States**Disclosures**Sizhe Huang: None. Qianbin Wang: None. Siyuan Rao: None.**Abstract**Neural circuits rely on the complex interplay of electrical, chemical, and mechanical signals atdifferent levels, making it challenging to measure or manipulate these diverse signalssimultaneously in vivo. Neural probes with multifunctional capabilities integrated into one devicehelp provide a holistic understanding of these complex neural activities. By utilizing semi-crystalline polyvinyl alcohol hydrogels, we developed soft optical probes with tunable refractiveindex (RI: 1.37-1.40 at 480 nm) and high light transmission (>96%). These probes also havetunable mechanical properties, including stretchability (139-169%), bending stiffness (4.6 ± 1.4N/m), and elastic modulus (2.8-9.3 MPa). The Control of Metamorphic Polymers' Amorphous-Crystalline Transition (COMPACT) strategy maintains the designed dimensions andminiaturization of the probes in vivo. Surface deposition of nanomaterials on hydrogel fibersfurther enhanced the stability of the hydrogels in vivo, with stable diameter changes (10.2 ± 5.8%)under accelerated physiological conditions (45°C, pH=7). By introducing high-aspect-ratioconductive nanofillers into a polymer matrix and using external stretching, we created electricallyanisotropic percolation pathways in hydrogel microelectrodes (specific impedance: 3.64-7.09kΩ·mm). The COMPACT strategy also allows the integration of multiple components into aminiaturized device, enabling bidirectional optical and electrical recordings. These soft neuralprobes have been validated through in vivo fiber photometry recording and mouse behavioralassays. They also facilitate electrophysiological recording of spontaneous neural activity (signal-to-noise ratio: 3.73) and light-evoked electrical signals in the brain, spinal cord, and hindlimbmuscles. The adaptability of these hydrogel neural probes to micro-motion permitted recording innaturally behaving mice.

Disclosures: S. Huang: None. **S. Rao:** None. **Q. Wang:** None.

Poster

PSTR310: Electrical Tools for Neuronal Probing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR310.12/X21

Topic: I.04. Physiological Methods

Support: CrossBrain project HORIZON-EIC-2021-PATHFINDERCHALLENGES-01- 02 (GA 101070908)
This work was carried out within the framework of the project "RAISE - Robotics and AI for Socio-economic Empowerment" and has been supported by European Union - NextGenerationEU

Title: Crossbrain: in-vivo validation of highly integrated circuits for self-standing implantable micro-scale devices (µBots)

Authors: *G. ANGOTZI[1], J. F. RIBEIRO[1], G. ORBAN[1], E. SALEMI[2], A. PERNA[1], J. LOCHE[3], F. BARREIRA[3], A. ABARCA[3], J. PITEIRA[4], L. BERDONDINI[5];
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Abstract: Recent years have witnessed remarkable strides in neural recording technologies, facilitating unprecedented insights into the intricacies of the brain's function. In this framework, circuit solutions that not only offer small form factors but also prioritize low-noise performance are paramount to access high fidelity neural recording and promote chronic stability. Furthermore, to gain insight into distributed neural computations underlying decision making and complex behaviors, advancements in experimental methods that also allow manipulation of neural population dynamics is necessary. Having the possibility of perturbing neural circuits at cellular level with sub-millisecond temporal resolutions would indeed permit to further elucidate properties of brain circuits and determine causal circuit roles.To meet these pressing needs, in CROSSBRAIN we aim at creating self-standing, microscale devices (µBOTS) measuring 100x100x100 µm³ that integrate circuits for neural activity sensing, and modulation, as well as miniaturized wireless power and communication technologies. Here we will report on ongoing activities related to the development of highly integrated front-end CMOS circuits for broadband recordings and neuro-modulation that fit the requirements of these microscale wireless µBOTS. Key features of our circuit solutions include optimized layout methodologies, and meticulous attention to component selection and sizing, resulting in a compact yet highly efficient neural frontend. Notably, our solution is characterized by its adaptability to large electrode-tissue DC offset (up to 500mV) without the need of large DC blocking input capacitors widely used in classical AC coupled architectures.A prototype integrating multiple instances of the proposed neural frontend was fabricated in a standard 180nm CMOS technology. We will report

comprehensive characterization of individual µBOTS. Finally, to assess performances to capture and modulate neural activity, a multichannel neural frontend solution was realized and used to interface multichannel silicon probes for in-vivo neural recordings in mice.

Disclosures: G. Angotzi: A. Employment/Salary (full or part-time):; Corticale Srl. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Corticale Srl. **J.F. Ribeiro:** None. **G. Orban:** None. **E. Salemi:** None. **A. Perna:** None. **J. Loche:** None. **F. Barreira:** None. **A. Abarca:** None. **J. Piteira:** None. **L. Berdondini:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Corticale Srl.

Poster

PSTR310: Electrical Tools for Neuronal Probing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR310.13/X22

Topic: I.04. Physiological Methods

Support: ChroMOS project H2020-MSCA-2019 (GA 896996)
This work was carried out within the framework of the project "RAISE - Robotics and AI for Socio-economic Empowerment" and has been supported by European Union - NextGenerationEU

Title: "Microwire-like" Cmos-based sinaps neural probe and its chronic performance

Authors: A. PERNA[1,3], G. ORBAN[1], C. STUBBENDORFF[1], M. VINCENZI[1], G. N. ANGOTZI[1,4], L. BERDONDINI[1], ***J. RIBEIRO**[2];
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Abstract: Achieving chronic mechanical and electrophysiological recording stability while maximizing the information gleaned from multiple brain areas in a single experiment are important challenges in the field of neurotechnology. Flexible substrates (e.g. polyimide or parylene) demonstrated the capability to enhance the long-term stability of intracortical implants by minimizing the mechanical mismatch between the implant and the hosting brain tissue. However, these devices display limitations in the maximum electrode density they can achieve. CMOS-based active neural probes can drastically increase the number of electrodes per sensing area. Further, this emerging technology enables to explore novel layouts of implants with optimal size and geometry to minimize acute tissue damage and Blood Brain Barrier (BBB) disruption to reduce foreign body reaction (FBR). In particular, SiNAPS probes technology was

shown to enable continuous recordings from up to 1024 densely packed electrodes (pitch <30 µm) at 20 kHz/channel. This is achieved by integrating into each electrode-pixel individual small area frontend amplifiers and on-probe time-division-multiplexing circuits, while keeping the shank width <90 µm.Here, we investigate very small cross-section CMOS-based SiNAPS probes with respect to their performances in enhancing chronic stability while maintaining a high number of electrodes per implant area. To address this challenge, we developed a "microwire-like" CMOS-based neural probe named ChroMOS probe. The realized probe prototype has a shank width and thickness of 26 µm. Although the very small shank size, we were able to integrate 64 electrode-pixels in a single column configuration. We will report chronic recordings performed with such ChroMOS neural probe and results of its mechanical stability. A comparison with commercial SiNAPS neural probes will also be presented and discussed in terms of recording performances and foreign body reaction (FBR) through histological studies.

Disclosures: A. Perna: None. **G. Orban:** None. **C. Stubbendorff:** None. **M. Vincenzi:** None. **G.N. Angotzi:** A. Employment/Salary (full or part-time):; Corticale Srl. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Corticale Srl. **L. Berdondini:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Corticale Srl. **J. Ribeiro:** None.

Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.01/X23

Topic: I.04. Physiological Methods

Support: JSPS Grant-in-Aid for Early-Career Scientists (JP19K17070)
Meiji Yasuda Mental Health Foundation
Mochida Memorial Foundation for Medical and Pharmaceutical Research

Title: Assessment of Postoperative Delirium Risk Using Resting EEG Phase-Amplitude Coupling

Authors: *N. ARAI[1,2], T. MIYAZAKI[3], S. NAKAJIMA[3], S. MORIYAMA[3], M. WADA[4,2], S. BOKU[1], M. TAKEBAYASHI[1], M. MIMURA[3], Y. NODA[3];
[1]Kumamoto Univ., Kumamoto-city, Japan; [2]Keio University School of Medicine, Tokyo, Japan; [3]Keio Univ. Sch. of Med., Tokyo, Japan; [4]Stanford Univ., Mountain View, CA

Abstract: Postoperative delirium (POD) is a risk factor for dementia and increased mortality. Preoperative prediction of POD would allow early and effective prevention of delirium. Phase-amplitude coupling (PAC) of electroencephalography (EEG) reflects the efficiency of

information processing through the interaction of neural activity over different frequency rhythms. It has been used to assess brain function in various neuropsychiatric disorders. This study investigated whether preoperative EEG PAC could predict POD. The present study was approved by Keio University School of Medicine Ethics Committee and was conducted in accordance with the Declaration of Helsinki. We recruited 71 patients scheduled for surgery. Participants underwent preoperative resting state EEG. EEG signals were segmented into δ to γ frequency bands. The modulation index (MI), a PAC indicator, was calculated for 10 phase-amplitude frequency pairs. The mean MI across 19 EEG electrodes for each pair was calculated and statistically analyzed. Corrections were made for multiple comparisons. Significant MI values were averaged and modeled using logistic regression to predict POD. The accuracy of the model was assessed using a receiver operating characteristic (ROC) curve. We compared MI between 18 subjects who developed delirium and 53 who did not. Among background factors, only years of education was significantly lower in the delirium group than in the non-delirium group. The δ-phase β-amplitude MI (δ-β MI) was significantly lower in the delirium group than in the non-delirium group. Further testing was performed on each electrode. A significant group difference was found for six of the 19 electrodes. As a result of ROC analysis, a model using the mean δ-β MI of these electrodes and years of education was able to predict POD with moderate accuracy. Since δ-β coupling may be related to stress and anxiety regulation and time prediction, the reduced δ-β MI may indicate a preparatory state for delirium. Significant changes at electrode sites Fp1, F3, F4, and F7 may reflect altered dorsolateral prefrontal cortex function, consistent with previous functional MRI studies that identified abnormalities in this region as a predisposing factor for delirium. The δ-β MI of resting state EEG in the prefrontal area may serve as a potential neurophysiological marker to predict POD preoperatively. Further validation in larger populations is needed.

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Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.02/X24

Topic: I.04. Physiological Methods

Support: Weill Neurohub Pillar Grant
Simons Collaboration on the Global Brain
Weill Neurohub RFP
NIH P51 OD010425
University of Washington Royalty Research Fund

Title: Measuring functional connectivity across days using optogenetic stimulation

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Abstract: Introduction: Estimating network connectivity is important for studying learning because connectivity can change during learning [1] and may influence learning computations [2]. However, currently available *in vivo* measurements are either limited in accuracy or statistical power. Correlations of activity across brain areas, for example, suffer from the common input problem, leading to inaccurate estimations. Network mapping using stimulation improves accuracy by directly measuring whether a signal at one site is received at another. However, current measurement techniques using electrical stimulation lack spatial specificity and have limited statistical power to detect weak but meaningful functional network connections. We address these limitations by introducing a phase-based analysis of micro-electrocoticogram (µECoG) activity during optogenetic stimulation. **Methods**: Two male rhesus macaques were implanted with chambers to co-register a 244-channel ECoG array with a 32-site fiberoptic assembly over frontal motor cortices. Spatial maps of significant connectivity were revealed using repeated optogenetic stimulation at a single site while recording ECoG array activity. To quantify connectivity, we introduce stimulation-locked imaginary coherence (SLIC), a phase-based measurement of directed connectivity. **Results**: Connectivity at rest was measured from all possible stimulation sites, each revealing a network of connected regions spanning multiple cortical areas. Significant and spatially non-gaussian connections were observed in most, but not all sites where optogenetic activity was present. We compared SLIC with an established method that uses signal detection to quantify responses to stimulation with non-zero latency [3]. We found clear agreement between SLIC and the signal detection method at sites with strong connectivity. We also observed that SLIC could better detect putative connections with fewer repeated stimulation or in sites with weaker stimulation-driven responses. We leveraged the stability of our setup to take repeated measurements across days, which revealed stable connection maps. We then explored the sensitivity of our measurements to changes in brain state. Connectivity maps were seen to increase in strength when visual stimuli were presented compared to rest. In summary, our method provides accurate and reliable network connectivity estimation that we will use to study how learning shapes networks in motor cortex. **References**: [1] Kleim, et al., 2004, J. Neuroscience 24(3), 628-633. [2] Sadtler, et al., 2014, Nature 512(7515), 423-426. [3] Banerjee, et al., 2010. J. Neurophysiology 104(6), 3705-3720.

Disclosures: L.R. Scholl: None. **R. Canfield:** None. **P. Rajeswaran:** None. **A.L. Orsborn:** F. Consulting Fees (e.g., advisory boards); Meta.

Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.03/X25

Topic: I.04. Physiological Methods

Title: Direct electrical stimulation to assess brain excitability and connectivity in freely moving mice

Authors: E. GRONLIER, C. ALLIOUX, B. CARABALLO, C. DUMONT, M. VILLALBA, C. ROUCARD, Y. ROCHE, ***C. HABERMACHER**, J. VOLLE;
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Abstract: The discovery and development of drugs for central nervous system (CNS) diseases remain challenging despite advances in technology. This high attrition is primarily driven by failure to show efficacy. Disturbance of neuronal activity, and in particular an imbalance in underlying excitation/inhibition (E/I), has been highlighted in multiple CNS disorders (e.g. Alzheimer disease), and can be regarded as forming a crucial link between structural brain pathology and cellular dysfunction. Although emerging methods aim to probe and influence this imbalance, the complexity of human brain dynamics has hindered the identification of an optimal approach. In this project, we developed a methodological approach that combines previous clinical work in the epilepsy field with event-related potential (ERP) methodology. We designed a novel process capable of electrically stimulating and recording mice in different regions of interest. This technique allows to infer mechanisms of action of drugs at the system level using electroencephalography (EEG) combined with neuropharmacology, such as performed in patients with pharmaco-TMS-EEG protocols.
Wild-type mice were implanted with bipolar intracranial depth electrodes, in the left hippocampus and the left ventro-medial prefrontal cortex. Electrical stimulation was applied between two electrodes and evoked responses were recorded at the other implanted sites. Data were processed offline using a pipeline comparable to human clinical studies. To demonstrate the pharmacosensitivity of this signature, we analyzed the effects of NMDA antagonists on evoked responses in the time domain but also on time-frequency components such as evoked power or inter-trial coherence (ITC).
We recorded evoked responses with high signal to noise ratio and reproducibility between animals. These responses comprised different components, depending on the stimulated and recorded sites, like human recordings. NMDA agents induced a temporary delay on several temporal components of the evoked response and an increase of the power and the ITC in the low frequencies (2-12Hz).
This project represents a step forward to the development of new tools to accelerate the drug development process. Our results indicate that this methodology can be transferred to preclinical research and applied to freely moving mice, achieving high translational relevance for future preclinical studies. This will be of interest to address various neuroscientific questions such as the inclusion of pathological animal models and the identification of impaired brain networks.

Disclosures: E. Gronlier: A. Employment/Salary (full or part-time):; SynapCell. **C. Allioux:** A. Employment/Salary (full or part-time):; SynapCell. **B. Caraballo:** A. Employment/Salary (full or part-time):; SynapCell. **C. Dumont:** A. Employment/Salary (full or part-time):; SynapCell. **M. Villalba:** A. Employment/Salary (full or part-time):; SynapCell. **C. Roucard:** A. Employment/Salary (full or part-time):; SynapCell. **Y. Roche:** A. Employment/Salary (full or

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Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.04/X26

Topic: I.04. Physiological Methods

Support: ANID, Fondecyt de Iniciación N.11190828

Title: Theta/beta ratio as an impulsiveness marker: an exploratory study with eeg

Authors: O. RODRÍGUEZ MUÑOZ[1,3], *C. SARACINI[2];
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Abstract: Impulsivity (or impulsiveness) is defined as a tendency to react quickly and unplanned to stimuli without considering the consequences. Evidence supports the role of the frontal lobe in controlling an individual's impulsive behaviour. Impulsiveness may involve at least two brain circuits working together: a circuit supported by the amygdala system responsible for emotion management, which is altered when impulsiveness is high, and a prefrontal system responsible for inhibitory control. Resting state electroencephalography (EEG) studies report patterns of brain activity characterised by increased activity in the slow frequency band (theta) and decreased activity in the fast frequency band (beta), a relationship expressed as the theta/beta ratio (TBR). The TBR is usually associated with attentional control (AC), but has also been proposed as a possible biological marker of impulsive behaviour, particularly in relation to the ADHD population. The aim of this exploratory study was to determine whether there are associations between TBR and Barratt's impulsiveness scores in a non-clinical population. The EEG of 32 neurotypical individuals was recorded during an 8-minute RS protocol. Impulsiveness was assessed using the Barratt Impulsiveness Scale (BIS-11). Theta and beta power density measures were obtained from three electrodes in the frontal region to calculate TBR. We found a significant negative correlation between TBR and BIS-11 "No-Planning" subscale scores. The present study provides evidence that TBR during RS could be considered as a biomarker of impulsiveness. Further research is needed to validate these findings with cognitive tests assessing executive functions related to impulsiveness and to explore their applicability in clinical contexts, where they could be useful in the early identification and treatment of impulsiveness-related disorders. From a translational perspective, our understanding of the neurobiological mechanisms underlying impulsive behaviour in the general population may benefit from the identification of functional correlates of cortical substrates associated with impulsiveness, which

is crucial not only from a neuroscientific perspective, but also from a social, clinical and therapeutic point of view.

Disclosures: O. Rodríguez Muñoz: None. **C. Saracini:** None.

Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.05/X27

Topic: I.04. Physiological Methods

Title: Microstate analysis of beta-theta and theta-beta neurofeedback training

Authors: *O. KOVALENKO[1], I. KUZNIETSOV[1], R. ROZENGURT[2], T. KACHYNSKA[3], O. ABRAMCHUK[3], O. ZHURAVLOV[3], N. KOZACHUK[3], D. LEVY[4], A. MENDELSOHN[5]; [1]Human and Animal Physiol., Lesya Ukrainka Volyn Natl. Univ., Lutsk, Ukraine; [2]Haifa Univ., Haifa, Israel; [3]Lesya Ukrainka Volyn Natl. Univ., Lutsk, Ukraine; [4]Reichman Univ., Herzliya, Israel; [5]Dept. of Neurobio., Univ. of Haifa, Haifa, Israel

Abstract: Understanding of the physiological mechanisms of neurofeedback training (NFT) still remains incomplete, partially because of high diversity of approaches and protocols used in this field. Brain electrical activity microstate analysis is a promising tool which may add extra light in understanding NFT mechanisms. 45 subjects participated in the study. Subjects were randomly assigned to one of three groups (15 subjects per group): beta-theta group, where subject had the task to increase beta- and decrease theta-EEG activity; theta-beta group, where subject had were instructed to increase theta- and decrease beta-EEG activity; and placebo (sudoku) group. The feedback stimulation was presented as a ball moving around the screen depending of theta- and beta-range spectral power density. Subjects were instructed to keep ball close to the goal, located in the middle top of the screen. Solving sudoku was used as a placebo activity in placebo group. EEG was recorded during 5 6-minutes sessions with 1 minute breaks between the sessions with 21-electrode EEG system, linked ears as reference. Loreta-KEY software was used to run microstate analysis for the first 30s of recording of the 1st and 5th sessions.

Most prominent changes were observed mainly in theta-beta group, where the duration of microstate 1 significantly increased during the NFT course. Transition rates significantly increased between the 1st and the 5th NFT sessions for the following transitions between microstates: microstate 2 to microstate 3, microstate 3 to microstate 1, microstate 4 to microstate 1. This effect was strongly expressed in theta-beta group. The microstate 1 exhibits a right-left orientation, corresponding to the conventional microstate B, which is supposed to reflect the activity of visual network. In our case, the source analysis of microstate B showed increased activity in frontal cortex (superior frontal gyrus). We suppose, that the increase in the

representation of this microstate in theta-beta group during the course of NFT may reflect the higher intensity of processing of visual feedback and stronger top-down control of relevant visual information processing in theta-beta group.

Disclosures: O. Kovalenko: None. **I. Kuznietsov:** None. **R. Rozengurt:** None. **T. Kachynska:** None. **O. Abramchuk:** None. **O. Zhuravlov:** None. **N. Kozachuk:** None. **D. Levy:** None. **A. Mendelsohn:** None.

Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.06/X28

Topic: I.04. Physiological Methods

Support: Templeton World Charity Foundation, Inc (TWCF0646)

Title: A multimodal protocol to inactivate user-selected portions of the cortex in head-fixed mice

Authors: *K. TAKAHASHI[1], S. PONTES QUERO[2], D. BENEDETTI[3], G. J. HUIS IN 'T VELD[1], R. YUSTE[2], C. M. PENNARTZ[1], U. OLCESE[1];
[1]Univ. of Amsterdam, Amsterdam, Netherlands; [2]Columbia Univ., New York, NY; [3]Neurosci., Univ. of California, San Francisco, San Francisco, CA

Abstract: Perceptual decision making involves the interaction of many cortical areas. Studying this process in a mechanistic way thus requires to jointly record and manipulate the activity of a large cortical network. To address this challenge, we have developed a novel multimodal experimental protocol to identify regions of the cortex that show significant responses during a perceptual decision-making task using Gabor patches and then optogenetically activate or inactivate functionally defined and arbitrarily-shaped portion of Gabor-patch activated neurons in the dorsal cortex of head-fixed mice.Our protocol uses wide-field calcium imaging to map the dorsal cortex and then enables users to optogenetically (in)activate functionally defined regions of the cortex via a projector, all done through a transparent Polydimethylsiloxane (PDMS) window. We first developed procedures to synthetize and surgically replace the skull covering half of a hemisphere with a PDMS window. This is required to chronically apply imaging and optogenetics across the whole dorsal cortex and, at the same time, to perform viral injections and electrophysiology through a flexible window. We successfully maintained clear transparency of the PDMS window in single mice over several months. Using a wide-field calcium imaging setup, we identified which region of visual cortex in GCaMP-expressing mice responds to Gabor-patch presentation on a monitor. Once the active regions of visual cortex were identified, we generated a binary map to decide which cortical region should be optogenetically (in)activated via a laser projector. The binary map consists of two colors: black (no illumination)

and any preferred color for optogenetics, depending on the opsin(s) being used (e.g., blue). The binary map was then projected on the brain across the PDMS window, (in)activating only regions where colored laser light was projected. Taking scattering into account, we obtained an illumination accuracy of 50 μm resolution. Thus, the precise anatomic subregion containing (in)activated cortical neurons can be identified and manipulated in a chronic head-fixed preparation via this multimodal protocol.Overall, our multimodal protocol provides a new approach to investigate causal roles of functionally defined neural populations by detecting the locations and sizes of active cortical regions and flexibly (in)activating them with high precision. Importantly, our approach can be applied over the course of several months and can be integrated with other techniques such as electrophysiology.

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Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.07/X29

Topic: I.04. Physiological Methods

Support: NSF 1922389

Title: Impacts of neuronal activation threshold in the presence of orthopedic plates on gradient-induced peripheral nerve stimulation under magnetic resonance imaging

Authors: X. YANG[1], L. YANG[1], J. ZHENG[2], H. YE[3], N. KAULA[4], ***J. CHEN**[1]; [2]Electrical and computer engineering, [1]Univ. of Houston, Houston, TX; [3]Biol., Loyola Univ. Chicago, Chicago, IL; [4]NOR, Arvada, CO

Abstract: Peripheral nerve (PN) axon excitation can occur via the electric fields generated by MRI gradient coils within the human body. Previous studies have shown that orthopedic implants, such as bone plates, can alter the electric field distribution and can potentially affect close by nerves' activation. Although the MRI electric field can affect the PN threshold in the presence of implants, little work has been done in this area. This study aims to quantify the potential variation in PN threshold due to the presence of orthopedic plate through the combined anatomical electromagnetic modeling and physiological neurodynamic simulations. First, electromagnetic fields are obtained in the Yoon-Sun human model, both with and without the presence of orthopedic plate implanted on the tibia bone. Next, simulated electric fields are projected onto nerves axons and integrated along the nerve fiber to obtain the effective electric potential along the nerve. Then, utilizing the physiological NEURON model, nerve responses are obtained in response to the electric potential along the nerve bundle with different fiber different

diameters. Finally, the PN activation threshold is captured by increasing the current strength in the gradient coils. The results show, that thresholds decrease up to 68% due in the presence of plate. One reason could be the rapid change of the electric field derivative on the fiber's positions near the tips of the orthopedic plate. This study provides evidence that orthopedic implants can affect the PN threshold during MRI. Metal implants are strongly suggested to be considered when evaluating the PNS of the high gradient MRI system to ensure the safety of the patients with implants.

Disclosures: X. Yang: None. **L. Yang:** None. **J. Zheng:** None. **H. Ye:** None. **N. Kaula:** None. **J. Chen:** None.

Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.08/X30

Topic: I.04. Physiological Methods

Support: FWO Grant 1272222N

Title: Unraveling drug-related electrophysiological footprints using high-density neuronal probes

Authors: *C. AYDIN[1], R. DE PLUS[2], S. HAESLER[1];
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Abstract: Clinical symptoms of neuropsychiatric and neurodevelopmental disorders arise from neurophysiological dysfunction in specific neural circuits. Progress has been made in understanding the brain regions and molecular factors responsible for these diseases. However, the main challenge in developing new treatments lies in the limited value of behavioral animal models. Although changes in behavior may indicate improvement in the clinical phenotype, they may not necessarily reflect improvement in the underlying circuit function; instead, they could be due to unrelated structural changes. Recent advances in electrophysiology enable the monitoring of single-unit activity at large scale and high density in freely behaving rodents, which might allow us to identify neural activity patterns under disease-causing circumstances. We combined high-density electrophysiology techniques with previously established drug-challenge assay to test this hypothesis. We used the candidate drug Dizolcilpine (MK-801), an uncompetitive N-Methyl-D-aspartate (NDMA) receptor antagonist. Using a single Neuropixel 2.0 probe implanted over the prefrontal cortex, we routinely record over 250 neurons simultaneously over multiple days. While injecting different concentrations of MK-801, we quantified how the population of neurons changes their activity over time. We observed significant differences between the control and each administered concentration group invariant

to animal behavior. Then, we asked whether electrophysiological footprints could be used solely to predict the administered concentration of dizocilpine. We used time-dependent individual neuron spike statistics changes and trained a multiclass classifier. Then, we correctly predicted the concentration of the administered drug over 70%. Ultimately, our current platform opens new avenues for drug development and personalized medicine.

Disclosures: C. Aydin: None. **R. De Plus:** None. **S. Haesler:** None.

Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.09/X31

Topic: I.04. Physiological Methods

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NSF CBET 1847315
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Title: Leveraging large-scale electrophysiology array recordings to identify neural biomarkers for improved closed-loop neuromodulation for chronic pain

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Abstract: Closed-loop, patient-specific neuromodulation with chronically implanted devices such as deep brain stimulation (DBS) and responsive neurostimulation (RNS) has become a practical and accessible approach for studying and treating an increasingly broad range of neuropsychiatric disorders in the clinical setting. Chronic pain is one such pathology affecting up to 1.5 billion people worldwide. An emerging body of work suggests that neuromodulation holds promise in providing treatment for numerous conditions, including chronic neuropathic pain. Given the safety and efficacy of current neuromodulation approaches, there is substantial opportunity to utilize closed-loop electrophysiological monitoring and targeted stimulation for patients with these conditions. Given constraints on performing high fidelity electrophysiological biomarker searches in human subjects, and limitations of retrospective datasets from patients with implanted recording devices for a primary cause unrelated to chronic pain, corollary work in mouse models presents an important opportunity to increase throughput in identifying electrophysiological biomarkers. A main limitation impeding integration of mouse studies into

human-applicable intervention, is the high dimensionality of neural recordings—including single unit spiking activity and broadband local field potential (LFP) recordings—and their poor translation to technology-limited clinical devices without prior identification of explicit parameters. Another challenge is alignment of subjective experiences in mice with electrophysiological readouts. Rapid development of neural control strategies toward clinical translatability is thus limited. To address this issue, we have developed a real-time system for parallelizing the search for brain state-correlated biomarkers. Using a neuropixels 1.0 probe, we are able to perform high temporal resolution recordings of LFP activity and single unit spiking activity from multiple brain structures. We then perform probe wide sweeps and characterize numerous spectral parameters (e.g. transient changes in oscillatory center frequency or relative power) in a region-aware manner to identify features of interest. We also demonstrate the ability to extract these features in near-real-time for subsequent closed-loop control strategy development. We show that this can be applied to complex neuropsychiatric disorders, such as mouse spared nerve injury (SNI) models of neuropathic pain. The result of this work is a method to scan hundreds of biomarkers—in parallel, across candidate regions of interest—and their latent relationships across multiple regions.

Disclosures: M. Sorrentino: None. **C. Slater:** None. **C. Kelley:** None. **B. Youngerman:** None. **Q. Wang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sharper Sense.

Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.10/X32

Topic: I.04. Physiological Methods

Title: Assessing Sleep Architecture and Pharmacodynamics: An EEG-Based Platform for Drug Testing

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Abstract: States of vigilance and sleep have always been a subject of interest in neurosciences due to the alterations in sleep architecture seen in many brain disorders, such as psychiatric disorders or neurodegenerative dirsorders. Drugs in themself can have positive or negative effects on sleep architecture and it is essential to evaluate their potential effects based on clinical indications. Increasingly, semi-automated analysis tools based on artificial intelligence are becoming available and are being adapted to current technologies to streamline and speed up workflows. We leveraged this to develop an analysis pipeline using an in-house EEG platform to

test drugs for their effects on states of vigilance and the corresponding pharmacodynamic modulations of frequency bands for each state, i.e. wakefulness, rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep. Indeed, EEG is a quantitative and objective tool that, when combined with EMG, allows us to distinguish between different states of vigilance and to study in detail the frequency bands in each state and their evolution over time. This platform can assess different classes of pharmacological compounds and serve as a reference for new drugs in development. Mice were implanted with parietal and nuchal electrodes to enable EEG and EMG recordings over 8 hours. We tested selected reference compounds to evaluate their effect on sleep architecture and on EEG pattern. The compounds were administered in a cross-over design, and the recordings were scored in 4-second epochs to identify sleep stages. We tested three different compounds from different classes and obtained their complete profiles with the platform. The procognitive agent donepezil showed no effect on sleep stages but increased power in the gamma band. The hypnotic agent suvorexant, an orexin receptor antagonist, had an impact on REM but without consistent modifications of EEG pattern. And zolpidem, a sedative agent, promoted both sleep states but differently impacted the frequency profiles of Wake, NREM and REM: it induced a shift towards lower frequencies of REM peak frequency and a reduction of delta activity (1-4Hz) during NREM. In conclusion, this new analysis pipeline based on an EEG-based platform focused on sleep architecture provides a tool to more quickly and objectively assess the effects of compounds in development and compare them to existing reference drugs for various indications.

Disclosures: C. Roucard: A. Employment/Salary (full or part-time):; SynapCell. **C. Habermacher:** A. Employment/Salary (full or part-time):; SynapCell. **E. Gronlier:** A. Employment/Salary (full or part-time):; SynapCell. **C. Dumont:** A. Employment/Salary (full or part-time):; SynapCell. **B. Caraballo:** A. Employment/Salary (full or part-time):; SynapCell. **C. Allioux:** A. Employment/Salary (full or part-time):; SynapCell. **M. Villalba:** A. Employment/Salary (full or part-time):; SynapCell. **Y. Roche:** A. Employment/Salary (full or part-time):; SynapCell. **J. Volle:** A. Employment/Salary (full or part-time):; SynapCell.

Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.11/X33

Topic: I.04. Physiological Methods

Title: A scalable and well defined human CNS co-culture platform, suitable for studying excitatory/inhibitory neuron imbalances and the discovery of drugs to treat associated diseases.

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Abstract: Neuronal circuits in the cortex consist of two main neuronal types, glutamatergic excitatory neurons and GABAergic inhibitory neurons (IN). The inputs of IN provide cortical networks with the ability to balance spontaneous and evoked excitatory activities, preventing runaway excitation. Abnormal IN function and excitatory-inhibitory imbalances are associated with various neurological diseases including autism, epilepsy and schizophrenia. Excitatory-inhibitory imbalances can also occur due to dysfunction of astrocytes as they have a critical role in the turn-over of the released glutamate in the synaptic cleft. Scalable approaches are needed to generate human in vitro models that can reliably recapitulate excitatory-inhibitory imbalances suitable for high-content drug screening to develop therapeutics to treat associated neurological diseases. We have used our deterministic cell programming technology opti-ox™ (optimised inducible overexpression) to generate the key cell types required to establish a platform for studying excitatory inhibitory neuron imbalances, namely GABAergic neurons (ioGABAergic Neurons™), glutamatergic neurons (ioGlutamatergic Neurons™) and astrocytes (ioAstrocytes™), from human iPSCs.
ioGABAergic Neurons form a highly pure population of GABAergic neurons that have undergone deep molecular characterisation by immunocytochemistry, RT-qPCR and single-cell RNA-sequencing, revealing cultures that consist of over 99% pure GABAergic neurons expressing the classical markers GAD1, GAD2, VGAT, DLX1, as well as DLX2 and are positive for GABA. Remarkably, SST was the only GABAergic subtype specific marker that was detected, further highlighting the purity of the ioGABAergic Neurons. Moreover, three independently manufactured ioGABAergic Neurons lots displayed highly equivalent transcriptomic profiles, confirming the consistency and scalability of the opti-ox technology. Functional assessment by MEA assays showed that ioGABAergic Neurons inhibit the excitatory activity of ioGlutamatergic Neurons in a ratio dependent manner, and that the inhibitory and excitatory balance can be further modulated by drugs targeting GABAergic signalling. Thus, the developed opti-ox driven co-culture platform can be used to accurately model complex CNS interactions in vivo, to study the principles underlying excitatory-inhibitory neuron imbalances to empower research and drug discovery for devastating neurological disorders, such as epilepsy.

Disclosures: T. Oosterveen: A. Employment/Salary (full or part-time):; bit.bio. **M. Ortiz:** A. Employment/Salary (full or part-time):; bit.bio. **G. Miller:** A. Employment/Salary (full or part-time):; bit.bio. **M. Rios de Anda:** A. Employment/Salary (full or part-time):; bit.bio. **R. Hickman:** A. Employment/Salary (full or part-time):; bit.bio. **P. Parac:** A. Employment/Salary (full or part-time):; bit.bio. **B. Klapholz:** A. Employment/Salary (full or part-time):; bit.bio. **S. Milde:** A. Employment/Salary (full or part-time):; bit.bio. **R. O'Reilly:** A. Employment/Salary (full or part-time):; bit.bio. **H. Garnett:** A. Employment/Salary (full or part-time):; bit.bio. **M. Iovino:** A. Employment/Salary (full or part-time):; CRL. **D. Magnani:** A. Employment/Salary (full or part-time):; CRL. **M. Raman Srivastava:** A. Employment/Salary (full or part-time):;

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Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.12/X34

Topic: I.04. Physiological Methods

Title: Investigating lysosomal ion channels using high throughput Automated Patch Clamp (APC) and Solid Supported Membrane electrophysiology (SSME)

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Abstract: Intracellular ion channels are known to play an essential role in various signaling pathways in health and disease. Over 80% of transport processes take place across intracellular membranes. Among the variety of organellar channels and transporters the proton leak channel transmembrane protein 175 (TMEM175), the transient receptor potential cation channel, mucolipin subfamily TRPML1, and the lysosomal two-pore channel (TPC) have received increasing attention in the field. This interest was sparked by genetic association of the corresponding transporter genes suggesting lysosomal (dys-)function to be a pathophysiological driver of conditions such as Parkinson's disease and cancer. Consequently, there is an increased interest in exploring intracellular ion channels and their pharmacology also by means of high-throughput electrophysiology. To this end, either patch clamp or solid supported membrane electrophysiology (SSME using the SURFE^{2}R 96SE), present the methodologies of choice. Until now, however, high-throughput patch clamp has lacked the possibility to collect data from native lysosomes. In this study, we describe two electrophysiological techniques for studying ion channels in native lysosomes. Lysosomes were isolated from WT or HEK293 cells stably expressing TMEM175 and recorded using both high throughput APC (SyncroPatch 384) and SSME (SURFE^{2}R N1 and SURFE^{2}R 96SE). Using different pH conditions currents were enhanced by DCPIB in both techniques. TPC2 was also recorded from lysosomes isolated from HEK293 cells and recorded using high throughput APC where currents were activated using the specific activator TPC2-A1-P. Last but not least, TRPML1 was activated by addition of K^+-containing solution, enhanced by PIP_2 and further enhanced by ML-SA5 using SSME.

Altogether these results demonstrate the potential to use high throughput electrophysiological techniques to record lysosomal ion channels in intact lysosomes or lysosomal membranes, using SyncroPatch 384 and SURFE^{2}R, respectively.

Disclosures: A.R. Obergrussberger: A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **M. Rapedius:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **A. Bazzone:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **R. Zerlotti:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **M. Paina:** A. Employment/Salary (full or part-time):; Axxam S.p.A. **R. Rizzetto:** A. Employment/Salary (full or part-time):; Axxam SpA. **A. Mondini:** A. Employment/Salary (full or part-time):; Axxam S.p.A. **Y. Lu:** A. Employment/Salary (full or part-time):; Nanion Technologies, Inc. **E. Dragicevic:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **M. Barthmes:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **N. Brinkwirth:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **C. George:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **J. Rolland:** A. Employment/Salary (full or part-time):; Axxam S.p.A. **N. Fertig:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH.

Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.13/X35

Topic: I.04. Physiological Methods

Title: Characterization of ion channels recorded from hiPSC-derived neurons after culturing in different conditions: an automated patch clamp study

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Abstract: The use of human induced pluripotent stem cells (hiPSCs) is becoming commonplace in biomedical research. Culture conditions for maturation of hiPSC-derived neurons are continuously being optimized, but these can be long in duration, and are not yet standardized. We have used hiPSC-neurons cultured under different conditions and compared the presence of different ion channels, along with their biophysical properties such as current amplitude and Vhalf using high throughput automated patch clamp (APC). We found that hiPSC-derived neurons could be used on APC with success rates exceeding 75% in some conditions and culture conditions had little effect on seal resistances and success rate. However, when hiPSC-neurons

were co-cultured with astrocytes, Na_V currents were detected in more neurons (100% when co-cultured, 59% when cultured alone) and amplitudes were significantly larger compared with those cultured alone (-3.3 ± 0.29 nA (n = 96) versus -1.0 ± 0.2 nA (n = 37)), whereas the V_{half} of activation and inactivation remained the same regardless of culture conditions. Similarly, K_V currents were detected in more cells (85% when cultured with astrocytes versus 57% when cultured alone) and amplitudes were also larger in hiPSC-neurons co-cultured with astrocytes compared with neurons cultured alone (1.5 ± 0.1 nA (n = 81) versus 0.85 ± 0.08 nA (n = 56). We also investigated the presence of different ligand-gated ion channels and could detect responses to acetylcholine, GABA, glycine and glutamate in neurons cultured in all conditions. A concentration response curve to GABA revealed an EC_{50} of around 20 µM. In summary, we show that co-culturing hiPSC-neurons with astrocytes does not affect sealing properties of cells on APC, but does affect the expression of Na_V and K_V channels. Co-culturing hiPSC-neurons with astrocytes, therefore, may be a reliable method for maturation of hiPSC-derived neurons for biomedical research and drug discovery.

Disclosures: N. Brinkwirth: A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **N. Becker:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **J. Neubauer:** A. Employment/Salary (full or part-time):; Fraunhofer-Institute for Biomedical Engineering (IBMT). **I. Karnatz:** A. Employment/Salary (full or part-time):; Fraunhofer-Institute for Biomedical Engineering (IBMT). **A.R. Obergrussberger:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **E. Dragicevic:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **E. Dragicevic:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH. **G. Okeyo:** A. Employment/Salary (full or part-time):; Nanion Technologies, Inc. **J. Wihan:** A. Employment/Salary (full or part-time):; Fraunhofer-Institute for Biomedical Engineering (IBMT). **N. Fertig:** A. Employment/Salary (full or part-time):; Nanion Technologies GmbH.

Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.14/Y1

Topic: I.04. Physiological Methods

Support: University of Wisconsin-Milwaukee and CalciGenix, LLC

Title: Modulation of signal-to-noise ratio in CA1 pyramidal neurons

Authors: *I. MORLEY[1], B. NATWORA[1], D. SAFARINI[1], J. R. MOYER, JR.[2];
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Abstract: Analogous to having a conversation amid the noise of a crowded room, the signal-to-noise ratio (SNR) is a pivotal feature in electrophysiological studies for discerning meaningful neuronal activity from background noise. In our experience, traditional methods for SNR calculation are challenged by the fact that variations in resting membrane potential or holding potential greatly distort signal or noise power. To circumvent these limitations, we present a novel methodological approach that utilizes the Poisson power spectrum excluding the zero hertz (0 Hz) bin to yield more accurate SNR measures in neuronal subpopulations. Visually guided whole-cell recordings (WCRs) were made from CA1 neurons from 400 µm hippocampal slices. Schaffer collateral stimulation was used to induce EPSPs and action potentials. To validate the sensitivity and reliability of this method in reflecting signal-to-noise ratio, we will apply a series of pharmacological agents known to modulate synaptic transmission (picrotoxin for GABAA receptor antagonism, CNQX for AMPA receptor antagonism, baclofen for GABAB receptor antagonism, and S-AMPA for AMPA receptor agonism). SNR is recorded before and after bath application of the various agonists/antagonists. The predictability of SNR changes in response to these drugs offers insights into the validity and reliability of this signal-to-noise measure. The resulting SNR is then compared to that obtained using other Fourier analysis approaches. This novel application of a Poisson power spectrum analysis excluding the 0 Hz bin substantially refines SNR calculations in whole-cell patch clamp studies. Initial experiments indicate that SNR predictably increases following bath administration of 10µM picrotoxin. Preliminary data indicate that this methodology proved sensitive to pharmacologically induced changes in signal and noise, validating SNR as a reliable measure of individual neuronal responses. This approach may have significant implications for understating the primary drivers of neuronal signal power and the excitatory/inhibitory balance in subpopulations of neurons.

Disclosures: I. Morley: None. **B. Natwora:** None. **D. Safarini:** None. **J.R. Moyer, Jr.:** None.

Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.15/Y2

Topic: I.04. Physiological Methods

Support: CONAHYT/CVU: 1102522

Title: Modulation of Ecitability of Medium Spiny Neurons in Nucleus Accumbens By Binge Type Intake

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Abstract: Overweight and obesity are mainly triggered by eating disorders such as binge-eating, which consists of an excessive consumption of high-calorie foods such as sugar and fats in a short period of time, approximately 2h (Corwin, 2006; DSM-V, 2013; Corwin et al., 2016). One of the nuclei that participate in the modulation of salience of stimuli such as palatable food is the nucleus accumbens (NAc). 95% of neurons of this nucleus are medium spiny neurons (MSNs). However, it is not known how binge eating can modulate the activity of MSNs. The objective of this study was to investigate the changes in the excitability of MSNs using a binge intermittent model in mice and electrophysiological registers with patch clamp. All experiments were conducted following the official Mexican standard of technical specifications for the production, care, and use of laboratory animals (NOM-062-ZOO-1999) and in accordance with the regulations of the Internal Committee for the Care and Use of Laboratory Animals (CICUAL) of the National Autonomous University of Mexico. The subjects were housed individually under controlled environmental conditions: a 12-12 h light-dark cycle, temperature 21 ± 2 ºC and humidity 70 ± 10%. The subjects were randomly assigned, and their initial weight was controlled. 21 C57BL/6 mice weighing between 19 and 22 g were used. Binge-type intake was evaluated using an intermittent model. Subjects were separated as follows: control group (CG) (n=7), intermittent group (IG) (n=7) and continuous group (COG) (7). For 28 days all subjects had *ad libitum* access to standard food and water. IG with binge-intake had access to palatable food 12 days (Monday, Wednesday, and Friday, for 4 weeks). COG had ad libitum access to M&M´s, standar food and water. We measured water consumption, standard foods, palatable foods, and weight every 22 and 24 h. The animals were deeply anesthetized with isuflurane and immediately decapitated. The brains were removed and placed in cold (5°C) artificial cerebrospinal fluid (ACSF). Coronal brain slices (250 μm thick) containing the NAc were obtained with a vibratome (Pelco 102, Ted Pella. INC) and stored in oxygenated ACSF at room temperature for at least 1 h before recordings. For the statistical analysis we used the Mann Whitney and Kruskal Wallis U trials. Meaningful differences were observed when $p < 0.05$. We found differences in the excitability of MSNs. IG had less excitability than CG and COG. Based on these data, we suggest that the projections of MSN in subjects with binge send fewer GABAergic projections to the VTA and therefore receive more dopamine, increasing the reward response to palatable food.

Disclosures: S. Ortega-Tinoco: None. **E. Barrera-Miranda:** None. **J. Garduño:** None. **S. Hernandez-Lopez:** None.

Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

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Program #/Poster #: PSTR311.16/Y3

Topic: I.04. Physiological Methods

Support: NIH Grant R01NS115707 and a diversity supplement to the parent grant
NIH Grant R03AG072218
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NSF CBET CAREER 1943906

Title: Conditional Knock-out of Fus Protein in Oligodendrocytes of Aged Mice Decreases Evoked Firing Rate Activity in the Visual Cortex

Authors: *C. GARCIA[1], S. WELLMAN[3], T. THAI[1], F. CAMBI[2], T. D. KOZAI[1];
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Abstract: Brain-computer interfaces (BCIs) hold promise for treating neurodegenerative diseases yet face challenges due to declining signal quality over time. Previously, our lab showed that depleting oligodendrocytes (OL) leads to a loss of recording performance but is rescued after pro-myelinating Clemastine administration. To decouple the effects from OL and off-target drug effects, we investigated the electrode recording performance of mice with Fused in Sarcoma conditional knock-out (FUScKO) of Fus proteins only in OL. These mice have thicker myelin, especially on small caliber axons relative to wild-type (WT) mice. FUScKO and WT mice (n=6 per group) were chronically implanted with 16-ch Michigan-style electrodes in the visual cortex. Response latency to visual stimulation and evoked signal-to-noise firing rate ratio (SNFRR) were quantified through electrophysiological recordings during drifting grating stimulation for 16 weeks and analyzed with two-way ANOVAs and Bonferroni corrected t-test. Given that myelin is correlated with higher neuronal signal conduction velocity, we hypothesized that FUScKO mice, would have lower response latency to visual stimuli than WT mice. No significant or consistent trends in response latency were observed among genotypes in the young cortex. However, aged FUScKO mice showed consistently higher latencies compared to aged WT mice, with a significant increase noted on day 77 ($p < 0.05$). While the differences in latency were not significant, the observed trend suggests that knocking out Fus in OL contributes to higher response latency and lower signal conduction velocity during aging. Previous studies show enhanced myelination improves SNFRR stability, so we next asked if SNFRR would increase in FUScKO mice. We anticipated higher ratios in FUScKO mice as myelin is associated with enhanced neuronal metabolic support. Surprisingly, SNFRR did not differ significantly between genotypes in young mice. The aged WT mice had greater SNFRR than the aged FUScKO mice at all time points, with significant increases at days 1, 4, 6, 8, 42, 63, 70, and 77 ($p < 0.05$). These findings propose a potential relationship between myelination and SNFRR stability. Future studies should investigate if this is related to metabolic exhaustion in aging or improved signal transduction efficiencies. Our findings showed that Fus conditional knock-out in OL does not significantly affect the latency or SNFRR value in young mice. In the aged group we only observed significantly greater SNFRR in WT mice compared to FUScKO mice. Future work aims to quantify chronic functional recordings from CA1, where preliminary analyses suggest longer recording performance.

Disclosures: C. Garcia: None. **S. Wellman:** None. **T. Thai:** None. **F. Cambi:** None. **T.D. Kozai:** None.

Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

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Program #/Poster #: PSTR311.17/Y4

Topic: I.04. Physiological Methods

Title: Evaluating anesthetic effects on simultaneous recording of pattern reversal electroretinograms and visual evoked potentials in cynomolgus monkeys

Authors: *A. MASUDA, M. UNNO, K. HAYASHIDA, T. ARAKI, Y. NUMATA;
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Abstract: Pattern visual evoked potentials (pVEPs) are an electroencephalography (EEG) recording from the visual cortex in response to patterned visual stimuli. Anesthetic drugs influence various stages of visual processing involved in pVEP. Simultaneous recording of pattern electroretinograms (pERG) during pVEP recording can differentiate between signal reception in the retina and the optic nerve to brain response. This study aimed to assess the nervous system effects of anesthetics on pVEPs by comparing pERGs from male cynomolgus monkeys anesthetized under different conditions. Three male cynomolgus monkeys (2-3 years old, 2-4 kg) were anesthetized using three different protocols: ketamine/xylazine (8.75 mg/kg, 0.5 mg/kg, i.m.), ketamine/medetomidine (10 mg/kg, 0.08 mg/kg, i.m.), and propofol (10 mg/kg, i.v.). Disc electrodes were attached to the scalp with gel and positioned according to the International 10-20 system (Oz for the active electrode and Fz for the reference electrode). Checkerboard pattern stimuli were presented at spatial frequencies of 1, 3, and 10 cycles per degree (cpd). Animals were placed so that their eyes were 55 cm in front of the monitor, and ERG contact lens were used to measure pERG, covering one eye with an eye patch. ERG parameters (implicit time and amplitudes of P50 and N95) and VEP parameters (P1, N1, and P2 latencies, as well as P1N1 and N1P2 amplitudes) were analyzed. Comparisons of pERG data in anesthetized monkeys by 3 protocols revealed no significant differences in the P50 or N95 latencies between the anesthetic conditions, indicating the anesthetic drugs have little effects on transmission speeds inside the retinal circuits. In pVEP results, however, propofol showed shorter P1, N1, and P2 latencies in pVEP than ketamine/xylazine or ketamine/medetomidine. The pVEP latencies under propofol were closed to those in awake monkeys or clinical standard values (P1: 75 msec; N1: 100 msec; P2: 135 msec), suggesting that ketamine/xylazine and ketamine/medetomidine anesthesia may delay nerve transmission in the pathway from the optic nerve to the brain. In both pERG and pVEP, larger amplitudes were found at 1 cpd in recording under ketamine/xylazine anesthesia than under other anesthetics. At 3 and 10 cpd, however,

pVEP amplitudes were larger under propofol than other anesthetics. Ketamine/xylazine anesthesia may be acceptable for specific condition such as visual stimulation at lower spatial frequency, but propofol may be useful for pVEP in a wider range of conditions.

Disclosures: A. Masuda: None. **M. Unno:** None. **K. hayashida:** None. **T. Araki:** None. **Y. Numata:** None.

Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.18/Y5

Topic: I.04. Physiological Methods

Support: Contract Research for Laboratory

Title: Effects of Violet Light Exposure on Reproductive Parameters in cynomolgus monkey

Authors: *Y. MITSUKURA[1], M. HIRANO[2], B. SUMALI[1], D. MORI[3], K. HAYASHIDA[3], K. TSUBOTA[4];
[1]Keio Univ., Kanagawa, Japan; [2]Keio Univ., Tokyo, Japan; [3]Shin Nippon Biomed. Labs., Ltd., Kagoshima, Japan; [4]Tsubota Lab., Inc., Shinjuku-ku Tokyo, Japan

Abstract: Objective: This study aimed to evaluate the impact of violet light exposure on testosterone levels, testicular size, and sperm motility in cynomolgus monkeys. Methods: Eight monkeys were divided into two groups: those exposed to violet light for two hours each morning and a control group without exposure. Over approximately one year, we measured testosterone 77 times from day 32 to day 298, and testicular size 43 times from day 1 to day 298. For correlation analyses between testicular size and testosterone, we used data from day 31 to day 277, adjusting for differences in measurement timings. Results: Monkeys exposed to violet light exhibited significantly higher testicular sizes and testosterone levels compared to both their baseline measurements and the control group. Sperm motility was also enhanced in the violet light group, correlating with testosterone changes. The correlation analysis was adjusted for differences in measurement schedules and only included matched data points within a maximum 5-day lag between testicular and testosterone measurements. Conclusions: Exposure to violet light significantly affects reproductive parameters in monkeys, indicating potential benefits in reproductive health management. Further studies are required to explore the mechanisms and applicability of phototherapy in reproductive medicine. Significance: These findings underline the potential of light exposure as a non-invasive method to enhance reproductive health, providing insights into its effects on hormonal and testicular adjustments.

Disclosures: Y. Mitsukura: None. **M. Hirano:** None. **B. Sumali:** None. **D. Mori:** None. **K. hayashida:** None. **K. Tsubota:** None.

Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.19/Y6

Topic: I.04. Physiological Methods

Title: The impact of anxiolytics on prefrontal cortex electroencephalographypatterns in cynomolgus monkeys

Authors: *K. HAYASHIDA[1], Y. HORIMOTO[2], T. UCHINO[2], Y. NUMATA[2], Y. MITSUKURA[3];
[1]Pharmacol., Shin Nippon Biomed. Labs., Ltd., Kagoshima, Japan; [2]Shin Nippon Biomed. Labs., Ltd., kagoshima, Japan; [3]Keio Univ., Kanagawa, Japan

Abstract: The prefrontal cortex (PFC) plays an important role in executive functions including stress control and emotions. In the PFC, higher electroencephalography (EEG) frequencies (beta band) represent concentration and nervousness, whereas lower EEG frequencies (delta, theta, and alpha bands) represent a tranquil state. Anxiolytics including 5-HT reuptake inhibitors and benzodiazepines have been used for decades to treat depression by reducing nervousness and anxiety. However, the impact of these anxiolytics on the EEG frequencies is not well understood. The present study examined the effects of anxiolytics on the PFC EEG patterns in cynomolgus monkeys. EEG electrodes, according to the international 10-20 system (Oz for the reference electrode and Fpz for the active electrode), were implanted and EEGs were recorded after oral administration of vehicle, paroxetine (5-HT reuptake inhibitor, 10 mg/kg) or etizolam (benzodiazepine, 1 mg/kg) in 6 male monkeys (5-8 years old). Both paroxetine and etizolam treatments decreased beta bands (paroxetine:22-24Hz, etizolam :22 and 24Hz) compared to the vehicle, indicating that both drugs reduced concentration and nervousness. The etizolam treatment also increased delta-theta bands (3 and 4Hz) compared to the vehicle, indicating that etizolam also induced a tranquil state. The present study demonstrates that paroxetine and etizolam affect the EEG patterns in cynomolgus monkeys by inducing a tranquil state and/or reducing concentration and nervousness, and indicates that the PFC EEG patterns may be a useful biomarker for evaluation of the efficacy of anxiolytics.

Disclosures: K. hayashida: None. **Y. Horimoto:** None. **T. Uchino:** None. **Y. Numata:** None. **Y. Mitsukura:** None.

Poster

PSTR311: Electrophysiological Recording of Neurons and Neural Networks

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR311.20/Web Only

Topic: D.02. Somatosensation – Touch

Support: NIH Grant P41 EB018783 (Wolpaw)
NYS Spinal Cord Injury Research Board C37714GG (Gupta)
NYS SCIRB C38338GG (Wolpaw)
Stratton Veterans Affairs Medical Center

Title: Single-trial decoding of somatosensory evoked potentials: Effect of stimulation frequency

Authors: *D. GUPTA[1,2], J. A. BRANGACCIO[3], H. MOJTABAVI[4], J. R. WOLPAW[5], J. HILL[6,2];
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Abstract: Objective: Single-trial decoding of somatosensory evoked potentials (SEPs) is important for developing Brain Computer Interface applications for rehabilitation post brain injury. However, it is unclear how SEP decoding is affected by stimulation parameters such as the stimulation frequency. We assess this with tibial nerve electrical stimulation at frequencies of 0.2 Hz, 1 Hz and 2Hz. **Method:** Nine healthy people (50.4$\pm$18.8y, 6m/3f) participated with informed consent (IRB #1584762). Referential electroencephalography (EEG) was acquired at 300 Hz (DSI-24, Wearable Sensing; BCI2000). Bipolar electromyography was measured from the soleus muscle (3200 Hz, AMT-8; EPOCS software). Biphasic 1-ms electrical stimuli were delivered to tibial nerve with a constant current stimulator, controlled with our EPOCS software. At each stimulation frequency, the soleus M-wave and Hoffman-reflex recruitment curves were obtained, followed by SEP measurement (75 trials), at a current intensity that elicited an M-wave of 10-20% Mmax. The 3 stimulation frequency blocks were repeated after a short break, presented randomly. EEG was notch and bandpass filtered (0.2 - 40 Hz) and denoised with independent component analysis. Preprocessed data were epoched (-50 to 400 ms); noisy epochs were removed using trial statistics. The SEP was obtained by averaging baseline-corrected epochs. The Friedman Test was used for repeated measure comparisons. Single trial classification was assessed with linear discriminant analysis (LDA) and 5-fold cross-validation. Classification performance was assessed with AUC (ROC curves). Decoding generalization was assessed by applying LDA models from set-1 recordings, to predict the SEPs in set 2. **Results:** SEP N_{70} latencies remained similar across stimulation frequencies ($p>0.05$), while the N_{70} peak

amplitude was significantly different (p= 0.0084); higher stimulation frequency elicited a smaller N_{70} peak. The N_{70} classification accuracy was 0.89, 0.79 and 0.80 for 0.2 Hz, 1 Hz and 2 Hz respectively. Generalization AUC scores were 0.81, 0.76 and 0.77 for 0.2 Hz, 1 Hz and 2 Hz, respectively. **Conclusion:** Results show an excellent classification accuracy for SEP N_{70}, elicited by tibial nerve stimulation at 0.2Hz, with a slight decrease in accuracy at higher stimulation frequencies. This may be attributed to attenuated SEP N_{70} at higher frequencies, possibly due to desensitization. Generalization scores show an expected decrease of accuracy at all frequencies, with the largest decrease at 0.2 Hz. Most importantly, all three frequencies appear to be able to support single-trial SEP decoding and may be usable for an SEP-based brain computer interface.

Disclosures: D. Gupta: None. **J.A. Brangaccio:** None. **H. Mojtabavi:** None. **J.R. Wolpaw:** None. **J. Hill:** None.

Poster

PSTR312: Electrophysiology and Electrode Arrays *In Vitro*

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR312.01/Y7

Topic: I.04. Physiological Methods

Title: Neural tri-culture on-a-chip: glutamatergic neurons, cortical astrocytes, and microglia show robust spontaneous and LPS & INF stimulated activity on microelectrode arrays.

Authors: *J. LAWSON[1], K. XU[5], C. PERRY[2], A. MASSMAN[3], J. A. HJELMHAUG[3], H. RUETH[4], K. REMONDINI[3], A. JOHNSON[3], D. HELD[6], K.-D. CHOI[4];
[1]Application Scientist, BrainXell, MADISON, WI; [2]Quality Control, BrainXell, Madison, WI; [3]BrainXell, MADISON, WI; [4]BrainXell, Madison, WI; [5]BrainXell Inc., Madison, WI; [6]Univ. of Wisconsin-Madison, Madison, WI

Abstract: Glutamatergic neurons, astrocytes, and microglia are involved in many neurodegenerative and non-neurodegenerative conditions including Alzheimer's disease (AD), Parkison's disease (PD), amyotrophic lateral sclerosis (ALS), epilepsy, schizophrenia, anxiety, and pain. However, their roles in these disorders have not been fully elucidated. Current preclinical screening platforms, although informative, are insufficient in that non-human primary models exhibit differences in key genomic and proteomic abundance and expression levels when compared to human counterparts. Immortalized cell lines become senescent and inadequate in expressing complex pathological pathways. The availability and viability of human cadaver tissue is limited. However, human induced pluripotent stem cells (hiPSCs)enable personalized medicine while also offering a more relevant and scalable screening assay. hiPSC scan be integrated into high-throughput mono-, co-, and tri-culture HTS screening platforms for novel drug discovery and development as well as molecular cell signaling studies. Here, we have developed atri-culture screening platform on Cytoview MEAs (Axion Biosystem) with our iPSC-

derived cortical glutamatergic neurons (GN), cortical astrocytes (CA), and microglia (MG) in two different medium recipes. GN and CA were cultured simultaneously over the electrode area as a co-culture and maintained for eight days. MG were seeded, on the eighth day directly over the co-cultures of GN and CA. Recordings were taken every other day for 29 days, and the neurons exhibited a robust mean firing rate (MFR), active electrode yield (AEY), bursting frequency (BF), and synchrony index (SI). Starting on Day 28, tricultures were incubated in lipopolysaccharide (LPS) and interferon-gamma (INF-γ) for 24 hours. MEA recordings showed an increase in neural activity in response to LPS and INF-γ. We then used an ELISA assay on the MEA plate to show cytokine levels in response to LPS INF-γ. Immunocytochemistry confirmed morphology of GN, CA, and MG of both the neural and neural inflammatory tri-culture models on the MEAs. This tri-culture model is a powerful tool that could be used to investigate neural responses to novel therapeutic compounds, molecular mechanisms involved in cell-cell signaling, neurodegenerative diseases, and non-neurodegenerative disorders.

Disclosures: J. lawson: A. Employment/Salary (full or part-time):; BrainXell. **K. Xu:** A. Employment/Salary (full or part-time):; BrainXell. **C. Perry:** A. Employment/Salary (full or part-time):; BrainXell. **A. Massman:** A. Employment/Salary (full or part-time):; BrainXell. **J.A. Hjelmhaug:** A. Employment/Salary (full or part-time):; BrainXell. **H. Rueth:** A. Employment/Salary (full or part-time):; BrainXell. **K. Remondini:** A. Employment/Salary (full or part-time):; BrainXell. **A. Johnson:** A. Employment/Salary (full or part-time):; BrainXell. **D. Held:** A. Employment/Salary (full or part-time):; BrainXell. **K. Choi:** A. Employment/Salary (full or part-time):; BrainXell.

Poster

PSTR312: Electrophysiology and Electrode Arrays *In Vitro*

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR312.02/Y8

Topic: I.04. Physiological Methods

Support: Commercialization Promotion Agency for R&D Outcomes(COMPA) funded by the Ministry of Science and ICT(MSIT) (RS-2024-00415902)
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Title: Flexible 3D microelectrode arrays for measuring electrophysiology of cortical organoids in vitro

Authors: *M. WILSON[1], Y. KIM[2], F. PUPPO[4], A. R. MUOTRI[5], J. H. LEE[3], D. KUZUM[4]; [1]Univ. of California San Diego, La Jolla, CA; [3]EE, [2]KAIST, Daejeon, Korea, Republic of; [4]UC San Diego, La Jolla, CA; [5]Pediatrics/Cellular Mol. Med., UCSD, La Jolla, CA

Abstract: Cortical organoids are revolutionizing the neuroscience field as models of brain development and dysfunction and offer promise as platforms for personalized drug screening, biological network analysis, and disease modeling. The primary benefit of organoids' complexity compared to 2D cultures comes from their 3D morphology. However, conventional electrophysiology devices were built to measure planar 2D cultures and are therefore not able to capture the entirety of organoid cellular activity. Here, we created a flexible 3D mesh which can deflect under organoid weight and encompass the organoid. This conformational, tight contact with organoids allows for thorough, longitudinal recordings of neural network electrophysiology. Serpentine shaped wires and thin-film parylene C encapsulation allow for high flexibility of mesh arms without any breakage. Characterizations of the array demonstrate low electrode impedances capable of capturing high SNR recordings. PDMS ports placed alongside the mesh recording chamber allow for perfusion of media and chemical exchange for drug studies with minimal perturbation to the organoid adhesion to array. When combined with two-photon imaging of organoids transduced with a calcium indicator, the mesh arrays permit simultaneous recordings which yields high-resolution information of the population and single-cell dynamics. This novel, multimodal recording setup for allows for holistic analysis of organoids in vitro and will yield insights into organoid network development and activity, furthering our understanding of the brain.

Disclosures: M. Wilson: None. **Y. Kim:** None. **F. Puppo:** None. **A.R. Muotri:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TISMOO. **J.H. Lee:** None. **D. Kuzum:** None.

Poster

PSTR312: Electrophysiology and Electrode Arrays *In Vitro*

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR312.03/Y9

Topic: I.04. Physiological Methods

Support: AMED Grant JP24be1004203h0003

Title: Functional analysis of synaptic network and single neuron in human iPS cell-derived neurons using HD-CMOS-MEA

Authors: *Y. ISHIBASHI, X. HAN, N. NAGAFUKU, N. MATSUDA, I. SUZUKI;
Tohoku Inst. of Technol., Sendai, Japan

Abstract: Human neurons and neural networks generated from human induced pluripotent stem (iPS) cells are greatly expected for wild applications including safety assessments, toxicity and side effect, in vitro disease modeling, and drug screening/discovery. However, it is necessary to evaluate functional maturity of cultured iPS neurons and networks before clinical translation. In the present study, we characterized the changes in electrophysiological and pharmacological properties of individual human iPS cortical neurons and neuronal networks using the field potential imaging technology. This method uses a high density complementary metal-oxide semiconductor microelectrode array (HD-CMOS-MEA) with 236,880 electrodes, which provides a cell-by-cell basis for functional analysis of iPS neuronal network. As a result, we successfully detected the response of both synaptic network bursts and single neuron bursts to seizurogenic compounds. Furthermore, we evaluated the synaptic connection strength between single neurons to show the difference between each compound. Therefore, the current field potential imaging provides considerable potential for accurate assessment of drug effects on iPS neuron cultures.

Disclosures: Y. Ishibashi: None. **X. Han:** None. **N. Nagafuku:** None. **N. Matsuda:** None. **I. Suzuki:** None.

Poster

PSTR312: Electrophysiology and Electrode Arrays *In Vitro*

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR312.04/Y10

Topic: I.04. Physiological Methods

Support: NEUREKA project, GA 863245, within the H2020 Framework Program of the European Commission.
HyVIS project, GA 964468, within the H2020 Framework Program of the European Commission.

Title: Next-generation electrophysiology for functional characterization of human neural organoids

Authors: *L. D'IGNAZIO, E. GUELLA, Z. LI, S. OLDANI, A. ORYSHCHUK, M. J. OBIEN;
MaxWell Biosystems, Zurich, Switzerland

Abstract: Human induced pluripotent stem cell (hiPSC)-derived neural models have emerged as invaluable tools for studying neurological disorders, such as epilepsy, Alzheimer's, and Parkinson's disease. Real-time, label-free measurement of electrical activity in self-organizing in vitro cellular models provides critical insight into the complexity of their neuronal networks. High-density microelectrode arrays (HD-MEAs) enable non-invasive electrophysiological

recordings from various electrogenic samples, including iPSC-derived neurons, retinal explants, brain slices, and neural organoids.In this study, we used MaxOne and MaxTwo high-density MEA platforms (MaxWell Biosystems AG, Switzerland), with 26,400 electrodes per well to record extracellular action potentials in neural organoids at different scales, ranging from cell population networks to single-cell resolution and subcellular levels. We showcased the flexible selection of electrodes for recording neural activity, increasing the reproducibility and statistical power of the data collected. Key metrics such as firing rate, spike amplitude, and network burst profile were extrapolated in a parallelized manner to capture even the smallest neuronal signals. Furthermore, we characterized axonal function and structure using the AxonTracking Assay, which allows measurement of action potential conduction velocity, latency, axonal length, and branching. This automated assay facilitates high-throughput characterization of disease models targeting axon initial segments, axonal branching, development, and conduction. MaxWell Biosystems' HD-MEA platforms, along with automatically generated plots and extracted metrics, provide a unique, user-friendly approach to identifying and isolating functionally active regions in 3D cultures. These powerful platforms enable long-term in vitro disease modeling and compound testing in acute recordings and/or longitudinal studies.

Disclosures: L. D'Ignazio: None. **E. Guella:** None. **Z. Li:** None. **S. Oldani:** None. **A. Oryshchuk:** None. **M.J. Obien:** None.

Poster

PSTR312: Electrophysiology and Electrode Arrays *In Vitro*

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR312.05/Y11

Topic: I.04. Physiological Methods

Support: NEUREKA Project, GA 863245
Hyvis project, GA 964468

Title: The importance of high-density microelectrode arrays for recording multi-scale extracellular potential and label-free characterization of network dynamics in iPSC-derived neurons

Authors: *E. GUELLA, Z. LI, F. MODENA, L. D'IGNAZIO, S. OLDANI, A. ORYSHCHUK, M. J. OBIEN;
MaxWell Biosystems, Zurich, Switzerland

Abstract: Advances in the development of microelectrode arrays (MEAs) for in-vitro electrophysiological recordings have enabled the characterization of multi-scale behavior in neuronal networks, ranging from subcellular level to network dynamics. Such devices are fundamental for studying the phenotype of neurological disorders and for drug discovery,

providing unique insights into the complexity of neuronal networks. Electrode density, spacing, and size influence the signal quality, noise level, and sensitivity. To properly characterize the full behavior of neuronal networks, MEAs must combine single-cell and subcellular resolution with high-throughput assays, while maintaining sensitivity to small extracellular action potentials to describe the full range of network dynamics. In this study, the MaxOne and MaxTwo high-density (HD) MEA systems (MaxWell Biosystems, Switzerland) were used to record activity from induced pluripotent stem cell derived neurons, demonstrating the advantages of having 26,400 electrodes per well, which is key to increasing the statistical power of data collected longitudinally. HD-MEA recordings were compared with simulated low-density recordings, in which larger, low-density electrodes were mimicked by clustering adjacent electrodes on HD-MEAs. Additionally, the AxonTracking Assay, an automated tool for recording and analyzing individual axonal arbors from many neurons in parallel, was used to characterize the function and axonal structure of recorded cultures. Results indicated that higher density and smaller electrodes provided greater sensitivity, enabling the detection of smaller spikes, and covering the full spectrum of network behavior. The high-resolution analysis of network dynamics, coupled with the AxonTracking Assay's subcellular insights, provide powerful insights into drug screening and disease modelling.

Disclosures: E. Guella: None. **Z. Li:** None. **F. Modena:** None. **L. D'Ignazio:** None. **S. Oldani:** None. **A. Oryshchuk:** None. **M.J. Obien:** None.

Poster

PSTR312: Electrophysiology and Electrode Arrays *In Vitro*

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR312.06/Y12

Topic: I.04. Physiological Methods

Title: Establishment and Characterization of a Novel 3D Glioma-Neuron Fusion Model: Functional and Structural Analysis

Authors: *S. OTEN[1], S. KRISHNA[2], T. PICART[3], A. G. DANIEL[4], C. NAVA GONZALES[5], S. HERVEY-JUMPER[6];
[1]Neurosurg., UCSF, San Francisco, CA; [2]Dept. of Neurosurg., UCSF, San Francisco, CA; [3]Dept. of Neurosurg., Hospices Civils de Lyon, Lyon, France; [4]Neurolog. Surgery, UC San Francisco, San Francisco, CA; [5]UCSF, San Francisco, CA; [6]Dept. of Neurosurg., Univ. of California San Francisco, San Francisco, CA

Abstract: The functional relationship between malignant glioma cells and non-neoplastic cells, including neurons, has been shown to modulate tumor proliferation and glioma growth. Various models have been developed to demonstrate the dynamics of this complex microenvironmental interplay. However, there is a growing preference for a high-throughput three-dimensional (3D)

cell culture model that could recapitulate intra-tumoral heterogeneity and the microenvironmental interactions involved in glioma cell invasion, resistance, and recurrence. In this study, an in vitro, self-assembled, scaffold-free, neuron-glioma spheroid fusion model was developed and used to investigate the electrophysiological and functional interplay between glioma and the brain. Primary mouse cortical neurons (MCN) were isolated from prenatal brain tissues and were cultured at 500,000 cells/spheroid on ultra-low attachment plates. Glioma organoids (WHO Grade 2-4) were developed from primary patient-derived tissue samples and were allowed to self-assemble for at least two weeks. After spontaneous spiking activity was detected in MCN spheroids, they were combined with glioma organoids in culture and allowed to fuse. Multielectrode array (MEA) was used to characterize the electrophysiological properties of the fusion model. Structural and functional characterizations were performed using immunofluorescence staining of proliferation, microglial, astrocytic, synaptic markers. Electrophysiological analysis of glioma-neuron co-cultures using MEA demonstrated a significantly increased network synchrony and firing rate in the fusion model when compared with the neuron-only condition, consistent with 2D models in the past. In correlation, the fusion model also demonstrated a significant increase in the Ki67 proliferation index across WHO grade 2-4 when compared to the glioma organoid-only condition. Although MCN neurospheres were established in an unguided protocol, they developed mature astrocytes, reactive microglia, and neuronal differentiation, as evidenced by the expression of GFAP, Iba1, and MAP2, respectively. Strikingly, the microglia integrated into the fusion neurosphere in a mosaic pattern. Together, these results offer a high-throughput 3D model for recapitulating the tumor-brain microenvironment for low and high-grade gliomas. The presence of active microglia is representative of the later stages of brain development, which may be an advantage for modeling the microenvironment of glioma, a generally late-onset disease. Future studies will focus on the use of this model for drug screening and the exploration of malignant transformation in glioma.

Disclosures: S. Oten: None. **S. Krishna:** None. **T. Picart:** None. **A.G. Daniel:** None. **C. Nava Gonzales:** None. **S. Hervey-Jumper:** None.

Poster

PSTR312: Electrophysiology and Electrode Arrays *In Vitro*

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR312.07/Y13

Topic: I.04. Physiological Methods

Title: Modeling Alzheimer’s disease with cocultures of human iPSC derived glutamatergic neurons and APOE E4 mutant astrocytes on a microfluidic DuaLink MEAs.

Authors: J. LAWSON[1], N. SALMAN[2], H. RUETH[1], J. A. HJELMHAUG[1], D. HELD[1], *S. HANSON[1], H. GAUTIER[2], B. MAISONNEUVE[2], T. HONEGGER[2];
[1]BrainXell, Inc., Madison, WI; [2]NETRI, Lyon, France

Abstract: Alzheimer's disease (AD) is an inheritable brain disorder that gets worse over time and is the most common form of dementia. Both glutamatergic neurons and astrocytes have been implicated in AD but their roles have not been fully defined. The development of human induced pluripotent stem cells (hiPSCs) has enabled disease modeling with specific cell types containing specific genotypes. While there have been several genes linked to AD, the most common gene variant linked to an increased risk for developing late-onset AD is apolipoprotein (APOE E4). However, there is more to developing AD than just having the genes; lifestyle and environment have been known to also play a role. Chronic inflammation can also contribute to AD development. Current established disease models typically involve only single cell monocultures or disorganized cocultures. Established in vitro cell culture plates struggle to accurately mimic physiologically relevant connectivity found in vivo and are unable to record phenotypic signaling activity. This is due to the plates lacking both microfluidic chambers while simultaneously housing microelectrode arrays (MEAs). In this study we utilize a DuaLink MEA (a 3-channel microfluidic MEA device from NETRI) with genetically modified astrocytes containing the APOE E4 gene variant, healthy astrocytes, and healthy glutamatergic neurons. The compartmentalized MEA device enabled cocultures of hiPSC derived healthy glutamatergic neurons with APOE E4 mutant astrocytes to synapse onto cocultures of healthy glutamatergic neurons with healthy cortical astrocytes. Recordings were taken every other day for 30 days. The cocultures with APOE E4 cells received 100ng/ml of LPS and 20 ng/ml of INF-y˙to induce chronic inflammation. The cocultures showed a robust spontaneous mean firing rate, active electrode, bursting frequency, network bursting, and synchrony index. Immunocytochemistry confirmed the morphology of neurons and astrocytes. This disease modeling system of DuaLink MEA with hiPSCs is a robust tool that enables noninvasive live cell monitoring for the life of the culture and can be used as a toxicity screen, novel drug screening platform, and cell-cell signaling studies.

Disclosures: J. lawson: A. Employment/Salary (full or part-time):; BrainXell, Inc. **N. Salman:** A. Employment/Salary (full or part-time):; NETRI. **H. Rueth:** A. Employment/Salary (full or part-time):; BrainXell, Inc. **J.A. Hjelmhaug:** A. Employment/Salary (full or part-time):; BrainXell, Inc. **D. Held:** A. Employment/Salary (full or part-time):; BrainXell, Inc. **S. Hanson:** A. Employment/Salary (full or part-time):; BrainXell, Inc. **H. Gautier:** 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.

Poster

PSTR312: Electrophysiology and Electrode Arrays *In Vitro*

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR312.08/Y14

Topic: I.04. Physiological Methods

Title: Advancing neurological assay readouts in drug discovery: CytoTronics' Pixel Electrical Imaging platform

Authors: *S. CHITALE[1], C. AMBROSI[2], J. ABBOTT[1];
[1]CytoTronics, Inc, Boston, MA; [2]CytoTronic, Inc, Boston, MA

Abstract: *In vitro* neurological models enable precise manipulation and high throughput screening of therapeutic compounds, advancing treatments for disorders such as Alzheimer's, Parkinson's, and epilepsy. A significant challenge in this area has been the lack of measurement systems for electrophysiological readouts of neural activity at scalable throughputs. Moreover, most systems can solely assay neural activity, in essence ignoring the contribution of structural changes in neurons and of supporting cells to disease and/or toxicity. CytoTronics' Pixel electrical imaging platform fills this crucial gap by offering non-invasive, label-free, live cell multiparametric readouts at scale. The platform comprises a high-density electrode array with 12.5 µm spatial resolution capable of electrophysiological recordings providing functional assessments of electrogenic cells. In addition, the same electrodes capture over 20 functional and morphological parameters, including tissue barrier integrity, cell-surface attachment, cell flatness, and motility via unique field-based impedance measurements. The platform facilitates real-time measurements at intervals ranging from minutes to hours, enabling the creation of electrical images and time-lapsed videos, providing longitudinal insights. Electrophysiological measurements facilitate spike detection and monitoring of network activity, crucial for understanding neuronal function. Furthermore, electrical imaging enables assessment of morphological features of cells at the population level. Alternately, spatial data can be utilized to evaluate single-cell behavior and assess cellular heterogeneity and multi-cellular system responses. The Pixel's high throughput capacity makes it well-suited for toxicity screening and disease modeling applications. Here, we show that the integration of impedance measurements, scalability, and high throughput capabilities presents a powerful combination for advancing neurological assay readouts. Utilizing impedance, morphological changes in neurons can be accurately detected, offering insights into cellular structure alterations. Moreover, the platform enables the identification of individual cell populations through impedance measurements combined with electrophysiological recordings, particularly in co-culture and tri-culture systems. CytoTronics' Pixel platform emerges as a versatile tool for advancing assay readouts in drug discovery, offering comprehensive insights into cellular behavior critical for therapeutic development.

Disclosures: S. Chitale: A. Employment/Salary (full or part-time):; CytoTronics, Inc. **C. Ambrosi:** A. Employment/Salary (full or part-time):; CytoTronics, Inc. **J. Abbott:** A. Employment/Salary (full or part-time):; CytoTronics, Inc.

Poster

PSTR312: Electrophysiology and Electrode Arrays *In Vitro*

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR312.09/Y15

Topic: I.04. Physiological Methods

Title: Towards more Physiological Assays: Using High Throughput Automated Patch Clamp for Compound Screening in Primary Hippocampal Neurons

Authors: *D. NAGY[1], K. BAMPALI[2], K. BODDUM[3], M. ERNST[4];
[1]Sophion Biosci., Bedford, MA; [2]Dept. of Drug Design and Pharmacol., Univ. of Copenhagen, Copenhagen, Denmark; [3]R&D, Sophion Biosci., Ballerup, Denmark; [4]Med. Univ. of Vienna, Ctr. For Brain Res., Vienna, Austria

Abstract: Neuroscience is a notoriously difficult therapeutic area - neuropharmacology programs have the lowest success rate within therapeutic approvals by the FDA. One of the key factors is the dearth of relevant disease models for testing and investigating pathophysiological mechanisms. Dissociated primary mice neurons are excellent modelling systems for neurobiological, biophysical, and pharmacological evaluations. The presence of a wide variety of ion channels and receptors ensures a physiologically relevant analysis of cell response and signaling. To date, patch clamp is the only technique that provides direct functional, temporal and spatial information of a cell's electrical and signaling properties. In addition, the Qube automated patch clamp (APC) platform enables the possibility of a high throughput screening (HTS) for thousands of compounds by recording 384 cells simultaneously. In this study, isolated primary hippocampal neurons from mice were patched on the Qube 384 APC system. Using an optimized cell dissociation protocol to obtain healthy cell membranes for patch-clamp, we obtained a whole-cell success rate of 65% ± 6.5%. Among these cells, 69% ± 21% expressed sodium (Na_v) currents and out of the latter group, 44% ± 6% of cells showed a >100 pA response to 100 µM GABA. Furthermore, action potential firings were recorded from 31% of the cells that passed the quality criteria filtering using the current-clamp mode of the system. Finally, in the GABA-responsive cells, we characterized the effect of 12 GABA receptor modulators on responses to sub-µM GABA concentrations (0.5 µM GABA).

Disclosures: D. Nagy: A. Employment/Salary (full or part-time):; Sophion Bioscience. **K. Bampali:** None. **K. Boddum:** A. Employment/Salary (full or part-time):; Sophion Bioscience. **M. Ernst:** None.

Poster

PSTR312: Electrophysiology and Electrode Arrays *In Vitro*

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR312.10/Y16

Topic: I.04. Physiological Methods

Support: NIH BRAIN Initiative Grant 1U19NS107464

Title: Studying cooperative effects in spike-timing-dependent plasticity (STDP) of neuronal networks through holographic optogenetic stimulation

Authors: *A. DE ZOYSA[1], S. J. GATES III[2], W. LOSERT[3];
[1]Univ. of Maryland - Col. Park, College Park, MD; [2]IPST, Univ. of Maryland Col. Park, College Park, MD; [3]Col. of Computer Mathematical and Natural Sci., Univ. of Maryland, College Park, MD

Abstract: This study utilizes holographic optogenetic stimulation to understand how group-to-group neuronal interactions enhance STDP compared to sequential stimulation of individual neurons. The hypothesis posits that STDP intensifies in group stimulations due to the redundancy of multiple pre-synaptic neurons increasing the likelihood of post-synaptic firing. Employing our open-source software platform NeuroART, we identify neurons for photostimulation under three criteria: selecting highly active neurons, those most correlated with the population, and a random selection uncorrelated with the group. The study progressively examines the impact of pairwise stimulation in neuronal groups ranging from pairs to trios, aiming to elucidate the influence of neighboring neurons' cooperative interactions on synaptic strength. This exploration extends to understanding the role of redundant pre-synaptic neurons in bolstering post-synaptic response efficacy. Utilizing real-time imaging and photostimulation facilitated by NeuroART (real-time processing up to 30Hz), the research promises insights into the complex dynamics of synaptic modulation within neuronal networks, highlighting the significance of cooperative neuronal activity. We have conducted our experiments in-vitro, imaging the calcium activity of primary rat embryonic hippocampal neuronal cells that were transduced using the bicistronic lentiviral vector, pLV[Exp]-Bsd-SYN1-jGCaMP8s-P2A-ChrimsonR-ST, that provides robust co-expression of the Calcium indicator (jGCaMP8s) and the opsin (stChrimsonR) used for holographic optogenetic stimulation. Furthermore, we study neural networks composed of mixtures of neurons and astrocytes, that provide insights into the complex dynamics involving both neurons and astrocytes during learning. The group to group photostimulation paradigms were tested on two different photostimulation setups, one which utilizes a Spatial light modulator (SLM) with voltage overdrive (with switching rates up to 1kHz), while the second setup utilizes a Digital Micromirror Device (DMD). Both of these setups operate at physiologically relevant timescales that enable millisecond precision control of neuronal activity to study STDP of living neuronal networks.

Disclosures: A. De Zoysa: A. Employment/Salary (full or part-time):; University of Maryland - College Park. **S.J. Gates:** A. Employment/Salary (full or part-time):; University of Maryland - College Park. **W. Losert:** A. Employment/Salary (full or part-time):; University of Maryland - College Park.

Poster

PSTR312: Electrophysiology and Electrode Arrays *In Vitro*

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR312.11/Y17

Topic: I.04. Physiological Methods

Support: AFOSR FA9550-23-1-0386
AFOSR-FA9550-23-1-0736
EU-Horizon MSCA-ASTROTECH-956325

Title: Electrical stimulation of distinct calcium signalling in brain astrocytes through graphene oxide-based interfaces.

Authors: *V. BENFENATI;
Cnr-National Res. Council, Bologna, Italy

Abstract: Astrocyte Ca^{2+} signalling is crucial for neurovascular coupling and synaptic transmission, and it is disrupted in the central nervous system diseases. Nevertheless, deciphering the biophysical mechanisms that underpin the diverse nature of astrocyte Ca^{2+} dynamics has remained a significant issue. In this study, we exploit the unique properties of graphene-oxide (GO) and reduced GO (rGO)-coated electrodes for controlling Ca^{2+} signalling in astrocytes via electrical stimulation. Using qRT-PCR, we determined the effect of electrical stimulation on GFAP expression on primary rat cortical astrocytes grown on indium tin oxide (ITO) coated with GO or rGO films. We found that GO/rGO are biocompatible coating interfaces, promoting astrocyte development while exhibiting no detrimental gliotic reactivity, even after electrical stimulation. Ca^{2+} imaging analyses performed in vitro and in brain slices obtained from GFAP/EGFP transgenic mice show. Unexpectedly, we discovered that electrical stimulation can cause distinct intracellular Ca^{2+} responses in astrocytes *in vitro and ex-vivo*, depending on the electrical properties of rGO/GO interfaces. Astrocytes stimulated by insulating GO electrodes show a slow, sustained Ca^{2+} response, mediated by external Ca^{2+} influx, mainly though TRPV4 and TRPA1 channels and involving IP3 and Gq G-Protein Coupled Receptor (GPCR) signalling pathway. Conversely, astrocytes stimulated by conductive rGO electrodes exhibit a fast, oscillatory Ca^{2+} response, exclusively due to Ca^{2+}release from intracellular stores through IP3Rs and Gi/o GPCR. Notably, comparative analyses in neurons and astrocytes revealed that astrocytes respond earlier and more strongly to the electrical stimulation than surrounding neurons. Accordingly, we propose a bioelectrical model, assuming that the different conductivity of the substrate influences the electric field at the cell/material or cell/electrolyte interfaces, depolarising astrocytes with different onset and respectively driving extracellular Ca^{2+} influx or Ca^{2+} release from cytoplasmic stores The model was validated by pharmacology, patch-clamp and voltage sensive dye imaging, calcium imaging and pharmacology. In conclusion, we present a simple tool for selectively activating distinct Ca^{2+} pathways in brain astrocytes[1], without the need of genetic modification[2].

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Disclosures: V. Benfenati: None.

Poster

PSTR312: Electrophysiology and Electrode Arrays *In Vitro*

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR312.12/Web Only

Topic: I.04. Physiological Methods

Support: NSF ECCS-1846659
AFOSR FA9550-23-1-0006

Title: Label free mid infrared photothermal microscopy for imaging of neurological samples

Authors: *P. SAMOLIS[1], P. DURGUN[1], V. BENFENATI[2], C. LAZZARINI[2], T. POSATI[2], A. KONSTANTOULAKI[3], G. CONTE[2], M. Y. SANDER[1];
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Abstract: Imaging neurological samples is a vital step for understanding fundamental mechanisms associated with physiological processes, differentiation and functionality. For biological applications, the molecular fingerprint region in the mid-infrared (mid-IR, 3 µm - 12 µm) has attracted interest since it contains characteristic vibrational resonances of many biophysically relevant compounds, including proteins, lipids, and nucleic acids. In mid-IR photothermal microscopy, targeting these resonances leads to imaging with high specificity and sensitivity with sub-diffraction limited resolution smaller than 1 µm. Intracellular signatures of neural cells can overlap with other mid-IR resonances, including their physiologically relevant environments, making their detection challenging. Unlike direct spectroscopy methods that solely investigate the absorption characteristics, our photothermal microscope can extract additional information for differentiation based on thermal diffusion properties, molecular structure and chemical content. With VIPPS (Vibrational Infrared Photothermal and Phase Signals) imaging, areas with different thermal diffusion properties can be detected with high contrast. Boxcar detection further enables time-resolved imaging to quantify the local transient thermal dynamics. This technique was applied to imaging axon bundles extracted from the main motor axon of the crayfish leg in a saline solution. Protein clusters and their characteristic thermal decay constants were identified, and were shown to vary between isolated axon bundles and those embedded in a neural tissue environment [1]. In addition, primary rat neocortical astrocytes were studied in vitro. Protein-rich areas and lipid accumulations were characterized as

well as membranous features consisting of alpha-helices or beta-sheets. This structural information provided insights into the differentiation processes and their corresponding molecular structure and morphology for cells on various substrates including poly-D-lysine and nanostructured hydrotalcite-like compounds capable to provide a molecular and functional differentiated astrocytes phenotype in vitro [2],[3]. Overall mid-infrared photothermal microscopy is a versatile and emerging technique that can shed more light on the biochemical content of neural cells and help address important neuroscience questions associated with the brain environment and its morphology, and the mechanisms through which neuroglia maintain homeostasis. [1]. Anal. Chem. 95, 45, 16514-16521 (2023). [2]. Sci Rep. 6, 31226 (2016). [3]. Cell Physiol Biochem. 55(S1),196-212 (2021).

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Poster

PSTR313: Flexible Electrode Arrays and Sensing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.01/Y18

Topic: I.04. Physiological Methods

Title: Improvements in flexible microelectrode arrays through process optimization and new techniques to achieve higher yield, higher density, and advanced device capabilities

Authors: *J. ZHOU[1], J. HERNANDEZ[1], J. GONZALES[1], A. M. YORITA[1,2], T. L. MASSEY[1,2], R.-U. HAQUE[1,2];
[1]Lawrence Livermore Natl. Lab., Livermore, CA; [2]Kavli Institue for Fundamental Neuroscience, San Francisco, CA

Abstract: Implantable electrode arrays are optimized for different use cases to improve parameters such as device performance for increased signal-to-noise or lifetime, capabilities like adding sensing elements for biomarker detection or modified electrode surfaces for better stimulation, durability for post-fabrication assembly, and size. Here, we have optimized process steps and utilized new techniques to advance capabilities that were previously otherwise difficult or inconvenient. Thus far, we have designed, fabricated, and distributed microelectrode arrays for a variety of species: rat, mouse, chinchilla, songbird, non-human primates, and human. The general process flow of our flexible implantable devices, fabricated using thin-film microfabrication techniques, is similar, allowing us to leverage process improvements on all of our designs. As our implantable technology moves towards higher channel densities and electrode counts, we are optimizing our processes to enable finer features while maintaining a small footprint and high yield. For example, a defining step in our process flow to achieve higher channel densities is the metal patterning step. To pattern a thick metal stack layer with relatively

tight pitch, we optimized the resist profile for our plasma etching process. We have also incorporated electron beam lithography into our process to achieve submicron features further pushing our capabilities to achieve more electrodes in a given volume than previously possible. Overall, optimizing process steps, introducing new fabrication techniques, exploring other device platforms, and producing high yield arrays are a few ways we seek to improve implantable technology development to better understand neural processes. Prepared by LLNL under Contract DE-AC52-07NA27344.

Disclosures: J. Zhou: None. **J. Hernandez:** None. **J. Gonzales:** None. **A.M. Yorita:** None. **T.L. Massey:** None. **R. Haque:** None.

Poster

PSTR313: Flexible Electrode Arrays and Sensing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.02/Y19

Topic: I.04. Physiological Methods

Support: Indian Council of Medical Research ITR grant (ID 2021-10828)

Title: Understanding onset and epileptic spread in a genetic mouse model of lafora disease using customised flexible polyimide electrodes

Authors: *G. CHAUHAN[1], D. CHUGH[2], K. KUMAR[4], K. DESHPANDE[5], S. GANESH[6], A. RAMAKRISHNAN[3];
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Abstract: Introduction:Understanding seizure initiation and its propagation remains a critical goal of epilepsy research. Exploring the properties of seizure-initiating circuits may enable efficient seizure control. To this end, we have utilised a genetic knock out mouse model of Lafora disease (LD), a rare and fatal neurodegenerative disorder, that manifests with epileptic seizures, myoclonus, ataxia, and cognitive decline. To gain a comprehensive understanding of the electrographic signatures underlying epileptogenesis in LD, we have used custom-developed, flexible neural probes to optimise spatial resolution, increase longevity, while providing high signal quality, for precise identification of seizure initiation zones.
Methodology:We performed acute and chronic (up to 14 days, n=6) electrophysiological recordings to characterise the epileptiform signatures of the LD mouse (C57BL/6). We employed three levels of recording from the brain using screw EEG from frontal and temporal cortices, and customised flexible 32 channel ECoG electrodes and 32-channel depth electrodes (flexible electrodes were provided by EYWA NEURO PVT. LTD, INDIA) to determine epilepsy onset

and spread in LD mice.
Results:In this study, we used flexible polyimide depth electrodes to elucidate neuronal firing properties during spontaneous seizures in LD mice across several days. The utilization of flexible polyimide electrodes at various levels of invasiveness, with high signal-to-noise ratio and continuous chronic recording capability, represents a significant advancement in the localization of epileptogenesis zones. Our preliminary results revealed age-dependent variations in the occurrence and frequency of spontaneous epileptiform discharges - like spike and wave discharge, polyspikes, sharp waves, and giant spikes. Notably, regional disparities were evident in the initiation of ictal events. Future investigations, incorporating chronic neural recordings with 32-channel depth electrodes, hold promise for deeper insights into unique neuronal firing dynamics in epileptic zones such as the penumbra, potentially paving the way for more effective seizure prediction strategies in LD and other forms of epilepsy. Multi-level neural recordings can reveal aberrant network connectivity patterns and identification of critical nodes of epileptic network by enhancing spatial and temporal resolution.

Disclosures: G. Chauhan: A. Employment/Salary (full or part-time):; Indian Institute of Technology Kanpur. **D. Chugh:** A. Employment/Salary (full or part-time):; Indian Institute of Technology Kanpur. **K. Kumar:** A. Employment/Salary (full or part-time):; Indian Institute of Technology Kanpur. **K. Deshpande:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eywa Neuro PLC. **S. Ganesh:** A. Employment/Salary (full or part-time):; Indian Institute of Technology Kanpur. **A. Ramakrishnan:** A. Employment/Salary (full or part-time):; Indian Institute of Technology Kanpur.

Poster

PSTR313: Flexible Electrode Arrays and Sensing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.03/Y20

Topic: I.04. Physiological Methods

Support: NIH 1DP1DK130673
NIH 1RF1MH123948

Title: Soft and flexible bioelectronics for brain-machine interfaces

Authors: *J. LIU;
Harvard Univ., Boston, MA

Abstract: Large-scale brain mapping through brain-machine interfaces is important for deciphering neuron dynamics, addressing neurological disorders, and developing advanced neuroprosthetics. Ultimately, brain mapping aims to simultaneously record activities from

millions, if not billions, of neurons with single-cell resolution, millisecond temporal resolution and cell-type specificity, across three-dimensional (3D) brain tissues over the course of brain development, learning, and aging. In this talk, I will first introduce flexible and soft bioelectronics with tissue-like properties that can track electrical activity from the same neurons in the brain of behaving animals over their entire adult life. Specifically, I will discuss the fundamental limitations of the electrochemical stability of soft electronic materials in bioelectronics and present our strategies to overcome these limitations, enabling a scalable platform for large-scale, long-term, stable brain mapping. Then, I will discuss the creation of "cyborg organisms", achieved by embedding stretchable mesh-like electrode arrays in 2D sheets of stem/progenitor cells and reconfiguring them through 2D-to-3D organogenesis, which enables continuous 3D electrophysiology during the development of human stem cell-derived brain organoids and animal embryonic brains. Next, I will highlight our current efforts that merge 3D single-cell spatial transcriptomics, machine learning, and electrical recording, enabling cell-type-specific brain activity mapping. In conclusion, I will envision the fusion of soft and flexible electronics, spatial transcriptomics, and AI for a comprehensive brain cell functional atlas to enhance future brain-machine interface applications.

Disclosures: J. Liu: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Axoft, Inc..

Poster

PSTR313: Flexible Electrode Arrays and Sensing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.04/Y21

Topic: I.04. Physiological Methods

Support: DARPA Contract N66001-17-C-4001
DoD CDMRP Contract HT9425-23-1-0758
NSF Grant 1546296
NIH Grant R01DC019498

Title: Stable, chronic in-vivo recordings from a fully wireless subdural-contained 65,536-electrode brain-computer interface device

Authors: *T. JUNG[1,2], N. ZENG[6,1], J. D. FABBRI[2], G. EICHLER[3], Z. LI[7], K. F. WILLEKE[7], K. WINGEL[8], A. DUBEY[8], R. HUQ[2], M. SHARMA[1], Y. HU[2], A. PARIHAR[2], H. YIN[2], D. OSWALT[8], G. RODRIGUEZ[7], C. NEALLEY[7], E. F. SPINAZZI[4], S. S. PATEL[7], D. YOSHOR[8], P. D. CANOLL[5], L. P. CARLONI[3], B. PESARAN[8], B. YOUNGERMAN[4], R. COTTON[9], A. S. TOLIAS[7,10], K. L. SHEPARD[1];
[2]Electrical Engin., [3]Computer Sci., [4]Neurolog. Surgery, [5]Pathology, [1]Columbia Univ., New York, NY; [6]Kampto Neurotech LLC, Lloyd Harbor, NY; [7]Ophthalmology, Stanford Univ.,

Stanford, CA; [8]Neurosurg., Univ. of Pennsylvania, Philadelphia, PA; [9]Shirley Ryan Abilitylab / Northwestern Univ., Chicago, IL; [10]Baylor Col. of Med., Houston, TX

Abstract: Micro-electrocorticography (μECoG) is a minimally invasive intracranial electrophysiological technique that holds promising potential for chronic applications in the clinical and prosthetics space. Existing devices, however, require connection to bulky, often external electronics which incurs surgical overhead and increases the risk of infection and tissue damage. Here, we developed a wireless, battery-free, mechanically flexible μECoG device that monolithically integrates electrodes, signal processing, data telemetry, and powering onto a single thin chip. The device has an array of 256×256 microelectrodes covering an area of 6.8mm×7.4mm and provides 65,536 recording and 16,384 stimulation channels, from which we can simultaneously record up to 1024 channels at a given time. At a thickness of less than 50 μm, the device can be fully implanted in the subdural space. A relay station positioned over the implant provides wireless powering and bi-directional communication to the implant from outside the body. Our device was validated through a series of proof of concept in-vivo recordings from different cortical regions of a porcine and non-human primate (NHP) models. From the porcine model, we took subchronic recordings of somatosensory evoked potentials (SSEPs) in response to peripheral stimulation. In the NHP, we took acute recordings from motor cortex while the subject performed asynchronous reach-and-grab tasks. In addition, we took chronic (up to two months) recordings from the visual cortex while the subject was presented with multiple sets of visual stimuli of different nature. In our presentation, we show the rich spatiotemporal dynamics of the recordings collected from each experiment. Some notable features of our recordings include resolving temporal dynamics of high-gamma band activity in the motor cortex and capturing long-term stable, high spatial resolution retinotopic map in the visual cortex. We also present the sensory, motor, and visual decoders trained from our recordings that yielded highly predictive models, indicating the high fidelity of the recorded signals. Our device is a brain-computer interface that delivers a high spatiotemporal resolution μECoG technology while making orders-of-magnitude improvements in volumetric efficiency and channel count over existing approaches.

Disclosures: T. Jung: A. Employment/Salary (full or part-time):; Kampto Neurotech LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Kampto Neurotech LLC. **N. Zeng:** A. Employment/Salary (full or part-time):; Kampto Neurotech LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Kampto Neurotech LLC. **J.D. Fabbri:** None. **G. Eichler:** None. **Z. Li:** None. **K.F. Willeke:** None. **K. Wingel:** None. **A. Dubey:** None. **R. Huq:** None. **M. Sharma:** None. **Y. Hu:** None. **A. Parihar:** None. **H. Yin:** None. **D. Oswalt:** None. **G. Rodriguez:** None. **C. Nealley:** None. **E.F. Spinazzi:** None. **S.S. Patel:** None. **D. Yoshor:** None. **P.D. Canoll:** None. **L.P. Carloni:** None. **B. Pesaran:** None. **B. Youngerman:** None. **R. Cotton:** None. **A.S. Tolias:** None. **K.L. Shepard:** None.

Poster

PSTR313: Flexible Electrode Arrays and Sensing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.05/Y22

Topic: I.04. Physiological Methods

Support: National Natural Science Foundation of China (62171254)

Title: Coupling of Cortex-wide Neurons to Populations Using Optical-electrical Multimodal Interface During Loss of Consciousness

Authors: *G. XIAO;
Tsinghua Univ., Beijing, China

Abstract: Electrophysiology and optical imaging are two complementary methodologies for acquiring multi-region neural activities across the wide cortex. Optical imaging provides high spatial resolution, while electrophysiological tools offer direct recordings with high temporal resolution. However, each method has its limitations, such as limited temporal resolution in optical imaging and relatively low throughput in electrophysiology. Combining these techniques through multimodal recordings can provide a more comprehensive understanding of neural activity. In this study, we introduce the development and implementation of our Whole-Cortex Optical-Electrical Multimodal (WHOM) neural interface. As the optical recording component, the WHOM neural interface enables acquiring neural activity of ~10,000 neurons from whole mouse cortex at a speed of 10Hz by integrating our previous reported RUSH imaging platform and calcium imaging technology. As for the electrical part, we developed a 32-ch transparent ECoG electrode array to complement the limitations of optical imaging in capturing the rapid propagation of neural activity without obstructing the imaging. Contributed from this combination, high temporal and spatial resolution recordings of whole-cortex neural activity is achieved. This multimodal recording setup can be maintained for over a month. Through the implementation of the WHOM neural interface, we studied the process of consciousness loss and recovery in mice under anesthesia, revealing the coupling of individual neurons to populations across a wide cortex. This work represents the first demonstration of such a comprehensive approach, opening up new possibilities for understanding neural activity in the cortex.

Disclosures: G. Xiao: None.

Poster

PSTR313: Flexible Electrode Arrays and Sensing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.06/Y23

Topic: I.04. Physiological Methods

Support: NIH Grant NIBIB DP2-EB029757

Title: Intracranial electroencephalogram (iEEG) microdisplay for neurosurgical applications

Authors: *S. FISHER[1], S. DAYEH[2], K. COFFEY[1], T. WU[1];
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Abstract: Every year millions of neurosurgeries are performed to treat a variety of conditions such as drug resistant epilepsy or glioma. A key element of these procedures is the determination of surgical boundaries, but the techniques for doing so have a coarse resolution and do not relay information to the surgeon in an efficient manner. We have addressed these shortcomings in surgical brain mapping by combining high resolution electrocorticography (ECoG) grids with LED microdisplays capable of displaying neural activity in real time. In our previous work, a proof-of-concept system composed of a 1024 channel ECoG grid and a dual color 2048-pixel LED array was successfully validated in a pig model. We have since pushed this technology forward by adding a surgical flap allowing the display to remain on the brain throughout the surgery, by scaling up the resolution to match the state of the art in display technology, and by implementing additional safety features critical for the eventual translation of this system into the operating room. In the proof of concept design the microdisplay covers the brain and would need to be removed during surgery. To further tailor this technology toward its neurosurgical application we have adjusted the design to incorporate a flap allowing the central portion of the LED array and ECoG grid to be folded back, exposing the brain. By optimizing the microfabrication techniques we developed for producing flexible LED arrays, we have achieved a pixel count of 102,400 resulting in a 423-dpi resolution comparable to that of a modern smartphone screen. Critically, our scaled up microdisplay is capable of capturing the data provided by our group's 4096 channel ECoG grids while being adaptable to the ongoing improvements in the channel count of our ECoG technology. The scaling of our display has also been accompanied by a full redesign of its backend circuitry. The updated LED driver board serves to accommodate the larger array by utilizing improved LED driver chips and connectorization scheme while adding live monitoring of leakage current from the microdisplay. This leakage current monitoring is accomplished by comparing the current being provided by the LED driver chips to the measured current returning from the microdisplay 1200 times a second, giving our system the capability to shut down immediately following the onset of current leakage out of the display. The implementation of these advancements to our microdisplay technology has maximized the potential utility of the system and lays the ground work for us to validate its efficacy in improving surgical outcomes through planned experiments in animal models.

Disclosures: S. Fisher: None. **S. Dayeh:** None. **K. Coffey:** None. **T. Wu:** None.

Poster

PSTR313: Flexible Electrode Arrays and Sensing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.07/Y24

Topic: I.04. Physiological Methods

Support: NINDS Grant RFA-NS-21-026

Title: Directional and scalable microelectrode array for epilepsy detection

Authors: *R. SHORES[1,2], J. C. MOSHER[3], N. TANDON[2], S. PATI[3], R. M. LEAHY[4], Y. S. VAKILNA[3], T. MEDANI[4], A. JOSHI[4], C. MATTHEWS[2], J. P. SEYMOUR[1,2];
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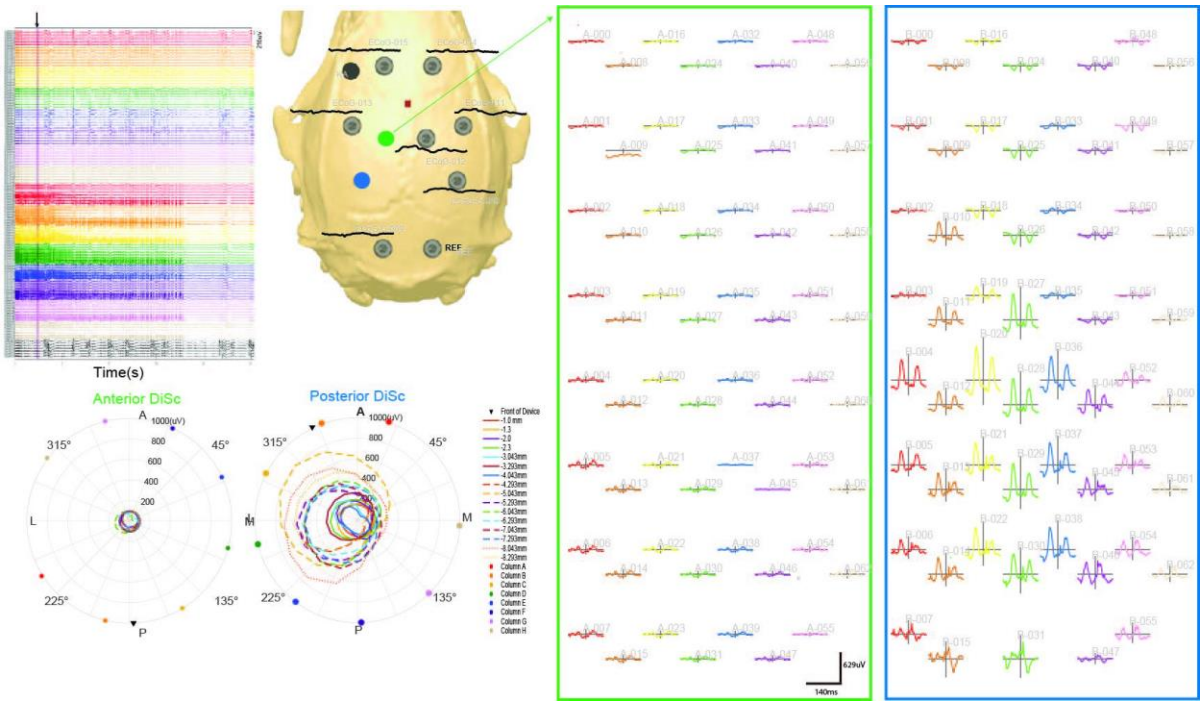
Abstract: Epilepsy diagnostic tools seek to identify hyper-synchronous neural substrates that form the seizure onset zone. Greater resolution of activity in putative foci and network nodes has the potential to help surgeons create more efficacious treatment plans and accelerate our understanding of the disease's etiology. As a technology demonstration, we have utilized a novel high-density stereo-electroencephalography (sEEG) electrode array in a rat kainic acid (KA) seizure model during status epilepticus to provide a direct comparison with virtual ring electrodes, which are the clinical standard of care. We implanted ECoG bone screws and high-resolution devices - directional and scalable (DiSc) electrode arrays - in a rat KA seizure model. Two DiSc arrays were implanted ipsilaterally into rat hippocampus and used to record electrical activity in vivo following a kainic acid injection into the anterior basolateral amygdala (BLA) sufficient to cause status epilepticus (n = 6). Magnetic Resonance Imaging (MRI) confirmed the location of the injection site and the trajectory of each device through the brain.
We previously demonstrated that an insulating substrate 0.8mm in diameter provides high-resolution circumferential information of neural activity in the rat barrel field and now hypothesize that DiSC can also reveal the seizure progression in sub-hippocampal regions. We have compared our signal-to-noise ratio to that of the virtual ring electrodes and demonstrate multiple, distinct sources that develop dynamically before and during status epilepticus. Current efforts include source localization via inverse modeling in BrainStorm software. The hardware and software tools developed here will allow epileptologists a means to study the pathogenesis and network connections in epilepsy models



Disclosures: R. Shores: None. **J.C. Mosher:** None. **N. Tandon:** None. **S. Pati:** None. **R.M. Leahy:** None. **Y.S. Vakilna:** None. **T. Medani:** None. **A. Joshi:** None. **C. Matthews:** None. **J.P. Seymour:** None.

Poster

PSTR313: Flexible Electrode Arrays and Sensing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.08/Y25

Topic: I.04. Physiological Methods

Support: NIH Grant UG3/UH3
Swebilius Grant

Title: Biosense - The utilization of neuroengineering to monitor neurological disorders

Authors: *D.-S. R. K. MURRAY;
Wu Tsai Inst., Yale Univ., New Haven, CT

Abstract: Increasing our understanding of our most critical organ is tantamount to providing clinicians with the appropriate information to adequately diagnose, monitor, treat and rehabilitate patients in a variety of care settings. One way to achieve this is by creating new bioelectronic tools that work not only in the lab but, have also been rigorously tested to be reproducibly used

in real-world scenarios, for example, during neurosurgery. These tools can help us monitor the spread and progression of a range of neurological injuries, diseases and disorders, enabling clinicians to provide the best standard of care for their patients. The development of such tools can result in better outcomes for a large patient population, especially those severely injured in neurocritical care. Crucially, with the development of such tools for monitoring, we can better understand the progression of diseases and injuries and better characterize the ailment and the best support/therapies needed. Driving forward this understanding is vital due to the growing global burden of neurological disorders. Neurological disorders are the second leading cause of global mortality and the leading cause of disability worldwide. The emergence of COVID-19 and its long-term impact on vasculature is also exacerbating the relative risk of neurologic injury. Here, I present my work on creating and integrating devices to monitor various brain injuries: including traumatic brain injury, stroke and glioblastoma. I focus on the use of physical, electrical and chemical modalities within brain implantable devices, emphasizing a concerted push to clinically translate such efforts for patient benefit.

Disclosures: D.R.K. Murray: None.

Poster

PSTR313: Flexible Electrode Arrays and Sensing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.09/Y26

Topic: I.04. Physiological Methods

Title: Enhancing Long-Term Viability of Neural Probes via Comprehensive Assessment towards Standardization

Authors: *C. SIELAFF, U. P. FRORIEP;
Fraunhofer ITEM, Hannover, Germany

Abstract: The current pre-clinical regulatory framework regarding the approval of intracortical neural probes is marked by a notable lack of depth, posing significant challenges in effectively addressing the multifaceted obstacles associated with their sustained performance over time. Existing guidelines exhibit considerable deficiencies in offering the comprehensive criteria required to adequately confront issues, such as conductive interface degradation and scarring, both of which profoundly affect the long-term functionality of neural probes. Moreover, the absence of standardized methodologies within these regulatory frameworks not only impedes a systematic comparison of emerging probe designs and materials but also creates substantial barriers to progress within the field, hindering innovation and slowing down advancements. While the primary regulatory focus predominantly revolves around biocompatibility assessments, there remains a gap in enabling a comprehensive understanding of neural probe performance over extended durations. Recognizing and acknowledging this limitation, ongoing

efforts to establish standardized quality assessments for neural implants are gaining significant traction. This entails the imperative to delineate and define clear and comprehensive quality criteria for material and device characterization, probe implantation and explantation procedures, as well as protocols for histological tissue analysis, electrical recording and stimulation experiments, and the subsequent analysis of collected data. In response to this need, we aim to bridge the regulatory deficit by laying the groundwork for a robust and expansive framework aimed at thorough evaluating the long-term performance of intracortical electrodes. This can pave the way for paradigm shift beyond conventional assessments, also striving significantly to reduce the reliance on animal testing by aggregating and standardizing vast repositories of data and facilitating the development of high-fidelity computational simulations. This in turn can reduce the need for laboratory animals, can refine the use of each animal that is still needed and can replace tests that then can be done in vitro instead. Through these efforts, the overarching objective is to significantly advance the long-term treatment outcomes for patients in need, thereby fostering a more comprehensive, ethical, and effective approach to the regulation and innovation of neural probes. This work was supported by the EU Horizon 2020 programme (GA 814654) and by the BMBF.

Disclosures: C. Sielaff: None. **U.P. Froriep:** None.

Poster

PSTR313: Flexible Electrode Arrays and Sensing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.10/Y27

Topic: I.04. Physiological Methods

Support: SNSF/Innosuisse BRIDGE Discovery grant Nr. : 40B2-0_211760
SNSF Sinergia grant Nr.: NCRSII5_198739/1
ERC Consolidator grant Nr.: 818179

Title: Mri-visible superparamagnetic ultraflexible electrodes for precision electrophysiology

Authors: *E. ÖZIL[1,2], P. GOMBKOTO[3], A. APOSTOLELLI[3], T. YASAR[3], A. VAVLADELI[3], M. MARKS[4], W. VON DER BEHRENS[3], M. F. YANIK[3];
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Abstract: Ultraflexible polymer dense electrode arrays can allow chronic single-neuron recording and stimulation in multiple brain areas with good electrode-tissue integration. Due to the high flexibility of such electrodes and their resulting deflections during insertion into the brain tissue, purely stereotaxic implantations do not allow the precise localization of the

electrodes post implantation. Therefore, it is important to identify the exact brain structures extracellularly recorded and/or stimulated. However, due to the material properties and dimensions of ultraflexible electrodes (i.e., 2um thick polyimide), it is challenging to localize them by standard noninvasive imaging techniques such as magnetic resonance imaging (MRI) or computed tomography. To address this challenge, we developed MRI-visible superparamagnetic ultraflexible electrodes. A thin film of superparamagnetic iron oxide nanoparticles (IONP) was deposited in between two polyimide layers of individual electrode wire segments, where the pattern and amount of IONP deposited were optimized to achieve specific MRI contrast patterns with high localization accuracy. Our electrode arrays were easily localizable (up to 60µm resolution) with a 7T MRI, while standard arrays without IONP-deposition were invisible. Two 64-channel multielectrode arrays with and without IONP-deposition were implanted bilaterally through the dorsal hippocampus (dHPC) and thalamus of rats (n=3). To validate the positions of electrode channels localized in MRI, we used electrophysiological landmarks of the dHPC (i.e., laminar amplitude profile of sharp-wave ripples, and theta oscillation). The positions of the IONP-deposited electrode channels could be measured immediately after implantation and were trackable in vivo over several months. Our electrodes were visible and easily localizable also with a clinical 3T MRI (300µm isovoxel). Our approach of making ultraflexible electrodes MRI-visible can enhance the interpretability of neural recordings and stimulation in clinical and preclinical studies by precisely identifying their localization even in minute brain structures such as sub-cortical nuclei or distinct cortical layers.

Disclosures: E. Özil: None. **P. Gombkoto:** None. **A. Apostolelli:** None. **T. Yasar:** None. **A. Vavladeli:** None. **M. Marks:** None. **W. Von Der Behrens:** None. **M.F. Yanik:** None.

Poster

PSTR313: Flexible Electrode Arrays and Sensing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.11/Y28

Topic: I.04. Physiological Methods

Support: National Key R&D Program of China (2021YFF0702200)
STI 2030-Major Projects (2021ZD0200401)
Key R&D Program of Zhejiang Province (2021C03001)

Title: Mr-compatible Microelectrode Array and SVD-FCNN Algorithm for Neural Signal Recovery in fMRI

Authors: *H.-Y. LAI[1,2,3,4,5], X. YU[6], Z. LYU[6,4], T. HE[7,2], B. QU[7,2], H. WANG[7,2], Z. TANG[6,4], M. YE[7,2], Y.-Y. CHEN[8];
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Abstract: Functional MRI (fMRI) infers neural activity via hemodynamic changes, yet the exact relationship between these changes and neural signals remains unclear. While simultaneous electrophysiological recordings and fMRI can reveal insights into neurovascular coupling, electromagnetic noise poses significant challenges. To address this, we developed an MR-compatible microelectrode array and a combined singular value decomposition and fully convolutional neural network (SVD-FCNN) to effectively reduce magnetic susceptibility artifacts and eliminate electromagnetic noise (EN) while preserving neural signal integrity. We evaluated the magnetic susceptibility artifacts of the microelectrode array both in vitro and in the brain of a rhesus monkey by using 7T MRI system. The SVD-FCNN, trained using simulated and tactile-evoked neural signals, demonstrated its ability to significantly reduce EN while maintaining consistent spike waveforms and retaining local field potential signals. In an experiment involving a rhesus monkey undergoing fMRI scanning, no significant loss of blood oxygen level-dependent (BOLD) signals was observed around the microelectrode array, and the SVD-FCNN showed superior performance. The spike loss rate was only 3% for SVD-FCNN, compared to 11% for FCNN and 77% for SVD, underscoring the SVD-FCNN's efficacy in restoring neural signals. Furthermore, SVD-FCNN accurately recovered tactile-evoked neural signals, which correlated with BOLD signals in beta-band neural oscillations. This research introduces a novel MR-compatible microelectrode array and a robust denoising solution using SVD-FCNN for simultaneous fMRI and neural recordings, providing a valuable tool for studying neurovascular coupling and brain function.

Disclosures: H. Lai: None. **X. Yu:** None. **Z. Lyu:** None. **T. He:** None. **B. Qu:** None. **H. Wang:** None. **Z. Tang:** None. **M. Ye:** None. **Y. Chen:** None.

Poster

PSTR313: Flexible Electrode Arrays and Sensing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.12/Y29

Topic: I.04. Physiological Methods

Support: JST JPMJMS2012
JST JPMJPR22S6
JSPS JP23H04978
NCNP Intramural Research Grant 5-8

Title: A wireless recording system for cortical-wide electrocorticography in freely behaving marmosets

Authors: *M. KOMATSU;
Tokyo Inst. of Technol., Tokyo, Japan

Abstract: A major neuroscience challenge is the lack of a method for recording brain activity across the entire brain during spontaneous behaviors. Primate experiments with animal restraints have yielded crucial findings, though they restrict access to neural mechanisms in their natural settings. Here, we developed a wireless cortical-wide electrocorticography (WicE) to simultaneously record animals' behaviors and neural activities over a hemisphere. WicE successfully collected data over 2 years from freely moving marmosets with stable data qualities. We investigated event-related neural activities for spontaneous movements in a cortical-wide manner. Classification of animal behaviors identified 6 types of behavioral states: sitting, standing, clinging, moving, scratching, and food manipulation. We focused on clinging states, since they involve goal-directed actions, jumping up (Jump Up) and jumping down (Jump Down), whose mechanisms are difficult to investigate on restrained animals and remain unclear. We proceeded to investigate the spatiotemporal neural activity across the cortex by mapping significant signal changes surrounding the onsets of actions. We found that the frontal, parietal, and temporal association areas exhibited notable neural activity prior to the onsets of both actions. Furthermore, we observed action-dependent patterns of neural activity in the frontal association areas; dorsal activation preceding 'Jump Up' and ventral activation preceding 'Jump Down'. Moreover, we identified pronounced pre-action signal changes in visual areas, particularly preceding 'Jump Up,' which were characterized as sustained suppressions. WicE provides a way to investigate cortical-wide neural mechanisms in freely behaving primates.

Disclosures: M. Komatsu: None.

Poster

PSTR313: Flexible Electrode Arrays and Sensing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.13/Y30

Topic: I.04. Physiological Methods

Support: NIH UG3/UH3 Grant
NSF Graduate Research Fellowship

Title: Fully wireless 4224-channel ECoG electrode recording of pig neuronal activities

Authors: *T. S. PORTER[1], J. LEE[1], W. JEON[1], H. LE[1], K. LEE[1], P. BOTROS[1], J. GU[1], K. FORSETH[2], S. FISHER[1], R. VATSYAYAN[1], A. BOURHIS[1], A. C. PAULK[3], S. S. CASH[3], E. HALGREN[4], S. BEN-HAIM[2], A. RASLAN[5], S. DAYEH[1];
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Abstract: Around 50 million people live with epilepsy worldwide, and over a quarter of all epilepsy patients experience drug-resistant epilepsy where the most effective paradigms for treatment become surgical intervention or electrical stimulation. Electrocorticography (ECoG) is the gold-standard mapping technique to identify epileptogenic zones and to delineate boundaries between pathological and healthy tissue. High spatiotemporal resolution with broad cortical coverage is crucial for precise localization to enhance our understanding of epilepsy, improve patient outcomes and reduce functional impairments and potential side effects of surgery. However, current clinical ECoG electrodes have low spatial resolution and channel count where individual contacts are from 2 to 3 mm in diameter and up to 10 mm in intercontact spacing. Building upon our first-in-human intraoperative brain mapping with 1024-channel microelectrode arrays on pathological tissue, we further scaled up micro-ECoG technology and advanced it toward wireless human implantation. To demonstrate stable, high-channel recording, we developed an ECoG grid with 4224 channels to cover a single hemisphere of the pig brain (3.2 x 1.3 cm^2), with a higher resolution grid (31 μm spacing) embedded within sparser contacts (130 μm horizontally; 600 μm vertically). Novel platinum nanorods (PtNRs) were utilized as electrode contacts for low impedance and long-term stability with parylene-C as the insulating material, which is transparent to the brain surface and conformal to its movements. By utilizing advanced multi-layer parylene-C fabrication methods, we achieved over 90% yield of recording contacts, with average impedance of 20 kΩ for 30 μm diameter contacts. In addition, we developed a fully wireless system that records and streams all 4224 data channels through WiFi to a recording desktop. The acquisition system and software were custom designed with IMEC chips and Open Ephys respectively. We used this fully wireless 4224-channel system in the anesthetized pig brain model to perform sensory mapping of the rostrum gyrus and to explore the functional limit of what can be resolved from the brain's surface. Contralateral sensory mappings were obtained through somatosensory evoked potential measurements by air-puff stimulation in precise locations on the pig's snout controlled by a programmable XYZ stage.
Overall, our results advance the scaling and development of high-channel clinical grids for a semichronic epilepsy monitoring platform with fully wireless data and power transfer, and also pave the way toward other applications in responsive neurostimulator systems and brain-machine interfaces.

Disclosures: T.S. Porter: None. **J. Lee:** None. **W. Jeon:** None. **H. Le:** None. **K. Lee:** None. **P. Botros:** None. **J. Gu:** None. **K. Forseth:** None. **S. Fisher:** None. **R. Vatsyayan:** None. **A. Bourhis:** None. **A.C. Paulk:** None. **S.S. Cash:** None. **E. Halgren:** None. **S. Ben-Haim:** None. **A. Raslan:** None. **S. Dayeh:** None.

Poster

PSTR313: Flexible Electrode Arrays and Sensing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.14/Y31

Topic: I.04. Physiological Methods

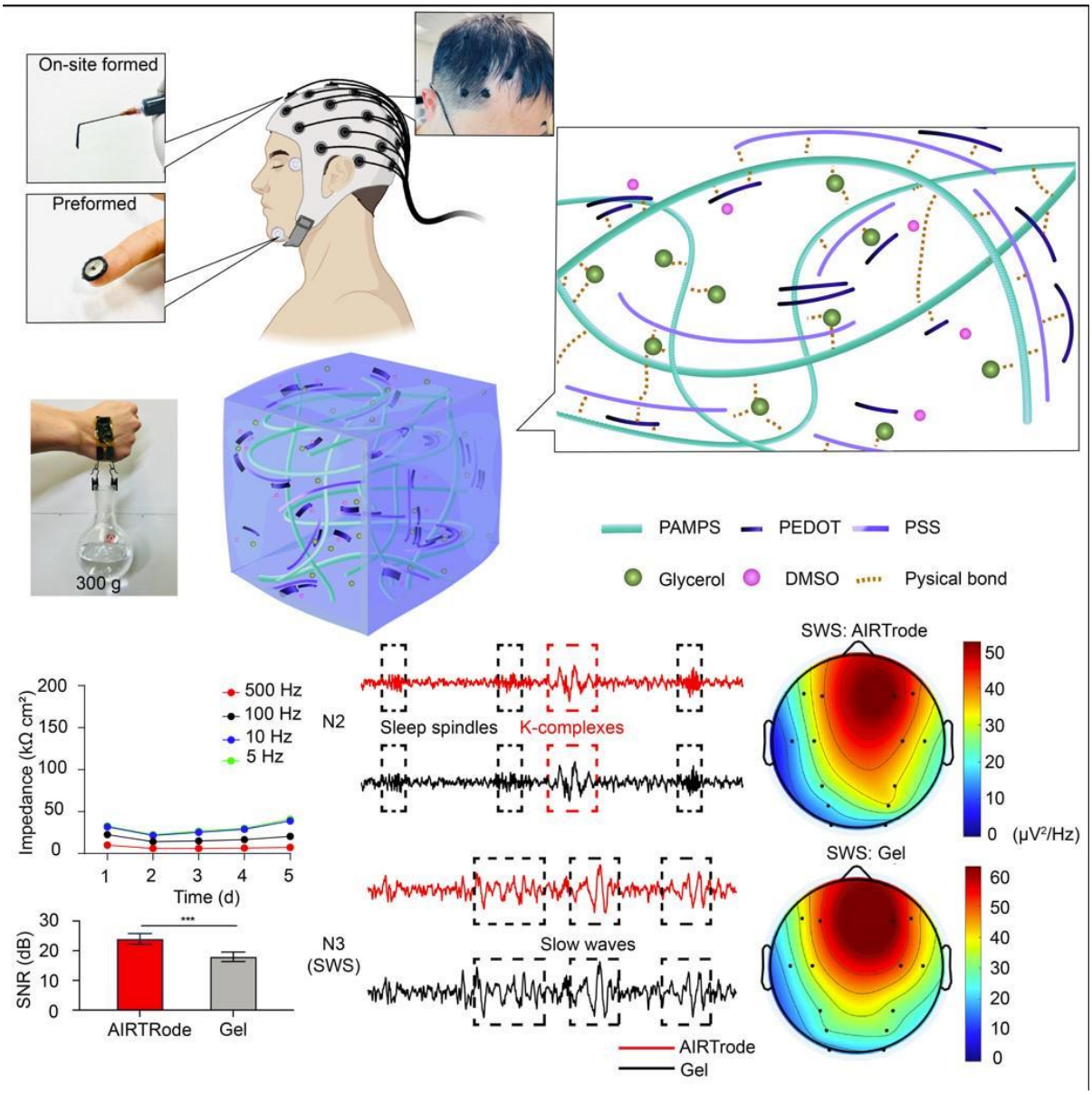
Support: University of Texas at Austin Startup Fund
UT Austin Proof of Concept Award
UT Austin Proof of Concept Award
Alzheimer's Association New to the Field (AARG-NTF) research grant
NIH Maximizing Investigators' Research Award (MIRA) (R35) grant
Whole Communities-Whole Health
Inspire Commercialization Grant from Texas Innovation Center at the University of Texas at Austin
Human Frontier Science Program Fellowship

Title: Long-term, high-quality sleep EEG monitoring with AIRTrode: A novel injectable and self-adhesive hydrogel EEG electrode

Authors: *J.-C. HSIEH[1], W. HE[1], W. WANG[1], J. JEONG[1], K. TANG[1], B. BAIRD[2], H. WANG[3];
[1]Biomed. Engin., [2]Dept. of Psychology, Univ. of Texas at Austin, Austin, TX; [3]Biomed. Engin., Univ. Of Texas At Austin, Austin, TX

Abstract: The need for long-term, stable electroencephalogram (EEG) monitoring in sleep medicine and neuroscience research underscores the necessity for advancements in more advanced EEG electrodes. Standard EEG gel (Gel) often suffers from issues such as poor adhesion and signal degradation over a short time. To address these challenges, we have developed AIRTrode, a novel hydrogel designed for prolonged and high-quality EEG recording. Our approach utilizes a unique blend of 2-acrylamido-2-methylpropane sulfonic acid, poly(3,4-ethylenedioxythiophene) polystyrene sulfonate, dimethyl sulfoxide, and glycerol to create AIRTrode. It is an injectable and self-adhesive hydrogel that spontaneously cross-links at room temperature without the presence of curing agents and treatments, allowing easy and direct application to the scalp. Thus, it achieves high signal fidelity and stability. Our study also demonstrated Airtrode's superior mechanical and electrical properties. AIRTrodes can record sleep EEG on hairy scalp regions with low impedance for > 8 hours of prolonged recording. The high adhesiveness of 0.92 N cm-1 with repeated attachment capability and long-term wearability are achieved. We applied AIRTrode to overnight sleep recordings and compared its performance with that of Gel. AIRTrode demonstrated a superior signal-to-noise ratio (SNR of 24 dB versus 18 dB for Gel). The robust adhesion of AIRTrode exhibited significantly reduced noise over extended monitoring periods. Furthermore, sleep stage classification using AIRTrode and Gel

have high correlation coefficients (Pearson's r = 0.91 ± 0.06) and Cohen's Kappa values (κ = 0.84 ± 0.06), confirming AIRTrode's reliability for research applications. The development of AIRTrode marks a significant step forward in EEG technology, providing a robust tool for sleep medicine and related fields. AIRTrode enhances the quality of EEG monitoring and expands its application in various long-term recording settings.
Original paper DOI: 10.1016/j.device.2023.100182



Disclosures: J. Hsieh: None. **W. He:** None. **W. Wang:** None. **J. Jeong:** None. **K. Tang:** None. **B. Baird:** None. **H. Wang:** None.

Poster

PSTR313: Flexible Electrode Arrays and Sensing

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.15/Y32

Topic: I.04. Physiological Methods

Support: Zeto, Inc.

Title: Technical Validation of the Zeto Wireless, Dry Electrode EEG System

Authors: *Z. NADASDY[1], A. FOGARTY[2], R. S. FISHER[3], C. PRIMIANI[4], K. D. GRABER[5]; [1]Univ. of Texas at Austin, Austin, TX; [2]Dept. of Neurol. and Neurolog. Sci., Stanford Univ., Palo Alto, CA; [3]Stanford Univ., Stanford, CA; [4]Epilepsy, NIH, Palo Alto, CA; [5]Neurol., Stanford Univ. Med. Ctr., Stanford, CA

Abstract: Wireless technology is transforming the practice of electroencephalography (EEG), but adoption of the technology is contingent upon uncompromised data quality. Therefore, technical validation of the reliability of measurements made by a new type of device is critical to proving the non-inferiority of new devices relative to conventional ones. We report four key results from testing the signal quality of the Zeto WR19 EEG system against a conventional EEG system conducted on patients in a clinical setting. We performed 30-minute simultaneous recordings using the Zeto WR19 (zEEG) and a conventional clinical EEG system (cEEG) in a cohort of 15 patients. We compared the signal quality between the two EEG systems by computing time domain statistics, spectral density, and signal-to-noise ratio. All the statistical comparisons resulted in signal quality non-inferior relative to cEEG. (I) Time domain statistics and the Hjorth parameters showed equivalence between the two systems, except for a reduction of sensitivity to electric noise in zEEG relative to cEEG. (II) The point-by-point waveform correlation between the two systems was acceptable ($r>0.6$; $P<0.001$). (III) Each dataset showed a high spectral correlation ($r>0.99$; $P<0.001$) and overlapping spectral density across all electrode positions, indicating no systematic signal distortion. (IV) The mean signal-to-noise ratio of the zEEG system exceeded that of the cEEG by 4.82 dB, equivalent to 16 % improvement. We concluded that the signal quality of the zEEG system is non-inferior to conventional clinical EEG systems concerning all relevant technical parameters that determine EEG readability and interpretability.

Disclosures: Z. Nadasdy: A. Employment/Salary (full or part-time):; Zeto, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Zeto, Inc.. **A. Fogarty:** None. **R.S. Fisher:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Zeto, Inc.. **C. Primiani:** None. **K.D. Graber:** None.

Poster

PSTR314: Network Computation: Theory and Modeling II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.01/Z1

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF Grant #2024607

Title: End-to-end modeling of the Drosophila early vision system with cascading divisive normalization processors

Authors: *B. BU[1], A. A. LAZAR[1], Y. ZHOU[2];
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Abstract: Large-scale connectomics studies have extensively revealed the connectivity of the neural circuit in the Drosophila early visual system. While the underlying mechanisms have been studied, a comprehensive characterization of the circuit's functional logic within this system remains elusive. Previous research, primarily relying on simple visual inputs and basic behavioral metrics, falls short in accounting for the complexities of Drosophila's visual environment and have yet to assess the system's capabilities and robustness. Moreover, divisive normalization, a canonical model of neural computation, suggests a more effective method of uncovering the functional logic of this system.

We modeled and evaluated the early vision system of Drosophila from phototransduction in the retina to motion detection in the Medulla and Lobula. We employed a unified theoretical framework known as divisive normalization processors (DNPs) to model the system as four cascading stages: phototransduction, contrast gain control, ON-OFF processing, and local phase information extraction in four cardinal directions. These stages collectively form an input/output-driven architecture that enables us to easily adjust the models and circuit configurations and visually evaluate the end-to-end capabilities of the model system in real-time. We employed high frame rate natural scenes as visually inspectable stimuli that capture the rapid changes and diverse luminance conditions arising in Drosophila's visual environments. The end-to-end responses are visual representations of detected motion in the ON and OFF pathways that reflect both the motion direction and velocity magnitude. In particular, contrast gain control from photoreceptors and amacrine cells interactions reliably improved motion detection by making it virtually luminance-independent, while the ON-OFF separation significantly enhanced the alignment of detected motion with the actual contours of moving objects in the visual field. Together, these cascading processing stages enable motion detection that is consistent and directionally precise, effectively extracting global and local motion patterns under varying velocities and lighting conditions.

We demonstrated that DNPs can efficiently process visual information in complex visual environments and robustly detect motion. The DNP architecture supports the flexible exploration of the functional logic of the Drosophila early vision system. By providing an open source

platform, we offer a valuable novel methodology for the theoretical and computational explorations of the functional logic of fruit fly brain circuits and beyond.

Disclosures: B. Bu: None. **A.A. Lazar:** None. **Y. Zhou:** None.

Poster

PSTR314: Network Computation: Theory and Modeling II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.02/Z2

Topic: I.06. Computation, Modeling, and Simulation

Support: SCGB AWD543027
NS104899
9R01DA056404-04
NIH NINDS R35 1R35NS111580-01
BRAIN NINDS R01 NS104899

Title: From connectome to effectome: learning the causal interaction map of the fly brain

Authors: *D. POSPISIL[1], M. ARAGON[2], S. DORKENWALD[5], A. MATSLIAH[6], A. STERLING[7], S.-C. YU[3], C. E. MCKELLAR[9], M. COSTA[10], K. EICHLER[11], G. JEFFERIS[12], M. MURTHY[4], J. W. PILLOW[8];
[1]Princeton, Princeton, NJ; [2]Princeton Neurosci. Inst., [4]Neurosci. Inst., [3]Princeton Neurosci. Inst., Princeton, NJ; [6]Princeton Neurosci. Inst., [5]Princeton Univ., Princeton, NJ; [7]Princeton Univ., Somerville, MA, ; [8]Princeton Univ., PRINCETON, NJ; [9]Janelia Res. Campus, Ashburn, VA; [10]Univ. of Cambridge, Cambridge, ; [11]Dept. of Zoology, Univ. of Cambridge, Cambridge, United Kingdom; [12]MRC Lab. of Mol. Biol., Cambridge, United Kingdom

Abstract: A long-standing goal of neuroscience is to obtain a causal model of the nervous system. This would allow neuroscientists to explain animal behavior in terms of the dynamic interactions between neurons. The recently reported whole brain fly connectome specifies the synaptic paths by which neurons can affect each other but not whether, or how, they do affect each other in vivo. To overcome this limitation, we introduce a novel combined experimental and statistical strategy for efficiently learning a causal model of the fly brain, which we refer to as the 'effectome'. Specifically, the effectome is the dynamics matrix, W_r, specifying the strength and sign of the causal effect each neuron has on every other neuron. We propose a consistent estimator of this dynamics matrix that uses stochastic optogenetic perturbation data to accurately estimate causal effects and the connectome as a prior on the effectome to drastically improve estimation efficiency. Even so, it would be infeasible in the fly—and most organisms—to independently stimulate and record from all neurons at once. The effectome thus would need to be gradually constrained across many experiments. It is unclear how to order experiments

such that insight into whole brain dynamics are achieved efficiently. We take the approach of analyzing the connectome to propose circuits that have the greatest total effect on the dynamics of the fly brain. Specifically, we perform the eigendecomposition of the matrix of the number of pre-synaptic contacts and inferred sign (inhibitory or excitatory). We discover that, fortunately, the dominant circuits (top eigenvectors) significantly involve only relatively small populations of neurons. Intriguingly, we find that this approach also re-discovers known circuits and generates testable hypotheses about their dynamics. Overall, our analyses of the connectome provide evidence that global dynamics are generated by a large collection of small circuits. This in turn implies that a causal model of a brain can be feasibly obtained in the fly.

Disclosures: D. Pospisil: None. **M. Aragon:** None. **S. Dorkenwald:** None. **S. Yu:** None. **C.E. McKellar:** None. **G. Jefferis:** None. **M. Murthy:** None. **J.W. Pillow:** None.

Poster

PSTR314: Network Computation: Theory and Modeling II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.03/Z3

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF Grant #2024607

Title: Modeling small object detection of Drosophila lobula circuits with divisive normalization processors

Authors: *S. SHUKLA[1], Y. ZHOU[2], A. A. LAZAR[1];
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Abstract: Insects evolved the ability to *robustly* detect small moving objects such as other insects and predators using visual cues. How the underlying neural circuits account for diverse natural lighting conditions and suppress background movement induced by complex mid-flight egomotion remains elusive.
In this work, we use the FlyWire connectome to analyze the connectivity graph of small moving object circuits in the fruit fly brain with (i) LMC neurons (L1, L2) in the lamina receiving retinotopic photoreceptor (R1-R6) inputs (ii) small-field CB neurons that receive LMC inputs in the lamina and project their outputs into the lobula (iii) wide-field mALC local neurons in the lobula that receive CB inputs (iv) LC21 neurons with inputs from CB and mALC neurons in the lobula, and have been experimentally demonstrated to detect small moving objects. We use the obtained insights to propose a three-stage model: (i) contrast gain control to account for both local and global lighting variations and increase motion sensitivity (ii) a novel phase based approach for background motion suppression and foreground motion enhancement (iii) a size-

tuned ON-OFF circuit for *small* moving object detection.
We then instantiate the proposed model end-to-end using *divisive normalization processors* (DNPs) and benchmark the DNP circuit on the (i) PESMOD drone videos dataset (ii) VISO satellite videos dataset. The datasets present challenging examples with multiple tiny (less than 10 square pixels) moving objects, local lighting changes due to specular reflections, and complex background motion due to 3D camera movement. We benchmark small moving object detection rates (precision and recall) and processing speed (frames per second) across both datasets, improving on previous fly-inspired models that do not perform contrast gain control and precise background motion suppression.
We thus demonstrate a DNP-based computational approach to exploring the functional logic of massively parallel and recurrent small moving object detection circuits in the early visual system of the fruit fly brain. Our large-scale computational model motivates the design of experimental methods to simultaneously record from large populations of neurons in the fruit fly brain, towards testing the functional predictions proposed in this work.

Disclosures: S. Shukla: None. **Y. Zhou:** None. **A.A. Lazar:** None.

Poster

PSTR314: Network Computation: Theory and Modeling II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.04/Z4

Topic: I.06. Computation, Modeling, and Simulation

Support: MH096881

Title: Computational analysis and multi-dimensional modeling uncover hyperbolic geometry in whole brain of C. elegans which aids in discovery of neural network states

Authors: *I. RUSU[1], Z. CECERE[2], K. QUACH[3], E. YEMINI[4], J. HOW[5], T. O. SHARPEE[6], S. CHALASANI[7];
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Abstract: Neural responses are influenced by both external stimuli and internal network states. While network states have been linked to behavioral and stimulus states, little is known about how sensory inputs are filtered by whole-brain activity to downstream motor neurons. Using calcium imaging in a Zeiss Airyscan 880, we recorded whole-brain activity of *Caenorhabditis elegans* (*C. elegans)* experiencing bacterial food stimuli and modeled how sensory inputs affect sensory and motor neurons in a network state dependent manner. We classified active neurons

into six functional clusters: two sensory neuron clusters (ON, OFF), and four motor/command neuron clusters (AVA, RME, SMDD, SMDV). We proceeded to analyze our multi-dimensional calcium trace data without losing the distance measures between points using a hyperbolic embedding technique, Hyperbolic Multidimensional Scaling (HMDS). We determined that there was a hierarchical structure among the neuronal populations. Bayesian information criteria analysis showed that our data can be optimally represented in 8-dimensional space. These dimensions correspond to the axes of 4 different sets of complementary neurons corresponding to the cell types we identified. Although neural computations performed by sensory neurons are linear due to their direct exposure to stimuli, the downstream neurons are often non-linear as they integrate inputs from multiple neurons. This non-linearity poses a challenge in interpreting downstream neural responses that correspond to their original input stimuli. Our goal is to analyze how input stimuli and sensory neurons affect downstream motor neural populations. We used low rank second order maximum noise entropy, which recapitulates the nonlinear filter dynamics within neural populations allowing us to identify specific states of the network that links sensory neuron activity with downstream motor neurons. Collectively, we present an interpretable approach for modeling network dynamics of neural populations.

Disclosures: I. Rusu: None. **Z. Cecere:** None. **K. Quach:** None. **E. Yemini:** None. **J. How:** None. **T.O. Sharpee:** None. **S. Chalasani:** None.

Poster

PSTR314: Network Computation: Theory and Modeling II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.05/Z5

Topic: I.06. Computation, Modeling, and Simulation

Title: Age-related differences in control energy of brain state transitions

Authors: *V. PARAKKATTU[1], L. LORENZO-LUACES[2], Y. JO[3], J. FASKOWITZ[4], A. PODSCHUN[5], S. MARKETT[5], R. BETZEL[6];
[1]Psychological and Brain Sci. - Clin. Sci., Cognitive Sci., Indiana Univ. - Bloomington, Bloomington, IN; [2]Psychological and Brain Sci. - Clin. Sci., Indiana Univ. - Bloomington, Bloomington, IN; [3]Psychological and Brain Sci. - Developmental Psychology, Cognitive Sci., Indiana Univ. - Bloomington, Bloomington, IN; [4]Psychological and Brain Sci., NIH, BETHESDA, MD; [5]Humboldt-Universität zu Berlin, Berlin, Germany; [6]Psychological and Brain Sci. - Computat. and Cognitive Neurosci., Cognitive Sci., Indiana Univ. - Bloomington, Bloomington, IN

Abstract: “Control energy” quantifies the effort needed to transition between different brain activation patterns based on the individual's connectome (Pasqualetti et al., 2014). This study aims to understand its variation across the human lifespan. Using the NKI-RS dataset (N = 458

with low-motion MRI data, 7-85 y/o; Nooner et. al., 2012), we reconstructed subject-specific connectomes from diffusion MRI (Tournier et al., 2019) and processed resting-state fMRI using fMRIprep (Esteban et al., 2019). Brain states were estimated by identifying amplitude peaks in brain-wide activity, clustering them using k-means (k = 6), and labeling each frame accordingly. The energy required to transition between each pair of states was calculated using subjects' connectomes and correlated with age after accounting for covariates (Fig 2c). The 6 clusters corresponded to activation and deactivation of known resting state functional networks, with control energy negatively correlated with activation pattern similarity (r = -0.93; Fig. 1). Regional and whole-brain control energies showed age-related correlations, particularly in sensorimotor systems (Fig 2), suggesting age-related differences in cognition/behavior may stem from navigating brain state transitions.

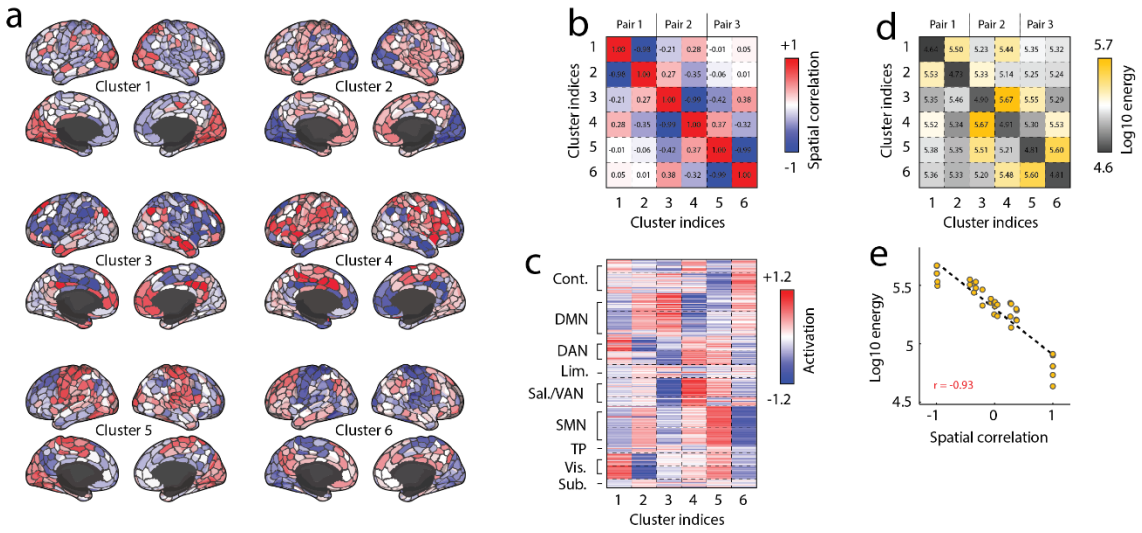

Figure 1: (a) k-means clustering algorithm produced 6 clusters, 3 pairs of near-perfect anti-correlates. (b) Spatial correlation grid for every cluster pair, indicating the three anti-correlated pairs: clusters 1 and 2, clusters 3 and 4, and clusters 5 and 6. (c) Clusters 1 and 2 correspond to activation/deactivation of the visual and dorsal attention networks. Clusters 3 and 4 correspond to activation/deactivation of the default mode with salience/ventral attention networks. Clusters 5 and 6 correspond to activation/deactivation of somatomotor and visual networks with the control network. (d) Grid indicating log of the control energy required to transition between every cluster pair, indicating highest values for energy transitions within anti-correlated cluster pairs. (e) The greater the spatial correlation between the two clusters, the lower the log of the control energy required to transition between those two clusters (r = -0.93).

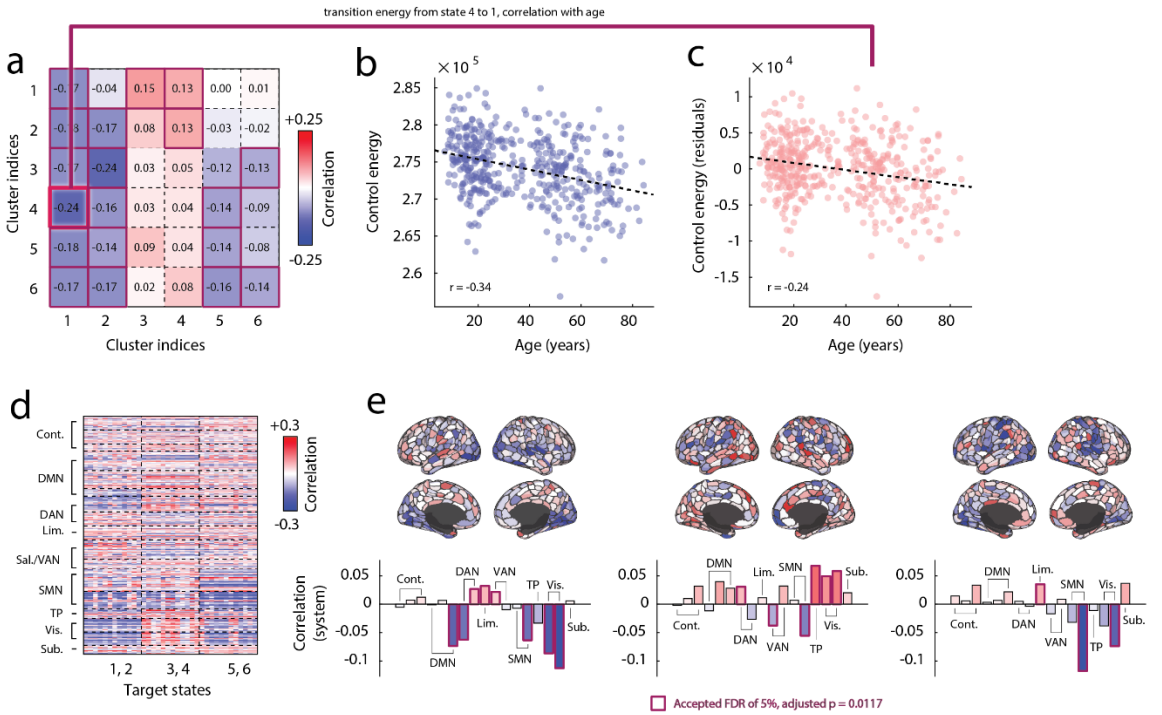

Figure 2: (a) Correlations between age and control energy required to transition between pairs of brain states. FDR-corrected statistically significant correlations highlighted with maroon border. (b) Representative correlation between age and control energy required to transition from Cluster 4 to Cluster 1. (c) Representative significant correlation between age and control energy required to transition from Cluster 4 to Cluster 1, adjusted for the effects of biological sex, intracranial volume, number of usable (low-motion) frames, as well as residual motion, after FDR correction. (d) Correlation between age and the control energy required to transition to the three paired clusters of target states (organized by functional network). (e) As age increased, transitions to Clusters 1 and 2 exhibited statistically significant decreases in activity in the default mode, dorsal attention, somatomotor, and visual networks as well as statistically significant increases in activity in the dorsal attention, limbic, and salience/ventral attention networks. As age increased, transitions to Clusters 2 and 3 exhibited statistically significant decreases in activity in the salience/ventral attention and somatomotor networks as well as statistically significant increases in activity in the dorsal attention, temporoparietal, and visual networks. As age increased, transitions to Clusters 5 and 6 exhibited statistically significant decreases in activity in the somatomotor and visual networks as well as a statistically significant increase in activity in the limbic network.

Disclosures: V. Parakkattu: None. **L. Lorenzo-Luaces:** None. **Y. Jo:** None. **J. Faskowitz:** None. **A. Podschun:** None. **S. Markett:** None. **R. Betzel:** None.

Poster

PSTR314: Network Computation: Theory and Modeling II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.06/Z6

Topic: I.06. Computation, Modeling, and Simulation

Support: JSPS KAKENHI [Grant Numbers JP21K21242 and JP23K16665]
funding agency: NEDO

Title: Spiking neuromusculoskeletal model toward simulation of pathological bipedal locomotion

Authors: *D. ICHIMURA;
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Abstract: Pathological bipedal locomotion is affected by variety factors-including environment, complex neural control, and compensatory behaviors. Although quantitative measurements of such locomotion are difficult and basic data are limited, various investigations are needed to prevent falls and other potential problems. For example, the pathogenesis of 'freezing of gait' observed in neurological diseases remains unclear, which limits development of a consistent treatment method. To tackle this issue, seamless investigation of both nervous system processing and bipedal motor control is required. In this study, we implemented a computer simulation of bipedal locomotion using a two-dimensional neuromusculoskeletal model. This model was driven by 18 muscle models triggered through activity of spiking neural models in a brainstem and spinal cord. The brainstem contained the pedunculopontine nucleus and cuneiform nucleus, which consisted of two leaky integrate-and-fire (LIF) model neurons. The spinal cord combined 12 LIF model neurons to generate the basic gait pattern. After optimizing 39 unknown parameters with a genetic algorithm, this model acquired a bipedal locomotion. Such computational simulations may provide insight into the pathogenesis of pathological bipedal locomotion caused by the nervous system, such as the brainstem and spinal cord.

Disclosures: D. Ichimura: None.

Poster

PSTR314: Network Computation: Theory and Modeling II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.07/Z7

Topic: I.06. Computation, Modeling, and Simulation

Support: SERB-DST India PDF/2021/000585
F.NO.K-15015/42/2018/SP-V
DBT India, BT/MED-III/NBRC/Flagship/Flagship2019

Title: Neuromolecular interactions guiding homeostatic mechanisms underlying healthy ageing

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Abstract: Aging impacts multiple scales of brain organization, ranging from microscopic level alteration in neurotransmitter levels, degradation in long-range white matter tracts, or, gray matter volume, to the macroscopic level changes in functional coordination causing cognitive and behavioral impairments. Because of such multiscale interactions, the effects of ageing on neural activity become complex, e.g., some functions of a healthy aging brain deteriorate largely, in contrast, others remain intact or even improve with age. We addressed whether the age-related markers can be captured by a framework considering multiple scales, e.g., large-scale brain dynamics and neurotransmitter kinetics.

Earlier studies have proposed that the dynamic working point is defined by the dynamics of constituent brain areas exhibiting metastability- resistance to convergence towards a single stable state. Building upon this concept, we hypothesized that healthy ageing, where fluid intelligence, language and other higher order cognitive functions are typically preserved, must also underlie an invariance of optimal dynamic working point indexed by metastability despite age-related structural decline. We identify the patterns of invariance in topological features, segregation and integration, applying graph metrics on empirical structural and functional connectivity (FC).

We deploy a biophysically inspired multiscale dynamic mean field model (MDMF) that incorporates the properties of empirically derived brain connectivity to simulate global brain dynamics at rest while preserving excitatory-inhibitory (E-I) balance. The two adjustable parameters, glutamate and GABA concentrations are estimated by constraining spatio-temporal features such as metastability and distance between simulated and empirical FC. The observed patterns of GABA/ Glutamate across healthy ageing was validated in three big data sets CamCAN, Berlin and NKI. Model performance is evaluated qualitative comparisons of topological characteristics between empFC and simFC.

The GABA/ Gutamate shifts extracted by MDMF reveal the compensatory mechanism behind preservation of various cognitive functions across lifespan ageing. Specifically, MDMF model inversion suggests that aging brain reduces glutamate level, without altering GABA significantly, to support network dynamics and shift dynamic working point of the brain. Thus, often observed re-organized functional connectivity with age is an outcome of this adaptive process.

Disclosures: S. Saha: None.

Poster

PSTR314: Network Computation: Theory and Modeling II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.08/Z8

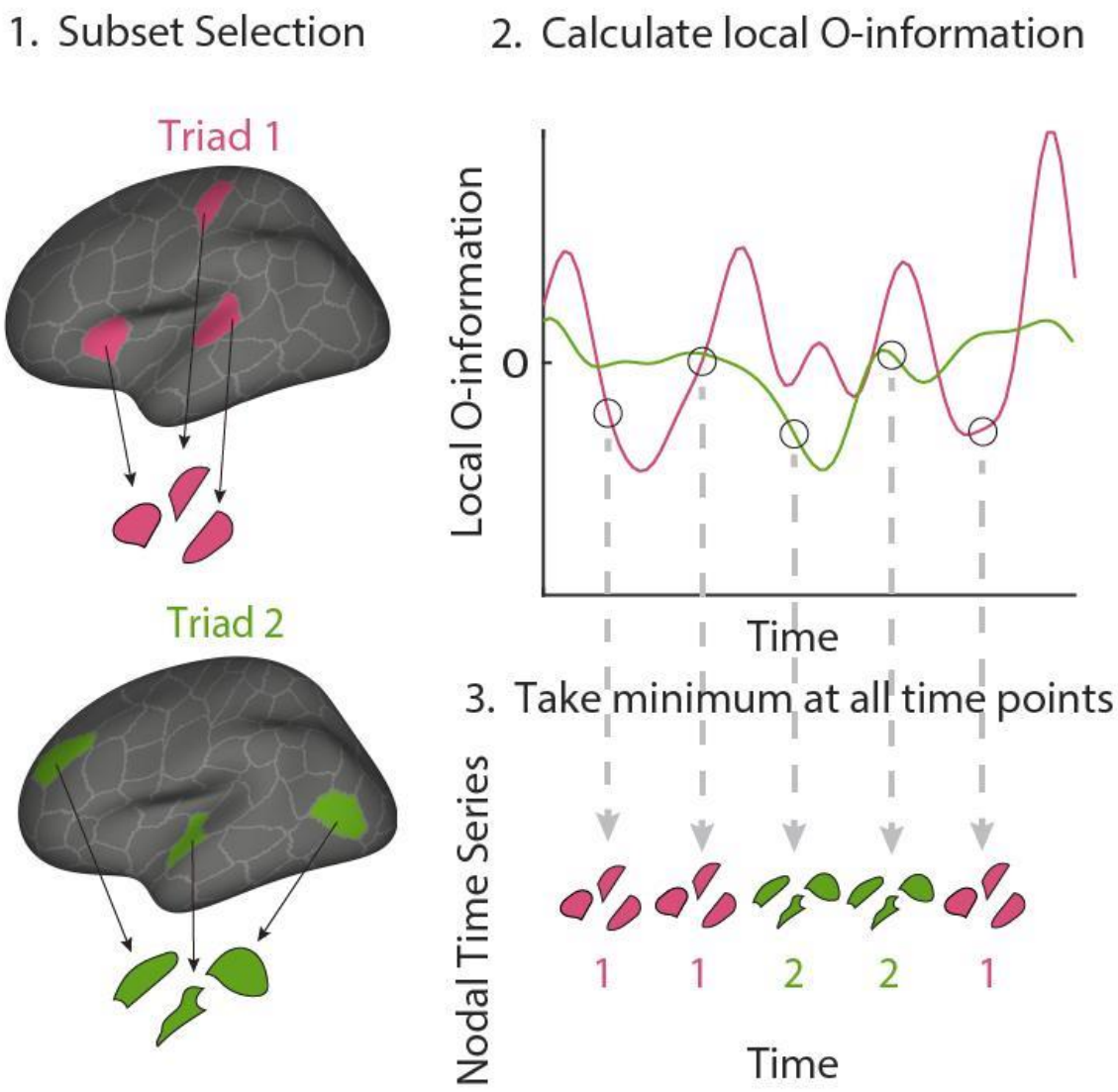
Topic: I.06. Computation, Modeling, and Simulation

Support: NSF Grant 2240777

Title: Time-varying synergy and redundancy dominance in the human cerebral cortex

Authors: *M. POPE[1], T. VARLEY[3], O. SPORNS[2];
[2]Psychological and Brain Sci., [1]Indiana Univ., Bloomington, IN; [3]Vermont Complex Systems Ctr., Univ. of Vermont, Burlington, VT

Abstract: Recent work [1,2] has emphasized the ubiquity of higher-order interactions in brain function. These interactions can be characterized as being either redundancy or synergy-dominated by the heuristic O-information [3]. Though the time-averaged O-information can be decomposed into local values to measure the synergy-redundancy dominance at each point in a time series, no such analysis of fMRI dynamics has yet been carried out. Here we analyze the moment-to-moment synergy and redundancy dominance of the fMRI BOLD signal during rest for 95 unrelated subjects from the Human Connectome Project. At the whole brain level, we find that synergistic moments are exceedingly rare, making up only 0.08% of time points. Randomly sampling subsets of 3-25 brain regions revealed that time-averaged redundancy-dominated subsets have the greatest dynamic range of local O-information values, and so experience both the most synergistic and most redundant time points. We calculated the most synergistic and most redundant triad and tetrad (set of three and four brain regions) at every time point. Both synergistic and redundant triads and tetrads are highly recurrent, and the synergy/redundancy dominance of the triad at a particular moment can be predicted by its relation to the long-term behavior of the triad. In particular, we show that a strongly synergistic moment is expected when a triad that spends a majority of time integrated becomes briefly disintegrated. Finally, we use a simulated annealing algorithm to find larger (n>4) subsets. This analysis indicates that synergistic subsets of many different sizes can be found at all time points, and that subsets of different sizes show distinct regional participation. We conclude that higher order interactions (both synergy and redundancy-dominated) are not only ubiquitous, but dynamic, existing at many subset sizes and incorporating almost all brain regions throughout the length of a scan.
[1] Varley and Pope et al. (2023). Nat. Comm. Biol., 6(1), 451. [2] Varley et al. (2023). PNAS, 120(30), e2300888120. [3] Mediano et al. (2019). Phys. Rev. E, 100, 032305.



Disclosures: M. Pope: None. **T. Varley:** None. **O. Sporns:** None.

Poster

PSTR314: Network Computation: Theory and Modeling II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.09/Z9

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF EFRI BRAID 2223793

Title: Modeling the parallel fiber of the ghost knife fish using synthetic nervous system

Authors: *S. JOHNSON[1], G. MARSAT[2], N. S. SZCZECINSKI[3];
[2]Biol., [3]Mechanical, Materials and Aerospace Engin., [1]West Virginia Univ., Morgantown, WV

Abstract: The *Apteronotus leptorhynchus* or ghost knife fish, is a weakly electric fish that hunts by emitting an electric field and detecting distortions caused by prey. As in many sensory systems, this process works because the fish can predict and filter out expected disturbances to the field, e.g., those due to its motion as it swims. Previous studies have shown that an important filtering mechanism relies on parallel fibers feedback inputs from the cerebellum onto primary sensory neurons [1]. The strength and relative timing of this feedback pathway is learned through local plasticity mechanisms, enabling the fish to adapt this pathway when its environment undergoes a persistent change, e.g., changes in body size as it grows. Our goal is to apply the mechanism of parallel fiber's cancellation function to other models of sensory processing, both to better understand how the cerebellum functions and to endow a robot with adaptive sensory processing. Because we are interested in robotic applications, we want our model to be tractable to analyze and fast to simulate. As a result, we have abstracted the system into a bank of resonators, each of which has a different resonant frequency. The frequency content of the sensory signal determines the relative activity of each resonator, each of which feeds back onto the sensory signal with a frequency-specific delay, canceling much of the sensory signal. Uncanceled transient information is sent to a higher-level decision center; ongoing uncanceled information triggers a behavioral change. This should enable a robot to determine what motor control is needed for a specific environment and adjust the force output necessary to overcome any perturbations it experiences without any adjustments from the user.

Disclosures: S. Johnson: None. **G. Marsat:** None. **N.S. Szczecinski:** None.

Poster

PSTR314: Network Computation: Theory and Modeling II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.10/Z10

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF Graduate Research Fellowship DGE2140743
NIH/NIGMS Grant T32GM008313
NIH/NINDS Grant R01NS116753

Title: The role of feedback in dynamic inference for spatial navigation under uncertainty

Authors: *A. CHEN, J. DRUGOWITSCH;
Dept. of Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Efficient behavior in our noisy and ambiguous world calls for the strategic use of the arising uncertainty by probabilistic inference. Whereas such strategic use has been demonstrated by humans and animals in static environments, we have less evidence for it in dynamic environments. Probabilistic inference in complex dynamic environments is particularly challenging because it not only requires tracking all the relevant latent variables coherently but also necessitates accounting for the interactions among them. As a result, a neural circuit encoding these variables would need to be recurrently wired to relay information between them via feedback connections. Given the additional cost of evolving and maintaining these feedback connections, we sought the circumstances under which the brain could get away with simpler circuits that omit some of the feedback connections necessary for optimal probabilistic inference. One example of inference in a dynamic environment is navigation, in which self-motion and landmark cues inform estimates of our velocity and position in space, respectively. Crucially, we can estimate our own velocity not only from observations of self-motion cues but also from successive observations of landmark cues, which are both considered by optimal dynamic inference. However, it remains unclear whether humans and animals use the latter in their estimation of velocity during navigation.

To assess the importance of a potential feedback connection relaying position information to velocity-coding brain areas, we developed a mathematical framework that allows us to compare the performance of optimal dynamic inference to that of scenarios where communication between some of the encoded variables is lacking. Applying this framework to navigation, we found that restricting feedback from position to velocity only causes non-negligible performance loss when the noise in observing self-motion is high but the noise in observing landmarks is low. By benchmarking inference performance against neural decoding accuracy from past rodent navigation studies, we further found that this performance deficit is, in fact, negligible at most biologically realistic noise levels. Therefore, across wide biologically relevant regimes, restricting feedback information flow from position to velocity has only a negligible impact on the nervous system's ability to accurately track position and velocity. Overall, our results suggest that simple circuits without all the necessary components for optimal inference could nevertheless support efficient navigation at lower developmental and energetic costs.

Disclosures: A. Chen: None. **J. Drugowitsch:** None.

Poster

PSTR314: Network Computation: Theory and Modeling II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.11/Z11

Topic: I.06. Computation, Modeling, and Simulation

Support: Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103449

Title: A Mathematical Model of Stage II retinal waves and their effect on the development of receptive fields of neurons in V1

Authors: *J. CRODELLE[1], W. DAI[2];
[1]Middlebury Col., Middlebury, VT; [2]Fudan Univ., Shanghai, China

Abstract: Spontaneous waves are ubiquitous across many brain regions during early development. Activity from waves occurring in the retina are propagated to downstream areas such as the thalamus and primary visual cortex (V1) and are hypothesized to drive the development of receptive fields (RFs). Disruptions to this pathway during development have been shown to underlie neurodevelopmental diseases and activation of this pathway can ameliorate symptoms of developmental deformation.
Different stages of retinal spontaneous waves coincide with the development of the retinotopic map, ON-OFF segregation, and orientation selectivity in the early visual pathway of mammals. However, the mechanisms underlying the influence of each retinal wave on RF refinement are not well understood. In this work, we build a biologically-constrained mathematical model of the development of the feed-forward RF of neurons in the primary visual cortex. These feed-forward synapses are driven by retinal waves using a spike-timing-dependent triplet plasticity rule. Using this model, we propose a possible mechanism that underlies a pruning process leading to different RF spatial structures. In particular, we quantify how key characteristics of the retinal wave, such as wave speed, width and presentation angle, affect the simulated pruning result and shape of the receptive field. We further elucidate potential mechanisms of learning through analysis of a reduced rate model. In particular, we find mechanisms for the formation of a periodic RF, which may help to understand related periodic RF development in other brain areas such as grid cells under spontaneous waves in the entorhinal cortex.

Disclosures: J. Crodelle: None. **W. Dai:** None.

Poster

PSTR314: Network Computation: Theory and Modeling II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.12/Z12

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF award #IIS-2123781
NSF Frontera OAC-1818253

Title: In vitro electrophysiology demonstration, exploring continuum oscillator dynamics with spatiotemporal variance to design near-critical behavior

Authors: *K. KAZEMI[1], S. KIM[1], G. UPADHYAY[2], Z. DOU[2], X. ZHANG[3], H. GRITTON[4], M. GAZZOLA[2];
[1]Univ. of Illinois, Urbana-Champaign, Urbana, IL; [2]Mechanical Sci. and Engin., Univ. of Illinois, Urbana-Champaign, Urbana, IL; [3]Mechanical Sci. and Engin., Univ. of Illinois, Urbana-Champaign, Urbana, IL; [4]Comparative Biosci., Univ. of Illinois, Urbana-Champaign, Urbana, IL

Abstract: The versatile and rich computational capability of biological neural networks likely stems from their ability to embed complex features within their dynamical states. According to the theory of criticality, an optimal network state can be achieved when neural activity is balanced during information encoding, transformation, and transmission. This physical phenomenon that has emerged from existing brain criticality studies provides hints on how *in vitro* neuronal cultures could be modulated to maximize computing capabilities. However, engineering guidelines to implement such systems are far from clear. Here, we present a framework for designing and manipulating near-critical population behavior *in vitro*. In this study, we primarily utilize the oscillator neuron model within large-population simulations, to provide a qualitative overview of the collective dynamics: macro-scale emerging behavior derived from the synchronization of micro-scale neurons. Our extended oscillator model incorporates spatial variance, connection topology, transmission delays, background stimulation/perturbation, and population-wise interactions to bring the simulated environment closer to experiments. Informed by our simulation, we develop key strategies for monitoring and fine-tuning criticality metrics and the population behavior. Further, we use *in vitro* neuron cultures to demonstrate the implementation of near-critical behavior in electrophysiology. Cultures are prepared from differentiated mouse embryonic stem cells grown on custom-made micro-electrode arrays (MEAs). To include controlled perturbation, we use genetically modified neurons that express Channelrhodopsin-2 (ChR2), enabling optical stimulation. By carefully designing culture layout, electrode configuration, and media chemical composition, we reproduce key characteristics and metrics that align with simulations.

Disclosures: K. Kazemi: None. **S. Kim:** None. **G. Upadhyay:** None. **Z. Dou:** None. **X. Zhang:** None. **H. Gritton:** None. **M. Gazzola:** None.

Poster

PSTR314: Network Computation: Theory and Modeling II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.13/Z13

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH F31 grant NS113407
Michigan Institute for Clinical & Health Research (MICHR) Translational Science grant UL1TR002240

Title: Using patient-specific computational models of spinal cord stimulation to analyze its physiological effects and mechanisms of action

Authors: *A. M. PORRAS LAURA[1], W. KYI[2], R. D. GRAHAM[3,4], E. MIRZAKHALILI[5,4], E. ROGERS[1], J. LOECHLI[1], P. G. PATIL[6], S. CHIRAVURI[7], S. F. LEMPKA[1];
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Abstract: Spinal cord stimulation (SCS) is an electric stimulation therapy that helps manage intractable chronic pain by implanting electrodes in the epidural space near the spinal cord. However, because we do not understand the mechanisms of action of SCS, it is difficult to optimize patient selection - leading to variability on effectiveness across patients. Therefore, we used a patient-specific computational modeling approach to investigate the variability of neural activation across patients.

To characterize neural activation profiles across patients, we constructed patient-specific computational models that accounted for inter-patient variability in anatomy, lead placement, and stimulation parameters. We used preoperative magnetic resonance imaging (MRI) and postoperative compute tomography (CT) imaging data, and co-registered them to build finite element method (FEM) models of the participant's unique anatomy and lead placement. We then used the FEM models to calculate the extracellular potentials to multi-compartment axon models distributed throughout the spinal cord. We compared model predictions of neural activation with each patient's clinical SCS settings and quantitative sensory data (QST), and patient-reported outcomes to investigate potential correlations.

We constructed patient-specific models for ten participants. Although the stimulation settings varied significantly across participants, the computational models predicted activation thresholds that mimicked the trends in participant-reported sensory thresholds and paresthesia coverage. We also observed that patient-specific characteristics, such as the amount of cerebrospinal fluid (CSF) and lead location, affected the pulse amplitudes required to activate dorsal column axons as well as the stimulation selectivity.

Patient-specific computational models are an effective tool for comparing neural activation profiles across patients. We are continuing to investigate the neural sources of stimulation-induced paresthesia and discomfort, and investigate how spatiotemporal neural activation profiles change in response to different forms of SCS (e.g., burst, 10 kHz). We will use the model predictions to establish potential correlations in the model-based neural recruitment profiles with the patient-reported outcomes and QST data. These correlations may provide insight into outcome variability as well as the mechanisms of action of SCS.

Disclosures: A.M. Porras Laura: None. **W. Kyi:** None. **R.D. Graham:** None. **E. Mirzakhalili:** None. **E. Rogers:** None. **J. Loechli:** None. **P.G. Patil:** None. **S. Chiravuri:** None. **S.F. Lempka:** 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.; Abbott Neuromodulation, Medtronic, plc, Neuromodulation Specialists LLC, Presidio Medical Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CereGate, Hologram Consultants LLC, Neuronoff Inc, Presidio Medical Inc. F. Consulting Fees (e.g., advisory boards); Ceregate, Presidio Medical Inc.

Poster

PSTR314: Network Computation: Theory and Modeling II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.14/Z14

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH U24 NS124001-01

Title: Open Source tools for modeling, analysis and simulation of large-scale brain networks: The Brain Modeling Toolkit (BMTK) and Visual Neuronal Dynamics (VND)

Authors: *K. DAI[1], B. ISRALEWITZ[2], X.-P. LIU[3], S. ITO[1], D. HAUFLER[1], J. SHARMA[4], E. TAJKHORSHID[4], A. ARKHIPOV[1];
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Abstract: As the field of neuroscience continues to explore the connectivity and dynamics of regional and whole-brain networks, it's increasingly important to also develop models that can test theories, replicate experiments, and make predictions. Similarly, the need to run simulations and computational analysis on such models, which is common with most physical sciences, is becoming an increasingly crucial goal for neuroscientists. To assist with such endeavor we have developed a suite of software tools for modeling, simulation, analysis and visualization of large-scale, realistic and heterogeneous brain network models; including the Brain Modeling Toolkit (BMTK) and Visual Neuronal Dynamics (VND).
In particular we will focus on the numerous additional features and improvements made in these tools in the past year. One particularly important enhancement is added integration with the Neurodata Without Borders (NWB 2.0) format, facilitating the merging of in-vivo recording and in-silico modeling and simulation. Additionally we have developed methods for incorporating even more diverse and realistic types of network stimuli, as well as enhanced abilities to record, visualize and analyze network dynamics across a range of different modalities. Both BMTK and

VND are open-source and free-to-use, and can be used along-side a wide-range of other neuroscience software and data formats.

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Poster

PSTR314: Network Computation: Theory and Modeling II

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.15/Z15

Topic: I.06. Computation, Modeling, and Simulation

Support: 1UF1 NS115821-01
R01 NS094184

Title: Task success in trained spiking neural network models coincides with emergence of cross-tuned inhibition

Authors: *Y. ZHU[1], T. JABRI[2], M. TANG[3], F. SCHERR[4], J. N. MACLEAN[5];
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Abstract: Spiking activity in neocortex constitutes computations which transform sensory inputs to appropriate behavioral outputs. Here we investigate the underlying mechanisms and ask how the specific structure and spiking dynamics of a recurrent network of excitatory and inhibitory neurons can yield circuit computations. We train spiking neural network (SNN) models of neocortex on a binary state change detection task, then identify the dynamical and architectural changes which yielded task computations. The two states of the task are defined by motion entropy, and the task mirrors behavioral paradigms that mice can perform. The models are composed of excitatory and inhibitory units with connection likelihoods and strengths matched to mouse neocortex. Throughout training, SNNs are constrained to maintain neocortical structure (sparse connections with lognormal weight distribution) and dynamics (low-rate, asynchronous spiking). After training, we discover that SNNs selectively adjust firing rates depending on motion entropy state, and that connectivity of the input and recurrent layers changed according to this rate modulation. Input channels that exhibit bias to one specific motion entropy level develop stronger connections to recurrent excitatory units during training, while channels that exhibit bias to the other entropy level develop stronger connections to inhibitory units. Furthermore, recurrent inhibitory units which positively modulated firing rates to one input strengthened their connections to recurrent units of the opposite modulation pattern. This scheme of cross-modulation inhibition emerged as the optimal solution when imposing Dale’s law during

training; removing this constraint led to the absence of this architectural solution. Our work highlights the critical role of interneurons and the specific architectural patterns of inhibition in shaping dynamics and information processing within neocortical circuits. By combining neurobiologically realistic network features and optimization of these network models on meaningful tasks, our approach can provide mechanistic insights into neocortical circuit computations.

Disclosures: Y. Zhu: None. **T. Jabri:** None. **M. Tang:** None. **F. Scherr:** None. **J.N. MacLean:** None.

Poster

PSTR314: Network Computation: Theory and Modeling II

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

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Topic: I.06. Computation, Modeling, and Simulation

Support: NIH-NINDS R01 NS121535
NIH-NINDS R01 NS125270
National Institute for Theory and Mathematics in Biology

Title: A general solution for understanding noise propagation through neural networks

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Abstract: Much work in theoretical neuroscience has been dedicated to understanding how noise affects ongoing processing. For example, the effects of noise have been analyzed in tasks such as integration of evidence, maintenance of working memory, judging timing, or combating error in motor control. However, a generalized understanding of how noise propagates over time through the dynamics of biological or artificial neural networks has remained elusive. Here, we derive a general solution. We begin by generalizing a recent idea we term ‘task manifold distortion’ - a description of how a parametric representation of a behavioral task is distorted in its representation by a neural population. Using this perspective, we derive coupled stochastic differential equations (SDEs) that predict how task information is degraded by firing rate noise. These SDEs describe three ways that noise can affect ongoing computations: perturbing the neural population state along the neural manifold, via dynamics shunting noise in off-manifold dimensions back on to the neural manifold, and influencing output dimensions directly. We present an analytic solution for these SDEs, and numerically demonstrate its predictive ability on recurrent neural networks (RNNs) trained on a variety of tasks. Finally, we derive general

predictions from these SDEs about how RNNs should optimize their activity and readouts to achieve noise-robust computations, and show that our predictions hold in RNNs trained with firing rate noise. This method opens the door to formal analysis of how neural networks are influenced by noise, and therefore both understanding network solutions and designing better artificial networks.

Disclosures: D.A. Sabatini: None. **H.A. Grier:** None. **M.T. Kaufman:** None.

Poster

PSTR314: Network Computation: Theory and Modeling II

Location: MCP Hall A

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Program #/Poster #: PSTR314.17/Z17

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF grant RET EEC-1801666

Title: Distinct microcircuit features underlie gamma and beta rhythms across cortical regions

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Abstract: Cortical neural rhythms in the gamma and beta bands are thought to be generated intrinsically by distinct microcircuits. These microcircuits involve different cell types and synaptic mechanisms that are engaged differentially by extrinsic inputs. We consider the pyramidal-interneuron gamma (PING) microcircuit that has been implicated in both gamma and beta rhythms, but with distinct interneurons and synaptic mechanism types. For instance, the gamma microcircuit has been linked to fast-spiking (FSI) interneurons and depressing synapses that are preferentially engaged by bursty or fluctuating extrinsic inputs. On the other hand, the beta rhythm has been linked to low-threshold (LTS) interneuron and facilitating synapse types that are engaged by steady or stable extrinsic inputs. Computational models have explored the role of the circuit parameters to the characteristics of neural rhythms (e.g., Cannon et. al, 2014). These studies have revealed that the strength of the extrinsic drive and of the connections, and the synaptic rise/decay times were all important in setting the peak frequency and bandwidth but their relative contributions have been difficult to parse in realistic conditions. Furthermore, although the peak frequency and bandwidth of these rhythms are known to vary across brain regions, the reasons are unclear. Here, we explored the mechanistic underpinning of the intrinsic gamma and beta rhythms using large-scale biophysical network models of rodent M1 and V1 cortices. These included multi-compartmental single cells with the intrinsic and synaptic channels constrained by neurophysiology data. The model local field potential trace revealed the

following spectral bands that matched in vivo data for both the regions: M1center frequency (bandwidth) - gamma 37 (±7) Hz, and beta 19 (±4) Hz; V1 - gamma 48 (±12) Hz, and beta 22 (±5) Hz. Naturalistic drives that fluctuated resulted in increased model gamma power in the LFP in both regions, while drives that were stable were seen to increase the beta power. Preliminary analysis of the underlying mechanisms revealed that the intrinsic excitability of the two interneuron types, convergence of the excitatory drives onto the FSI and LTS populations, the decay time constant of the PN-ITN connection and the strengths of the FSI-FSI and LTI-LTS connections were predictors of the variation in spectral peak for both rhythms across the regions. Ongoing work focuses on sensitivity analyses, and comparisons with three other regions. Quantifying the dependence of the rhythms on the microcircuit parameters and extrinsic drive has potential relevance to understanding inter-areal.

Disclosures: G. Glickert: None. **K. Choudhry:** None. **Z. Chen:** None. **D.B. Headley:** None. **S.S. Nair:** None.

Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.01/Z18

Topic: I.06. Computation, Modeling, and Simulation

Title: Comparing methods for quality control of spike sorting results

Authors: *J. COLONELL[1], C. MANAGAN[1], R. ARRUDA[2], K. BOONE[1], A. DEV[1], M. LAY[1], E. TENSHAW[1], X. YUAN[3], S. CHEN[4], T. HARRIS[1];
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Abstract: A persistent challenge in the analysis of extracellular electrophysiology data is the interpretation of spike sorting results. What thresholds should be set on quality metrics to select acceptable units? Would manual curation have a significant impact on the yield of acceptable units? The answers to these questions are specific to the data (brain region, conditions of the recording and pattern of electrodes on the probe) and the analysis path (sorting algorithm and manual curation protocol); in this work, we present results from diverse datasets and illustrate protocols for assessing the impact of curation. We compare the unit sets identified by Kilosort 2 plus filtering with quality metrics, Kilosort 2 plus manual curation, and ‘consensus’ runs of Kilosort 2 and Kilosort 4. ‘Consensus’ refers to running the sorter’s fit step from different starting template sets. The intersection of units found across these runs are easily isolated units. The union of all units found across runs can be interpreted as a broader exploration of template space. The datasets examined are all from awake mice, 14 recordings from Neuropixels 1.0 and 2.0 probes, spanning five brain regions, and a set of > 40 Neuropixels Ultra recordings from

seven brain regions. We compare unit yield, distributions of unit quality metrics and characterize the differences (extra merges/splits; false positives/negatives) between approaches. We find that quality metrics are a good predictor of which units are consensus units. In some recordings, manual curation with corrections yielded up to 30% more potential good units than simple filtering by quality metrics. However the gain was very variable, covering the full range of 0-30% in the set of NP1.0 and NP2.0 recordings we examined. Using the larger set of Neuropixels Ultra recordings, we characterize the correlation of gains from curation with brain region and unit density.

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Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.02/Z19

Topic: I.06. Computation, Modeling, and Simulation

Support: The Grass Foundation

Title: An open-source solution to spike sorting analysis in neurophysiology

Authors: *A. J. MALIK, U. M. RICOY;
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Abstract: The specialized field of neuroscience research characterized by extensive financial prerequisites occludes underrepresented students. Several studies have developed more accessible tools to spike sorting analysis in electrophysiology (Torres et al., 2021). While those are still locked behind significant costs of entry, the development of a free and open-source script for spike sorting analysis still remains. In this particular study we create a script in the open-source programming language Python, to offer an alternative for extracellular spiking data analysis in research programs lacking financial capabilities. We obtained electrophysiological recordings of mechanical stimulation from the hissing roach, Gromphadorhina portentosa, ventral nerve cord and femur through low-cost Backyard Brains Neuron SpikerBox amplifiers and the accompanying SpikeRecorder. Our analysis script requires the user to define parameters for spike sorting analysis including a voltage threshold, beginning, and end time interval. Next, our program filters the data to contain only spikes that both surpass the voltage threshold, and represent biphasic extracellular action potential waveforms. Feature extraction is done on each individual spike in the analysis interval to extrapolate a subset of characteristics. The spikes are subsequently scored through principal component analysis dimensionality reduction to group and

cluster similar neuron spikes in a two dimensional clustering algorithm. We find our resulting script, available for free and open-source through Python, to successfully spike sort by distinguishing between different units in a two dimensional clustering space. The interspike interval and frequency of each neuron are calculated and displayed along with a waveform comparison for in-depth analysis and consideration in students' studies. The accessibility of this script detracts from the significant financial inequality in neuroscience burdened by underrepresented rural communities (Ramadan & Ricoy, 2023), providing a meaningful avenue for neurophysiological research across a widened span of backgrounds.

Disclosures: A.J. Malik: None. **U.M. Ricoy:** None.

Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.03/Z20

Topic: I.06. Computation, Modeling, and Simulation

Support: U01AG076804

Title: Isadora: microscopy image processing task orchestration

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Abstract: Performing post-acquisition image processing for volumetric microscopy is a complex and resource-intensive endeavor. To mitigate this complexity, at UCLA BRAIN we developed Isadora, an image-processing task orchestration pipeline that adds flexibility and remote execution to the post-aquisition workflow. Isadora leverages the Prefect task orchestration development libraries to implement a deployment framework. Specifically, Isadora provides the means for managed execution of image processing on available computational infrastructure, including command line interaction, and launch via a website.
Isadora is agnostic to the underlying back-end image processing workflow, and could interface any code running on the desired architecture. In this work Isadora has been instantiated with SmartSPIM Post Processing (SmartSPIMPP) software libraries in collaboration with Life Canvas Technologies. UCLA BRAIN extended SmartSPIMPP by porting it to Linux, and adding support for parallel GPU execution. Isadora specifically instantiates SmartSPIMPP objects inside a Prefect deployment, which can be run remotely.
Key contributions of Isadora include:
1) Providing a microscopy lab with the means to execute the SmartSPIMPP workflow on Linux in addition to Windows.
2) Facilitating utilization of all available hardware, removing idle times.

3) Providing a computational lab functionality for collaborating with a microscopy lab to process an otherwise dormant image archive -- Isadora greatly eases the mechanics of a computational lab performing image processing for a microscopy lab -- ideal for institutional academic collaboration.
UCLA BRAIN has released Isadora as free and open-source software.

Disclosures: I. Bowman: None. **W. Guan:** A. Employment/Salary (full or part-time):; LifeCanvas Technologies. **L.H. Garcia:** None. **H. Dong:** None.

Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.04/Z21

Topic: I.06. Computation, Modeling, and Simulation

Support: Shanahan Fellowship

Title: Spatial transcriptomic evidence for the self-avoidance hypothesis in the cortex

Authors: *Y. WANG, C. KOCH, U. SÜMBÜL;
Allen Inst., Seattle, WA

Abstract: Neurons display remarkable diversity in their anatomical, molecular, and physiological properties. While stereotypy across features in subsets of neurons, such as their morphology, projection targets or proteins they express, has been a pillar of neuroscience since its early days, a key complication is high variability, in particular in the spatial distribution of cells. This obscures the extent to which neurons can be classified into a discrete number of cell types. In the retina, a laminated and spatially extended tissue, it is known that neuronal cell types avoid close proximity to each other. The extent to which this holds in neocortex remains unknown.
We provide evidence for such a non-random spatial distribution by developing a statistical point process framework for spatial transcriptomic data. We studied a recent whole-brain MERFISH dataset in the laboratory mouse where over 1000 genes are profiled in space and the transcriptomic identity and location, within the common coordinate framework (CCF), of more than two million cells have been determined. We examined the sources of noises in this dataset for neocortical brain slices and conducted formal statistical tests to assess spatial randomness. To analyze local organization in the point pattern of soma locations in a 2-D slice, we first identify a set of regions covering the points belonging to the transcriptionally defined cell type of interest, to avoid falsely assigning significance to unstructured patterns. We carefully designed our test for complete spatial randomness to consider exclusion zones that account for the fact that cell bodies are non-penetrating with finite size.

We demonstrate self-avoidance behavior within pure cell types and its disappearance in cell type mixtures for 16 selected excitatory and 10 inhibitory types. At the cell type level, we observe cell distribution patterns that can be well captured by point processes with soft-core repulsion zones, with an interaction range of around 20 microns for excitatory types, and around 60 microns for inhibitory types. On the other hand, we observe segregation effects for excitatory clusters at the subclass level, and clustering effects at near distances for inhibitory subclasses. Our findings elucidate a long-standing hypothesis concerning the organization of neocortical circuitry and could potentially offer gold standard metrics for evaluating the purity of cell types.

Disclosures: Y. Wang: None. **C. Koch:** None. **U. Sümbül:** None.

Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.05/Z22

Topic: I.06. Computation, Modeling, and Simulation

Title: A Deep Learning Framework for MRI Brain Tissue Segmentation across Species, Ages, and Modalities

Authors: *Z. LI[1,2], L. SUTKUS[1,2], P. SENTHIL[1], S. SINGH[3], S. HAN[3], D. J. MILLER[3], B. SUTTON[4], F. LAM[4], R. N. DILGER[5];
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Abstract: Magnetic Resonance Imaging (MRI) is important in comparing neurodevelopment across species for its non-invasive nature and detailed insights into brain structure and function. A critical component of MRI data processing is the segmentation of whole brain tissue. The development of an automated brain segmentation tool allows the high-throughput processing of MRI datasets. However, tools currently tailored for humans fall short in their ability to generalize across different species, ages, and imaging modalities. Deep learning-based approaches have demonstrated impressive performance in numerous biomedical image analysis problems, with the ability to identify complex patterns from multidimensional imaging data. This study proposes a novel deep learning-based framework for the automated segmentation of whole brain tissue from MRI data across species, age, and modalities. Our approach involves stacking three adjacent slices into the RGB channels of an image, which permits usage of encoders pre-trained on large public datasets of natural images, as well as spatial correlation from adjacent slices. We propose a trio of 2D U-Nets with EfficientNetB5 encoders, each dedicated to the segmentation of slices along one spatial orientation. Predictions from all three models were combined using a

voxel-wise majority voting ensemble technique to generate the final brain mask. This 2.5D method offers a tradeoff between the extensive spatial information of 3D and the constraints of data scarcity. We initially trained our base model on a dataset comprising 144 domestic pig MPRAGE volumes. Utilizing transfer learning, we retrained models on MRI data across various ages, species, and modalities, including 34 MPRAGE volumes from 8-week-old pigs, 162 DTI volumes from 4-week-old pigs, and 28 MPRAGE and 28 DTI volumes from adult humans, sourced from the MGH HCP diffusion dataset. Our preliminary results demonstrated high dice coefficients: 0.957 for adult human T1-weighted images, 0.963 for adult human diffusion tensor images, and 0.971, 0.966, and 0.968 for pig T1-weighted images at 4 weeks, 8 weeks, and pig diffusion tensor images at 4 weeks, respectively. Additionally, the intersection over union scores were equally robust, with values of 0.929 for adult human T1-weighted, 0.937 for adult human diffusion tensor images, 0.943 for 4-week-old pig T1-weighted, 0.935 for 8-week-old pig T1-weighted, and 0.939 for 4-week-old pig diffusion tensor images. This framework demonstrates the viability of a generalizable unified framework for MRI brain tissue segmentation.

Disclosures: Z. Li: None. **L. Sutkus:** None. **P. Senthil:** None. **S. Singh:** None. **S. Han:** None. **D.J. Miller:** None. **B. Sutton:** None. **F. Lam:** None. **R.N. Dilger:** None.

Poster

PSTR315: Computational Tools: Analytical II

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Program #/Poster #: PSTR315.06/Z23

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant 1RF1MH128778-01
NIH Grant U01NS132267-01

Title: Multimodal integration of transcriptomic, electrophysiological, and morphological neuronal data using coupled variational autoencoders

Authors: *I. CONVY[1], F. BAFTIZADEH[2], S. DORKENWALD[3], Y. MARGHI[2], U. SÜMBÜL[3];
[1]Brain Sci., Allen Inst., Seattle, WA; [2]Allen Inst. For Brain Sci., Seattle, WA; [3]Allen Inst., Seattle, WA

Abstract: The recent proliferation of multimodal neuronal datasets has fueled demand for models that can integrate different physical profiles of a cell into a consistent, low-dimensional representation. A promising model type for this task is the multimodal autoencoder, which assigns an encoder-decoder pair to each modality and then connects them via a correlated latent space. Leveraging data from the Patch-seq platform, we construct a multimodal autoencoder model that incorporates joint transcriptomic, electrophysiological, and morphological

measurements of individual cells. In contrast with previous work, this model assigns distinct latent variables to each modality and links them together via conditional distributions that are variationally optimized. These probabilistic mappings allow for the quantification of cross-modal uncertainties and can provide insight into the variance that exists within latent cell-type clusters. To support the inclusion of morphological data, which is often absent in other multimodal models, we utilize an arbor density featurization that transforms the raw neuronal images into coarse-grained histograms. We demonstrate the utility and robustness of this representation by performing cross-platform inference on unimodal morphological data taken from high-resolution electron microscopy images.

Disclosures: I. Convy: None. **F. Baftizadeh:** None. **S. Dorkenwald:** None. **Y. Marghi:** None. **U. Sümbül:** None.

Poster

PSTR315: Computational Tools: Analytical II

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.07/Web Only

Topic: I.06. Computation, Modeling, and Simulation

Title: M-ECG: extracting heart signals with a novel computational analysis of brain magnetoencephalography data

Authors: *A. IZADYSADR[1], H. BAGHERZADEH[2,3], J. R. STAPLETON-KOTLOSKI[1], D. W. GODWIN[1,2,3];
[1]Neurol., [2]Wake Forest Univ. Sch. of Med., Winston Salem, NC; [3]The W.G. (Bill) Hefner VA Med. Ctr., Salisbury, NC

Abstract: Magnetoencephalography (MEG) measures the magnetic fields generated by neural activity. MEG is a powerful tool for understanding brain dynamics due to its high temporal and spatial resolution. Because of its focus on brain activity, other biopotentials, including muscle artifacts and heart signals, are typically filtered or rejected. However, heart rate analysis may relate to the state of the brain in several cognitive conditions, such as posttraumatic stress disorder (PTSD). In the current study, we explored the feasibility of extracting heart signals from MEG data, which is termed “Magnetoencephalographic Electrocardiogram” (M-ECG; in contrast to the electrocardiogram, or ECG). Using the publicly available Brainstorm MEG auditory dataset - CTF, we developed a novel algorithm that combines independent component analysis (ICA) and MEG reference gradiometer sensors to accurately extract M-ECG signals and compute heart rate variability (HRV), including metrics such as RR intervals (the time between successive heartbeats), from MEG data. We employed signal processing methods, analytical tools, and statistical techniques to demonstrate the similarities between the computed M-ECG and HRV from MEG data with those from the recorded ECG signal across time, frequency, and

time-frequency domains. Our results indicate significant alignment in RR intervals (Pearson's correlation = 0.99; Kolmogorov-Smirnov test p-value = 0.99; Mann-Whitney U test p-value = 0.99) and frequency power characteristics between M-ECG and ECG signals, suggesting a promising degree of similarity and correspondence. Our findings highlight the feasibility of extracting M-ECG and HRV directly from raw MEG data. These insights hold the potential to enhance multimodal neuroimaging methodologies and further elucidate the intricate interplay between brain activity and cardiovascular function. Furthermore, the potential of HRV as a biomarker for brain disorders could improve diagnostic accuracy, prognostic assessment, and therapeutic strategies, especially in disorders affecting the autonomic nervous system, such as epilepsy, PTSD, Parkinson's disease, and certain mood disorders.

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Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.08/Z24

Topic: I.06. Computation, Modeling, and Simulation

Title: Characterization of the Gray Matter-White Matter Distribution: An Automated Approach with Demonstration of Age-Related Decline

Authors: *J. SONG[1], K. YE[1], R. FLEYSHER[2], M. L. LIPTON[3];
[1]Albert Einstein Col. of Med., Bronx, NY; [2]Columbia Univ., New York, NY; [3]Dept Radiology, Columbia Univ., New York, NY

Abstract: Background: Neuroimaging techniques offer valuable insights into the structural characteristics of the brain. A salient feature of the cerebrum is the distinct transition of voxel intensity at the gray matter-white matter interface (GWI). Leveraging this inherent difference in tissue composition - lower gray matter (GM) and higher white matter (WM) signal on T1-weighted (T1W) MRI--we introduce a novel metric and demonstrate its efficacy in capturing age-related effects. **Methods:** 169 participants (98 female; mean age 55.6; SD 21.5; range 18-91 years) were studied between 2019 and 2023. Each participant underwent 3 Tesla MRI including field map and 3D T1W whole brain MRI (MP-RAGE, 1mm^{3}isotropic voxels). We first performed whole-brain extraction and segmented the GWI region into orbitofrontal, frontal, occipital, temporal, parietal, and cingulate subregions within 5mm of the Freesurfer-defined cerebral GWI. Next, we quantified peak distance, computed as the difference in means of two fitted Gaussian distributions of T1-weighted voxel intensities, scaled by their common standard deviation. For each participant, whole-brain peak distance using all brain voxels was calculated. The mean peak distance was then computed for each subregion. We fit a linear model to the

whole brain and each peak distance as a function of age, with biological sex included as a covariate. **Results:** Greater whole-brain peak distance was associated with older age (estimate = -.07 ± 0.0083 per decade, p-value = 8.89e-15), indicating a decline in WM-GM distribution sharpness with advancing age . Among the six regions, the cingulate region exhibiting the sharpest decline with age (effect size = -0.12 per decade, p-value = <2e-16). Similarly, peak distance at the GWI declines with age in the orbitofrontal (effect size = -0.07 per decade, p-value =2.93e-14), frontal (effect size = -0.07 per decade, p-value = 9.27e-15), parietal(effect size = -0.07 per decade, p-value =<2e-16), temporal (effect size = -0.05 per decade, p-value = <2e-16) and occipital(effect size = -0.04 per decade, p-value = 1.35e-13) regions. **Conclusion:** GM-WM peak distance derived fromT1W MRI is sensitive to age-related changes across the whole brain and serves as a marker of cerebral GWI sharpness, with implications for understanding neurodevelopmental trajectories and identifying pathological deviations from expected aging processes

Disclosures: J. Song: None. **K. Ye:** None. **R. Fleysher:** None. **M.L. Lipton:** None.

Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.09/Z25

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant U24EB029005
NIH Grant U24EB029005-S1
NSF Grant 1935749
NSF Grant 1935771

Title: Neuroscience Gateway - Software Dissemination and Large Scale Modeling and Data Processing

Authors: *A. MAJUMDAR[1], S. SIVAGNANAM[2], K. YOSHIMOTO[3], N. T. CARNEVALE[4]; [1]Univ. of California San Diego, LA JOLLA, CA; [2]San Diego Supercomputer Ctr., UCSD, La Jolla, CA; [3]UCSD, Phoenix, AZ; [4]Neurosci., Yale Univ., New Haven, CT

Abstract: Since 2013 the Neuroscience Gateway (NSG) has been providing a platform for software developers to disseminate their software and for the neuroscience community to do large scale modeling and data processing on high performance computing (HPC), high throughput computing (HTC) and accelerator (GPU) computing resources. NSG is free and open to any academic institution and non-profit organization. NSG provides a software dissemination page where description of the disseminated neuroscience software is provided along with input and corresponding output files or results generated from running the software on NSG;

description of science and HPC/HTC parameters needed for the software are also showcased on this webpage from NSG's GUI form that users use to specify these parameters. These allow new users of a neuroscience software to get started with using the software on NSG. NSG provides an easy to use web interface and a programmatic REST interface to run jobs or process data on compute resources. Many of the computational neuroscience work requires HPC for large scale neuronal modeling. Neuroscientists involved in data processing are also utilizing the NSG and as a results NSG provides multiple new features related to data transfer, data management, data sharing and HTC. The neuroscience software is optimally installed on supercomputers located at multiple national academic supercomputer centers. The NSG team acquires over 30,000,000 core hours per year (in recent years) on these academic supercomputers, via a peer reviewed allocation proposal process and the supercomputer time is fairly used by the NSG user community for modeling and data processing using the neuroscience software and tools provided by NSG. NSG is used in large number of training activities by software developers and also used in classroom teaching of neuroscience and biology by faculties. The NSG team hosts yearly workshops at SfN, Organization of Computational Neuroscience and other neuroscience conferences where software developers and users of NSG give presentation on their software and research. Use of NSG results in large number of MS and PhD thesis work and publication and presentations in prestigious journals and conference. NSG is also used by BRAIN Initiative funded projects such as NeuroElectroMagnetic data Archive and tools Resource (NEMAR) and the Human Neocortical Neurosolver (HNN). This poster will describe the NSG platform and how it is enabling dissemination of neuroscience software and neuroscience research, education and training.

Disclosures: A. Majumdar: None. **S. Sivagnanam:** None. **K. Yoshimoto:** None. **N.T. Carnevale:** None.

Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.10/Z26

Topic: I.06. Computation, Modeling, and Simulation

Support: R01NS39600
RF1MH128693
R01NS86082

Title: Accelerating the continuous community sharing of digital neuromorphology data

Authors: *C. TECUATL, L. SHEN, Z. LI, G. A. ASCOLI;
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Abstract: The tree-like morphology of neurons and glia is a key cellular determinant of circuit connectivity and metabolic function in the nervous system of essentially all animals. To elucidate the contribution of specific cell types to both physiological and pathological brain states, it is important to access detailed neuroanatomy data for quantitative analysis and computational modeling. NeuroMorpho.Org is the largest online collection of freely available digital neural reconstructions and related metadata and is continuously updated with new uploads. The database currently contains over 264,000 cell reconstructions from 94 species and more than 70 brain regions. These data are openly accessible both by humans through a user-friendly web portal and by machines via an Application Programming Interface (API). Advances in imaging resolution, new labeling techniques, and automated tracing algorithms, together with improved disposition towards data sharing, have together resulted in a rapid increase in the potential availability of neural reconstructions, creating the need to process more data in less time. We are continuously refining and automating our pipeline to improve efficiency and release datasets without delay. Neural reconstructions downloaded from NeuroMorpho.Org have yielded hundreds of published research results by independent labs in diverse scientific fields. The overall scientific impact of NeuroMorpho.Org is summarized by ~4000 peer-reviewed publications: 2264 describing data available through the database, 864 using downloaded reconstructions, 54 publications about the project itself, and 894 additional references citing NeuroMorpho.Org, often as an exemplary resource in neuroscience data sharing.

Disclosures: C. Tecuatl: None. **L. Shen:** None. **Z. Li:** None. **G.A. Ascoli:** None.

Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.11/

Topic: I.06. Computation, Modeling, and Simulation

Support: UZIMA NIH GRANT 5U54TW012089-03

Title: Multimodal Data Integration for Accurate Psychosis Diagnosis

Authors: *M. NAKABUYE;
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Abstract: Introduction: Psychosis, a severe mental health disorder, affects millions of people worldwide. However, the prognostic prediction of psychosis remains a challenge due to the reliance on singular data types, leading to misclassification and suboptimal treatment outcomes. **Objective:** I aim to examine whether the combination of heterogeneous data types (clinical assessments, sociodemographic factors, patient and medical histories, and genetic data) and supervised and unsupervised machine learning (ML) algorithms could improve clinical risk

prediction and personalize treatment decisions for individuals with psychosis. **Methodology:**This work will leverage data from the NeuroGAP-Psychosis Cohort. Data types will include demographic data (age, gender, ethnicity, and family history of mental illness), biomarker data, genetic data (variants previously associated with psychiatric outcomes, and genetic risk scores aggregated from variants), previous diagnosis of other mental disorders (presence or absence of bipolar disorder, schizophrenia), clinical assessments (measurements of cognitive functioning and symptoms of psychosis), and history of physical health problems (hypertension or cardiovascular disease history). I will train unsupervised ML (PCA, UMAP, t-SNE) models to identify clusters of individuals who share characteristics, and supervised ML for prognostic prediction. The target variable of interest will be clinically diagnosed psychosis. Logistic regression will be used to assess statistical associations between the resulting clusters, and the outcome. I will utilize algorithmic fairness approaches to mitigate model biases and ensure fair prediction across population subgroups based on sensitive attributes (e.g., biological sex and age). For model interpretability, I will employ the SHapley Additive exPlanations (SHAP) technique to investigate various features' contributions to the predictive models. In predictive modeling, I will adhere to the TRIPOD-AI statement.**Outcome:**Findings from this study will offer insights into psychosis subtypes and the practical clinical implications of machine learning in psychosis diagnosis and treatment.

Disclosures: M. Nakabuye: None.

Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.12/Z27

Topic: I.06. Computation, Modeling, and Simulation

Title: Molecular Dynamics Characterization of Connexin-47 and the Pathogenesis of Two Amino Acid Variants

Authors: *D. GONG[1], D. KUMAR[3], Y. L. LUO[4], M. FREIDIN[5], C. K. ABRAMS[2]; [1]Biomed. Engin., [2]Neurol. & Rehabil., Univ. of Illinois at Chicago, Chicago, IL; [3]Dept. of Biotech. and Pharmaceuticals Sci., [4]Biotech. and Pharmaceut. Sci., Western Univ. of Hlth. Sci., Pomona, CA; [5]Neurol. & Rehabil., Univ. of Illinois At Chicago, Chicago, IL

Abstract: Connexins comprise the family of gap junction-forming membrane proteins which provide electrical, chemical, and metabolic coupling between apposed cells. Genetic variants of connexin-47 (Cx47), specifically expressed by oligodendrocytes, are implicated in hereditary leukodystrophy (Pelizaeus–Merzbacher-Like Disease 1) and spastic paraplegia-44. However, the mechanisms of pathogenesis of these disorders are not well understood. We performed molecular dynamics simulations of the wild-type Cx47 hemichannel and two disease-causing mutants,

p.Gly40Ser and p.Arg244Pro, in order to probe for biophysical etiologies of disease while also gauging the utility of molecular dynamics for variant effect prediction. Our trajectory analyses reveal that p.Gly40Ser introduces a secondary structure disturbance in the pore-lining α-helix, TM1. In addition, the N-terminal helix, suspected to be an integral component of the connexin gating mechanism, shows increased occupancy of the channel lumen. This would result in channel permeability reduction which is in agreement with our electrophysiological measurements. In contrast, our simulations of p.Arg244Pro demonstrate the forgoing of electrostatic interactions between the Arg244 side chain and lipid headgroups in the plasma membrane as well as the prohibition of β-sheet formation for multiple residues in Cx47's extracellular domains. The resulting conformational changes may inhibit docking of apposed hemichannels which could explain the experimentally observed loss of gap junction plaque formation which is specific to p.Arg244Pro. Our results show that molecular modeling is capable of differentiating between distinct mechanisms of pathogenesis, raising the possibility of using molecular dynamics as a prognostic tool for classifying undetermined connexin variants.

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Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.13/Z28

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant R00 NS119787

Title: Identifying interpretable latent factors within and across brain regions

Authors: *A. W. ULMER[1], A. J. ZIMNIK[2], D. YU[1], A. A. RUSSO[2], V. SUSOY[3], L. DRISCOLL[4], X. AN[1], A. KENNEDY[1], M. M. CHURCHLAND[2], J. I. GLASER[1]; [1]Northwestern Univ., Chicago, IL; [2]Columbia Univ., New York, NY; [3]Harvard Univ., Cambridge, MA; [4]Allen Inst., Seattle, WA

Abstract: Animal behavior results from the interplay of many neural computations spread across brain regions, and understanding how a behavior is generated requires delineating these computations from one another. Traditionally, identifying these ‘computational building blocks’ requires that researchers impose structure on the data: different task periods are analyzed separately or population-level structure is encouraged using supervised dimensionality reduction methods. While these approaches can be successful, they have obvious shortcomings - behaviors cannot always be separated into distinct epochs, and it is often unclear, particularly in exploratory data analyses, what type of structure to look for in neural data. Unsupervised

methods, like principal component analysis (PCA) identify large neural signals, yet often do not provide interpretable low-dimensional representations of the data. Moreover, existing approaches fail to dissect the role separate brain regions play within a given low-dimensional computation. Here, we present multi-region sparse component analysis (mSCA), an unsupervised dimensionality reduction method that produces interpretable, low-dimensional representations within and across neural populations. Our method builds upon the finding that distinct neural computations are dissociable in time from one another, and thus aims to find sparsely occurring latent factors. We first demonstrated the power of encouraging sparse factors within a single neural population across diverse datasets, including monkey motor cortex during reaching and C. elegans during mating. Our approach found not only structure previously reported using supervised methods, but also novel structure, such as posture-related signals distinct from movement-related signals. We then examined how mSCA, when applied to recordings from primary motor cortex and supplementary motor area during a cycling task, found interpretable factors that were unique to, or shared across multiple neural populations. We further validated mSCA using realistic synthetic datasets generated using trainable spiking neural networks. Finally, mSCA accurately learns time-delays at which individual factors affect different neural populations, allowing users to demix neural computations into interpretable factors, and understand their flow across populations.

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Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.14/Z29

Topic: I.06. Computation, Modeling, and Simulation

Support: National Eye Institute Grant R01 EY032125

Title: qPRF: An Efficient Method for Population Receptive Field Estimation

Authors: *S. C. WAZ[1], Y. WANG[2], Z.-L. LU[1];
[1]Ctr. For Neural Sci., New York Univ., New York, NY; [2]Sch. of Computing and Augmented Intelligence, Arizona State Univ., Tempe, AZ

Abstract: Patterns of BOLD response can be decoded using the population receptive field (PRF) model to reveal how visual input is represented on the cortex (Dumoulin & Wandell, 2008). The time cost of evaluating the PRF model is high, often requiring days to decode BOLD signals for a small cohort of subjects. We introduce the qPRF, an efficient method for decoding that reduced

the computation time by a factor of 1436 when compared to another widely available PRF decoder (Kay et al., 2013) on a benchmark of data from the Human Connectome Project (HCP; Van Essen et al., 2013). With a specially designed data structure and an efficient search algorithm, the qPRF optimizes the five PRF model parameters according to a least-squares criterion. To verify the accuracy of the qPRF solutions, we compared them to those provided by Benson et al. (2018). Both hemispheres of the 181 subjects in the HCP data set (a total of 10,753,572 vertices, each with a unique BOLD time series of 1800 frames) were decoded by qPRF in 23.2 hours on a 3.50GHz Intel Xeon E5-1650 v3 CPU. The absolute difference in R^2 reported by Benson et al. and achieved by the qPRF was negligible, with a median of $5.8x10^{-4}$ (R^2 units being between 0 and 1). The qPRF yielded a better fitting solution on 46.2% of vertices. Based on the time savings, we show that statistical characterizations of the PRF model (e.g., confidence intervals) are possible using numerical methods. With the qPRF, more advanced models can be built atop the PRF framework and novel clinical applications can be explored.

Disclosures: S.C. Waz: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent interest. **Y. Wang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent interest. **Z. Lu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent interest.

Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.15/Z30

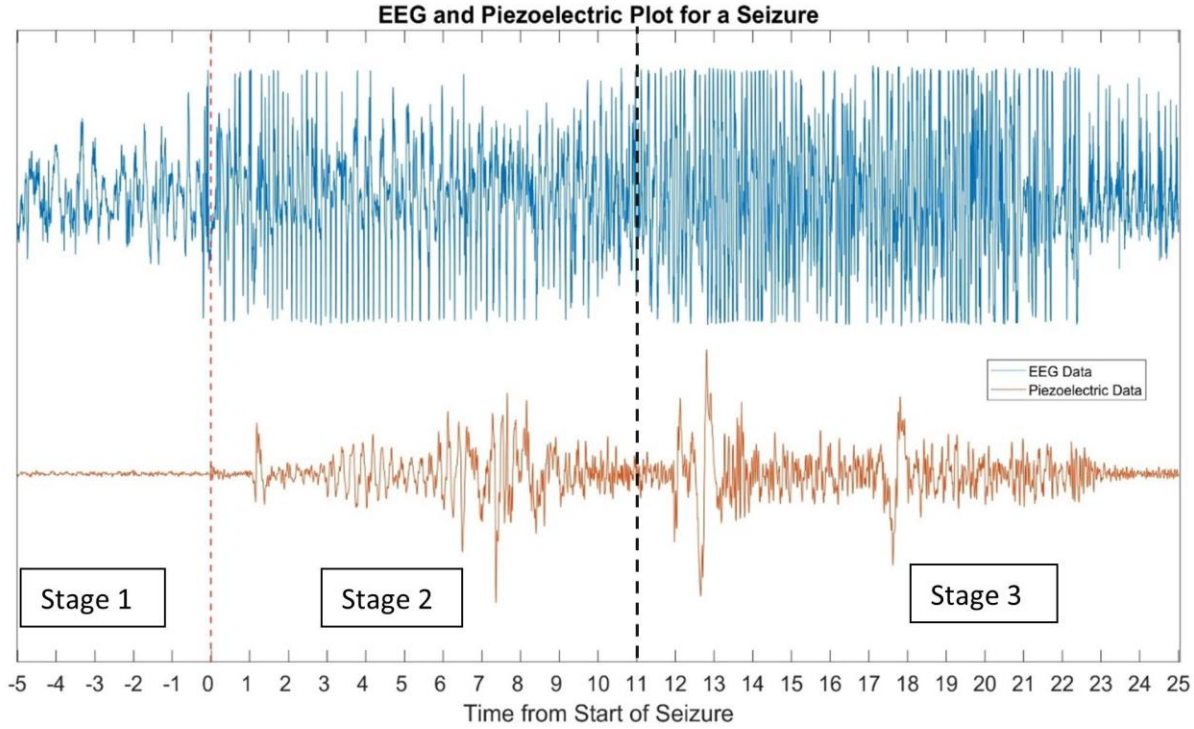
Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant NS107148

Title: Complementing EEG with Piezoelectric Motion Signals for Improved Seizure Detection and Characterization in Mouse Epilepsy Models

Authors: *M. LAVIN[1], D. HUFFMAN[2], S. SUNDERAM[3];
[1]Univ. of Kentucky, Lexington, KY; [2]Signal Solutions, LLC USA, Lexington, KY; [3]Dept. of Biomed. Engin., Univ. of Kentucky, Lexington, KY

Abstract: Electroencephalography (EEG) is an invaluable tool in preclinical epilepsy research. However, it does not by itself convey explicit information about seizure-related motor behavior, which is commonly used to grade seizure severity. Non-invasive motion measurements can help but are likewise limited in the information they convey about epileptiform activity in the brain. Here we examine EEG measurements in combination with piezoelectric ('piezo') motion signals to correlate patterns of cortical activity with overt behavior. Mice (n=6; 1-2 months old) were treated with pilocarpine to induce acute status epilepticus and then monitored for several weeks for signs of spontaneously recurring tonic-clonic seizures using surgically implanted EEG hardware (Pinnacle Tech.) and piezoelectric pressure sensors on the cage floor (Signal Solutions, LLC). All procedures were approved by the Institutional Animal Care and Use Committee of the University of Kentucky. Piezo signal analysis and manual video review were used to find a preliminary sampling of seizure events (n = 161). The provided figure shows EEG and piezo signals for a sample episode that has been split into three stages based on the observed EEG patterns: Stage 1, is the baseline pre-seizure state associated with sleep, corelated with low amplitude, rhythmic piezo signals; Stage 2 is most likely the tonic phase of seizure characterized by large population spikes that evolve in time and frequency and correlates with intense rhythmic piezo signals; Stage 3 is the clonic phase of the seizure, with variable high-frequency spiking in the EEG and less rhythmic piezo signals related to convulsive behavior. These qualitative observations will be verified through video review and the piezo behavior patterns modeled using Long-Short Term Memory neural networks and Hidden Markov Models to produce noninvasive seizure detection algorithms with higher specificity to seizures. Such models will also increase the value of motion measurements as sources of objective quantitative descriptions of seizure severity in preclinical epilepsy models.



Disclosures: M. Lavin: None. **D. Huffman:** A. Employment/Salary (full or part-time):; Signal Solutions, LLC. **S. Sunderam:** None.

Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.16/Z31

Topic: I.06. Computation, Modeling, and Simulation

Title: Unveiling disease trajectories with OPTIVIS digital twins: from MCI to Alzheimer's disease

Authors: *S. HONG[1], S. BAEK[2];
[1]oprimed inc., Suwon-si, Korea, Republic of; [2]Sungkyunkwan Univ. Sch. of Pharm., SUWON-SI, Korea, Republic of

Abstract: Alzheimer's Disease (AD), a complex neurodegenerative condition, demands precise monitoring and analysis across its severity spectrum, particularly at early stages like Mild Cognitive Impairment (MCI). In response to this need, our study leverages the innovative machine learning approach OPTIVIS to construct Digital Twins for AD patients. These Digital Twins, built on OPTIVIS algorithms, are virtual replicas of patients, encapsulating detailed clinical outcomes based on initial patient data and standard care scenarios. Our approach utilizes a comprehensive dataset from both observational studies and control arms of clinical trials, marked by a diverse array of both present and missing data points—a common challenge in AD research. Through a newly developed OPTIVIS model architecture, we adeptly handle this data variability, offering a robust tool for predicting disease progression. The efficacy of OPTIVIS is validated against an external test set, illustrating its remarkable capability to accurately reflect the progression of critical endpoints in clinical trials for a range of AD severities, from MCI to mild-to-moderate AD. This work not only enhances our understanding of AD progression but also opens new avenues for optimizing clinical trial strategies and patient care.

Disclosures: S. Hong: A. Employment/Salary (full or part-time):; Oprimed.inc. **S. Baek:** A. Employment/Salary (full or part-time):; Oprimed.inc..

Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.17/Z32

Topic: I.06. Computation, Modeling, and Simulation

Title: Deriving connectivity from spiking activity in biophysical cortical microcircuits

Authors: *F. MOGHBEL[1,2], A. T. GUET-MCCREIGHT[1], E. HAY[1,2,3];
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Abstract: Inferring detailed cortical microcircuit connectivity is essential for uncovering how information is processed in the brain. A common method *in vivo* uses short-lag spike correlations to derive putative monosynaptic connections, but inactive neurons and correlated firing can hinder the derivation accuracy. Previous studies that developed derivation methods from cross-correlations in ground-truth simulated data used simplified or small network models that did not address these key confounds of physiological large-scale networks. We tested connectivity derivation methods on ground-truth spiking data from detailed models of human cortical microcircuits in different layers and between key neuron types. We showed that physiological oscillations in the large-scale microcircuits imposed confounds on derivation, and we developed methods to overcome the confounds. We then showed that connection derivation was poor in cortical layer 2/3 microcircuits compared to layer 5, due to low firing rates and inactive neurons. General activation strategies for layer 2/3 microcircuits led to only a moderate improvement in derivation performance, due to a trade-off between the proportions of inactive neurons and overactive neurons, indicating the need for more refined strategies. Lastly, we showed that inhibitory connections from somatostatin interneurons targeting distal dendrites required derivation over a longer timescale of cross-correlation lags. Our results elucidate key physiological challenges and methods to improve accuracy in deriving connections from spiking activity in large-scale neuronal microcircuits.

Disclosures: F. Moghbel: None. **A.T. Guet-Mccreight:** None. **E. Hay:** None.

Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

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Program #/Poster #: PSTR315.18/Z33

Topic: I.06. Computation, Modeling, and Simulation

Support: This work was funded by the Bundesministerium für Bildung und Forschung BMBF, Förderkennzeichen: 01ZZ2016

Title: A large-scale computational exploration of cervical transcutaneous spinal cord stimulation to facilitate upper limb motor function

Authors: *A. ALASHQAR[1], V. GEMAR[1], Z. HU[1], S. DIAZ[2], E. NEUFELD[3], N. KUSTER[3], A. ROWALD[1];
[1]ProModell Group, Chair of Digital Hlth., Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany; [2]Simulation & Data Lab. Neurosci., Inst. for Advanced Simulation, Jülich Supercomputing Ctr., Forschungszentrum Jülich GmbH, Jülich, Germany; [3]Fndn. for Res. on Information Technologies in Society, Zurich, Switzerland

Abstract: Transcutaneous spinal cord stimulation (tSCS) applied over the cervical spine facilitates upper limb motor function after paralysis. Given significant variations among commonly applied tSCS protocols, there is no clear consensus on the definition of effective and safe stimulation parameters. Here, we present an extensive in-silico exploration of tSCS stimulation parameters on newly established efficacy and safety metrics. We investigated the activation of axon fibers present in the dorsal column, peripheral nerves and spinal roots extending over the brachial plexus and down to the muscles using a newly developed anatomically accurate, multi-scale computational model of the cervico-thoracic body. A model variant including an osteosynthesis implant, commonly implanted after spinal cord injury, was developed to assess the implant's impact on efficacy and safety. Our simulations reveal that commonly applied tSCS protocols differ substantially in their neural activation sites, ranging from the spinal root entry zone to the brachial plexus and down to the peripheral nerves. Safety assessments revealed concerns, notably vagus nerve co-activation with neck anode placements, while the neck anode performing exceptionally well in efficacy across tested configurations. The inclusion of osteosynthesis implants generally reduced the predicted efficacy while simultaneously reducing co-activation of the vagus nerve, resulting in improved safety metrics. We provide guidelines for adjusting stimulation polarity and pulse width to optimize spinal segment recruitment order and selectivity between afferent and efferent fibers. While targeting specific spinal segments remained challenging across tested tSCS protocols, our results indicate that precise activation of individual brachial plexus nerves is feasible through strategic electrode configuration over the ventral clavicle region.

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Poster

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Program #/Poster #: PSTR315.19/Z34

Topic: I.06. Computation, Modeling, and Simulation

Support: This work has been partially supported by the Army Research Laboratory Cooperative Agreement No W911NF2120186.
Brain and Behavior Research Foundation

Title: Decoding dynamic drug effects in mouse brain with convolutional neural networks and functional ultrasound imaging

Authors: *J. DEIGHTON[1], S. ZHONG[2], K. A. AGYEMAN[3], D. J. LEE[4], C. LIU[4], V. N. CHRISTOPOULOS[3], V. MAROULAS[1];
[1]The Univ. of Tennessee, Knoxville, TN; [2]Neurosci., [3]Bioengineering, Univ. of California Riverside, Riverside, CA; [4]USC, Los Angeles, CA

Abstract: Functional ultrasound imaging (fUSI) is an emerging neuroimaging technology that measures changes in cerebral blood volume (CBV) with high spatiotemporal resolution, high spatial coverage, and sensitivity. This novel technology has been employed in preclinical drug development studies to elucidate the mechanisms of action of various drugs targeting the central nervous system. However, previous studies predominantly focus on predetermined regions of interest (ROIs), potentially ignoring relevant neural activity outside these specific areas and leading to biased results. In the current study, we combined convolutional neural networks (CNNs) with fUSI to understand the pharmacokinetic process of MK-801 (Dizocilpine), a potent and selective NMDA receptor antagonist, in the mouse brain (n = 10). Mice receiving saline vehicle injection were used as a control group (n = 13). This approach allows for a comprehensive analysis of drug effects on the entire brain, without the reliance on predetermined region specification. The results show that CNNs, combined with class activation mapping (CAM), reveal the spatiotemporal effects of MK-801 in the brain and the influence of anesthesia. CNNs successfully captured the dynamic changes in neural activity induced by MK-801 administration, which initiated in the prefrontal cortex and propagated to the hippocampus, demonstrating its ability to detect drug effects over time. We also assessed the impact of anesthesia on mouse brain spatiotemporal hemodynamics throughout the recordings using CNNs and CAM. CNNs revealed distinct patterns of hemodynamic changes during the earlier and later stages of anesthesia. The combination of fUSI and CNNs offers a powerful tool for gaining deeper insights into the spatiotemporal dynamics of drug action in the brain, thereby accelerating the development of new therapies and providing a more comprehensive and unbiased assessment of drug effects on brain function in neuropharmacological studies.

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Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

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Program #/Poster #: PSTR315.20/Z35

Topic: I.06. Computation, Modeling, and Simulation

Title: Unveiling the structural landscape of highly regulated miRNAs in ischemic stroke - Implications for biomarker discovery and therapeutic targeting

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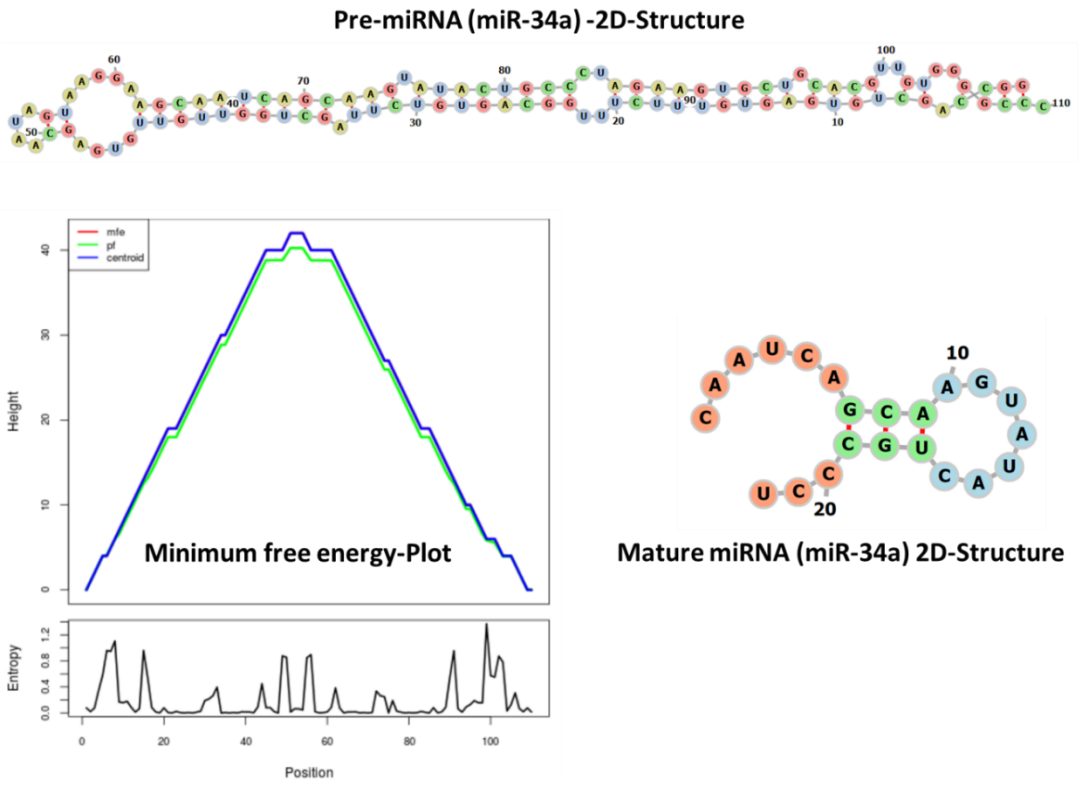
Abstract: The intricate role of microRNAs (miRNAs) in the pathology of ischemic stroke has been a focal point of research. In this study, we explore the significance of highly regulated miRNAs, shedding light on their pivotal roles through structural modeling. To achieve this goal, computational tools such as RNAfold, miRBase, 3DRNA and miRnet were used to elucidate precursor and mature miRNAs 2D and 3D structural information. We identified 40 highly regulated miRNAs and 60 genes associated with ischemic stroke using a network-based approach. These genes span various classes, including BDNF, Caspase, Redox Enzymes, Chemokines, interleukins, TNF-Receptor superfamily, MAP-kinases, Transcription factors, and matrix metallo-proteins. Further, the miR-mRNA molecular hybridization interaction mechanism to form a thermodynamically stable miR-mRNA hybrid complex suggest that the seed sequence complementary, canonical base pairing, absence of G-U pairings and minimum free energy were the essential key parameters that determine the efficacy of the hybrid complex in the disease condition. This structural analysis provides insights into their stability, interactions with target mRNAs, involvement in regulatory gene cellular networks which would help foster develop small therapeutic molecules. Understanding the structural intricacies of highly regulated miRNAs unveils their pivotal roles in influencing crucial pathways implicated in ischemic injury, neuroinflammation, and neuroprotection during stroke condition. In conclusion, this approach facilitates the rational design of miRNA-targeting therapeutics, fostering the translation of novel interventions from bench to bedside.
Referenceshttps://mirbase.org/http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgihttps://www.mirnet.ca/Secure/MirNetView.xhtmlhttps://bibiserv.cebitec.uni-bielefeld.de/rnahybridhttp://biophy.hust.edu.cn/new/3dRNA
Figure 1: Secondary Structure of miR-34a pre and mature sequence.



Disclosures: P. Avti: None. **J. Singh:** None.

Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.21/Z36

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH R01EB026936
NIH U19 NS123717
INCF MATLAB Community Toolbox (MCT) Training Project 2022

Title: Fast and accessible morphology-free calcium imaging analysis

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Abstract: Optical calcium imaging is a versatile modality for recording neural activity, enabling data capture from the micron scale of dendrites and spines to cortex-wide images through two-

photon and widefield microscopy. The analysis of functional calcium imaging hinges on the extraction of temporal fluorescence fluctuations from neuronal components such as cell bodies, dendrites, or brain regions in video data. Current methods heavily rely on spatial information, such as the compact shape of somas, to extract regions of interest and their corresponding temporal traces. This dependency can bias time trace estimation and limit generalizability across different morphologies and spatial scales.
Our approach shifts the focus from spatial to temporal analysis in favor of identifying the temporal traces present in the dataset. We map each pixel (and its time trace) to a node in a graph, where graph edges represent temporal correlations between pixels. Thus, neighboring pixels on a graph are likely to represent the same component regardless of spatial morphology. By casting the problem of extracting time traces, we can apply a dictionary learning framework, where the dictionary comprises the temporal traces and the spatial mappings are represented as sparse coefficients. While our Graft Filtered Temporal Dictionary Learning (GraFT) algorithm enables effective segmentation at different scales, the added computational cost of the graph-regularized l1 optimization can be prohibitive for larger datasets.
We build on the foundation of GraFT and introduce a more efficient solver for the l1 optimization that accelerates spatial coefficient calculations. We also further enhance its parallel processing capabilities by integrating principles of compressive sensing through the employment of random projections. Through these advancements, we reduce the computational demands in optimizing weighted lasso spatial coefficients and updating the temporal dictionary, which enhances the algorithm's speed and broadens its utility across increasingly complex and voluminous datasets. Finally, to ease access to the GraFT algorithm, we offer a stand-alone graphical user interface for easy data preprocessing and GraFT execution, improving user accessibility and efficiency.

Disclosures: A. Estrada Berlanga: None. **G. Kang:** None. **G. Mishne:** None. **A. Charles:** None.

Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.22/Z37

Topic: I.06. Computation, Modeling, and Simulation

Support: NIBIB R01EB026549

Title: Representational geometry, not topography, best characterizes human neural function in transmodal brain areas

Authors: *B. PETRE[1], H. JUNG[1], M. LINQUIST[2], T. D. WAGER[3];
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Abstract: At fine scales in the human brain coarse functional gradients and boundaries give way to highly idiosyncratic functional topographies. Multiple studies argue these are different implementations of common representations and computations shared across individuals. This rests on the superiority of high dimensional (hyper)alignment of topographies, approximately a spatial shuffling of signals that preserves latent category distinctions, relative to 2D and 3D warp alignments. However, no study has directly performed a controlled systematic comparison of these models. Here we compare representational correspondence between individuals to isomorphic correspondence of functional topographies throughout the brain.
Using unrelated participants (N = 278) from the Human Connectome Project (HCP), we estimated functional connectomes from resting state BOLD activity and sequentially diffeomorphically warped and hyperaligned pairs of connectomes (N = 139). Both alignments were estimated on matched data, and hyperalignment was performed parcel-wise without model averaging, enabling a nested model comparison. Alignments were applied to independent data from 7 tasks (emotion, language, motor, theory of mind, gambling, working memory and abstract feature matching) and evaluated by comparison of pairwise between subject correlations (BSC) of aligned evoked responses.
Warping significantly (α = 0.05, Holm-Sidak corrected) and consistently improved BSCs throughout the neocortex and cerebellum (24%-32% over baseline anatomical alignment). Further reprojecting common representations between subjects using hyperalignment also yielded significantly and consistently improved correspondence of task evoked responses in these structures (23%-32% over diffeomorphic alignment). Changes in BSC were small (Δr = 0.01-0.03), but so were baseline BSCs (r = 0.05-0.1). However, improvements systematically increased from established unimodal to transmodal areas (p < 1e-30, t_{138} = 15.3, mixed effects Satterthwaite df), and the largest changes in transmodal brain areas were more dramatic, revealing representations that were several times more similar than topographies would otherwise suggest (e.g. original, warped and hyperaligned BSCs for left Area 46: r = 0.03, 0.10 and 0.22; for left TPOJ2: r = 0.02, 0.07 and 0.14; HCP MMP labels).
These results attribute substantial interindividual variability in functional topographies to spurious but predictable implementation differences that obscure shared representations and computations. This motivates direct study of representational geometry over topography, especially in transmodal brain areas.

Disclosures: B. Petre: None. **H. Jung:** None. **M. Linquist:** None. **T.D. Wager:** None.

Poster

PSTR315: Computational Tools: Analytical II

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.23/Z38

Topic: I.06. Computation, Modeling, and Simulation

Title: Multimodal stereotaxic rat brain atlas for automated image analysis and data integration in 3D

Authors: S. BARRADO-BALLESTERO[1], J. PERENS[2], H. VILA-MERKLE[3], M. CORRAL-BOLAÑOS[4], H. KLEVEN[6], C. GUNDLACH[5], T. B. LEERGAARD[7], T. B. DYRBY[8], ***J. HECKSHER-SØRENSEN**[9];
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Abstract: Laboratory rat is a model organism in neuroscience research, often favored over mouse for specific experimental procedures owing to their larger brain size, greater behavioral complexity, extended lifespan, and metabolic resemblance to humans. Until recently, acquiring high-resolution and high-quality datasets of molecular markers in intact rat brains posed significant challenges. However, recent advancements in tissue clearing protocols for large organs and improvements in the working distance of light sheet microscopes (LSFM) have opened an opportunity to explore disease pathologies and therapeutic outcomes within the whole central nervous system of rats in unprecedented detail. Presently, the Waxholm Space atlas version 4 stands as the most advanced rat atlas, featuring 222 annotations of anatomical structures. This atlas is built upon a structural and diffusion-weighted magnetic resonance imaging (MRI) dataset acquired from a single Sprague Dawley rat brain. To facilitate analysis and integration of datasets from diverse 3D brain imaging modalities, as well as to standardize the reporting of experimental findings, we have developed a multimodal 3D rat brain atlas framework. This framework comprises population-averaged structural MRI and LSFM brain templates, population-based tractography featuring connectivity, delineations of regions according to the Waxholm atlas, and a stereotaxic coordinate system derived from the micro-CT-imaged skull. Acknowledging morphological disparities between MRI and LSFM-imaged brains resulting from tissue-clearing agents, the brain templates are maintained in their respective morphological spaces, with accompanying deformation fields provided for translating experimental data between atlas spaces. The rat brain atlas framework aims to enrich opportunities in neuroscience research by offering a versatile resource for multifaceted applications. It not only bridges the gap between the *in vivo* and *ex vivo* rat brain imaging but also facilitates data collection, sharing, and comparison across multiple various experiments within the same space. This framework enables the utilization of signal contrasts from multiple modalities and markers to enhance the precision of region delineations of the atlas. Integrated skull-derived coordinates ensure precision during stereotaxic surgeries, informed by findings from previous 3D imaging experiments. Moreover, the framework is designed to accommodate

extension, allowing for the inclusion of additional brain templates based on different signal generation mechanisms, as well as representations of diverse ages, strains, or genetic phenotypes.

Disclosures: S. Barrado-Ballestero: A. Employment/Salary (full or part-time):; Gubra A/S. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Gubra A/S. **J. Perens:** A. Employment/Salary (full or part-time):; Gubra ApS. **H. Vila-Merkle:** None. **M. Corral-Bolaños:** None. **H. Kleven:** None. **C. Gundlach:** None. **T.B. Leergaard:** None. **T.B. Dyrby:** None. **J. Hecksher-Sørensen:** A. Employment/Salary (full or part-time):; Gubra ApS. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Gubra ApS.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.01/AA1

Topic: I.07. Data Analysis and Statistics

Support: Kavli Foundation
NIH R24MH117295

Title: Nwb guide: simplifying the conversion of neurophysiology data to nwb format

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Abstract: Neurodata Without Borders (NWB) is a data standard that packages neurophysiology data with the metadata necessary for reanalysis. The NWB format allows data to be human- and machine-readable and enables data to be aggregated across many labs. The neuroscientists who want to use NWB have diverse data formats, and many of them have limited programming experience. To approach this problem, we have developed the NWB Graphical User Interface for Data Entry (GUIDE) to provide a simple entrypoint to the NWB ecosystem for any lab interested in adopting the standard.
Researchers must often convert their data from common proprietary formats such as Intan, SpikeGLX, TIFF, etc. It can be challenging to map these different data formats, each with its own unique structure, to NWB. This challenge has been overcome with the use of NeuroConv, a library for automatically handling the data mapping for 40+ proprietary formats spanning the modalities of intra- and extra-cellular electrophysiology, optical imaging, and behavior. However, the use of NeuroConv requires experience with Python and is poorly suited for those

unfamiliar with programming.
The first official release (v1.0.0) of NWB GUIDE is now available as a cross-platform desktop application that walks users through all the requirements for converting their data to the NWB format and uploading datasets to the DANDI Archive. NWB GUIDE streamlines the data conversion experience, walking users through input file specification, metadata extraction and curation, efficient handling of large datasets, synchronizing between multiple data streams, intuitive configuration of file compression parameters, and finally uploading to the DANDI Archive. We look forward to working with members of the NWB community to test and improve the platform based on user needs and feedback.

Disclosures: C. Baker: None. **G. Flynn:** None. **R. Ly:** None. **O. Ruebel:** None. **B. Dichter:** None.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.02/AA2

Topic: I.07. Data Analysis and Statistics

Support: NIH U24NS120057
The Kavli Foundation

Title: Advancing the NWB data standard and software for efficient integration with new technologies

Authors: M. AVAYLON[1], J. MAGLAND[2], R. LY[1], C. BAKER[3], G. FLYNN[4], S. M. PRINCE[1], B. DICHTER[5], ***O. RUEBEL**[6];
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Abstract: NWB continues to serve as a widely adopted data standard and software ecosystem to store complex neurophysiology data, allowing for data sharing and data reuse. Benefiting from the modularity of the NWB framework, the research community has adapted NWB technologies to create an expanding collection of community software, providing a variety of data exploration, analysis, and management tools. With the increasing integration across neurophysiology domains and applications, we have developed NWB GUIDE as a user-friendly graphical user interface for creating NWB files that supports converting data from 30+ neurophysiology data formats to NWB and uploading data to the DANDI data archive. We are also developing a C++ API to more easily store data with NWB by enabling researchers to record data directly into the NWB

format during data acquisition.
With the number of public neuroscience datasets growing, it has become increasingly important to have standardized methods to attach contextual metadata for relating datasets. We have expanded the HDMF External Resources Data (HERD) standard to encompass a spectrum of tools to more easily create and manage NWB data compliant with controlled sets of terms, ontologies, and external resources.
As researchers extend the boundaries of neuroscience, NWB needs to adapt to meet the requirements of the community. We have worked with the community to help them build NWB extensions to support the storage of events, probes, pose estimation, and multichannel volumetric imaging from C. elegans. In addition, we have worked to integrate NWB with the Behavioral Task Analysis & Building Language (BAABL) to store behavioral task programs and the states, events, and actions recorded by such programs.
As more and more NWB data are stored in the cloud, e.g., on the DANDI Archive, there is a need to evaluate the performance of reading NWB data from the cloud and optimize it. We have developed a new framework, "nwb_benchmarks", for running timing, memory, and network benchmarks for accessing NWB data using various interfaces and stored in various file formats, such as HDF5 and Zarr. We have also developed LINDI (Linked Data Interface), a new JSON-based representation of NWB data where the large data chunks are stored separately from the main metadata and can be accessed efficiently on demand.
Overall, these advancements to the NWB data standard and infrastructure enable researchers to convert neurophysiology data and metadata to NWB more easily, represent metadata more precisely, store a broader variety of data types, and interact with data stored in cloud repositories more efficiently.

Disclosures: M. Avaylon: None. **J. Magland:** None. **R. Ly:** None. **C. Baker:** None. **G. Flynn:** None. **S.M. Prince:** None. **B. Dichter:** None. **O. Ruebel:** None.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

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Program #/Poster #: PSTR316.03/AA3

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant R24MH117295
Amazon Web Services Open Data Program

Title: DANDI: An open ecosystem for neurophysiology and immunostaining data sharing

Authors: *K. GUNALAN[1], R. CHOUDHURY[2], B. DICHTER[3], C. BAKER[3], N. DEHGHANI[1], H.-I. IOANAS[4], D. JARECKA[1], A. KANZER[1], D. LAMANNA[2], A. MACDONALD[4], J.

NESBITT[2], M. SALVI[2], I. C. TO[4], M. VANDENBURGH[2], Y. O. HALCHENKO[4], S. S. GHOSH[1];
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Abstract: As an NIH BRAIN Initiative project, DANDI aims to provide data contributors, data explorers, scientists, and engineers with the tools to share and reuse data from scientific studies. DANDI is intended to play a central role in projects to manage data throughout the research lifecycle from data acquisition to publishing complete datasets alongside manuscripts. Importantly, DANDI allows users to explore and access over 800 TB of raw and processed data together with associated metadata. The open-source architecture leverages a cloud object storage which is supported by the Amazon Web Services (AWS) Open Data Program. DANDI's cloud storage allows for data access to scale as usage increases while maintaining performance. DANDI also includes a Web interface, an application programming interface (API), a JupyterHub instance, DataLad-versioned datasets, and a WebDAV interface. Data is shared on DANDI according to community-driven data standards such as NWB, OME-Zarr, and BIDS. Using the API, the neuroinformatics community has additionally integrated applications for data upload, visualization, analysis, and tutorials. This ecosystem includes tools such as NWB GUIDE, Neurosift, Neuroglancer, Dendro, and OpenScope Databook. In this work we present the open-source design, describe how data sharing follows the FAIR (Findable, Accessible, Interoperable, Reproducible) principles, provide examples of how the community has extended DANDI, and discuss new features of DANDI.

Disclosures: K. Gunalan: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DataJoint. **R. Choudhury:** A. Employment/Salary (full or part-time):; Kitware. **B. Dichter:** A. Employment/Salary (full or part-time):; CatalystNeuro. **C. Baker:** A. Employment/Salary (full or part-time):; CatalystNeuro. **N. Dehghani:** None. **H. Ioanas:** None. **D. Jarecka:** None. **A. Kanzer:** None. **D. LaManna:** A. Employment/Salary (full or part-time):; Kitware. **A. Macdonald:** None. **J. Nesbitt:** A. Employment/Salary (full or part-time):; Kitware. **M. Salvi:** A. Employment/Salary (full or part-time):; Kitware. **I.C. To:** None. **M. VanDenburgh:** A. Employment/Salary (full or part-time):; Kitware. **Y.O. Halchenko:** None. **S.S. Ghosh:** None.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.04/AA4

Topic: I.07. Data Analysis and Statistics

Support: NIMH 1R24MH117295
NIMH 2P41EB019936
NIH 2R24MH117179

Title: Bids 2.0

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Abstract: The Brain Imaging Data Structure (BIDS) is a community standard that defines how to organize and describe neural data and associated metadata. Initially designed to facilitate easier sharing, analysis, and preservation of anatomical and functional MRI data, BIDS has expanded organically to multiple data modalities (e.g., EEG, MEG, DTI, microscopy) through numerous BIDS Extension Proposals (BEPs), continuously incorporating community-driven improvements while maintaining backward compatibility with previous versions. Given the expanded scope of BIDS resulting from its success and adoption for multiple data modalities, some of the fundamental concepts initially developed for MRI data will need to be changed. With the introduction of BIDS 2.0, we are preparing to implement significant modifications to further enhance the structure's functionality and user experience. The forthcoming version proposes changes such as
- support for dataset-specific layouts, allowing for a directory hierarchy that best suits each dataset;
- enhanced modularity, enabling for instance individual subject session folders to function as fully-fledged BIDS datasets that can be processed or shared independently;
- addressing several quirks that have emerged over time, like having "participants.tsv" but "sub-" entity, etc.
These changes aim to streamline data management in neuroscientific research and ensure that BIDS remains at the forefront of data standardization efforts. We will present the core concepts of BIDS 2.0, discussing the rationale behind the pivotal updates and the expected impact on the neuroimaging community, and to seek feedback.

Disclosures: Y.O. Halchenko: None. **F. Pestilli:** None. **E. Earl:** None. **A.S. Rokem:** None. **C. Markiewicz:** None.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

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Program #/Poster #: PSTR316.05/AA5

Topic: I.07. Data Analysis and Statistics

Title: Neurozip: an approach to spike sorting long-term electrophysiology recordings using batch subsampling

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Abstract: Spike sorting is a critical process in neural data analysis that aims to identify and classify the actional potential activity ("spikes") of individual neurons from extracellular electrophysiological recordings. Traditional recordings might last 8 hours, but to capture long-term brain changes critical to mental health, multi-day or multi-week recordings will be necessary. Current spike sorting methods such as KiloSort rely on processing whole recordings, making the processing of longer recordings very computationally expensive - thereby preventing sorting of the longest recordings. We present a novel method for spike sorting that utilizes KiloSort's template-matching algorithm while introducing subsampling to allow the handling of larger datasets. In particular, rather than spike-sorting multiple individual segments of 12-24 hours, followed by subsequent spike-matching, which can introduce error, we sought a means to sort across the full span of a recording to avoid this post-hoc processing. To do this, we spike sort on only sections of the data that are spaced evenly across the full recording - for example, one minute out of every ten. Initial findings indicate that this approach yields high-quality replications of full dataset spike sorting. Detailed quantifications of optimal parameters are ongoing. In a second approach, we seek to select data segments that are optimal for spike template development due to having the highest information content. We then plan to focus computational resources on these selected data segments. Overall, these approaches open the door to reliably and efficiently spike sorting datasets spanning days or weeks.

Disclosures: D. Kirca: None. **D. Kim:** None. **M. Ruckstuhl:** None. **B.O. Watson:** None.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

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Program #/Poster #: PSTR316.06/AA6

Topic: I.07. Data Analysis and Statistics

Support: Wellcome Trust studentship (219872)
Mathworks Grant

Wellcome Trust (223144)
ERC/UKRI grant 101097874

Title: Bombcell: A toolbox for quality control of high-density probe recordings

Authors: J. FABRE[1], ***S. DODGSON**[1], E. H. VAN BEEST[1], A. J. PETERS[2], M. CARANDINI[1], K. D. HARRIS[1];
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Abstract: Introduction. Recent advances in high-density neural probes, such as Neuropixels, have revolutionized electrophysiology. However, they produce large, complex datasets that require extensive quality control. This is typically done through manual curation, which is time-consuming, subjective, and lacks standardization.
Methods. Here we introduce Bombcell (https://github.com/Julie-Fabre/bombcell) , an open-source, user-friendly toolbox that streamlines quality control for spike sorting outputs. Bombcell integrates five established and ten novel quality metrics, becoming the first toolbox to address the wide range of errors that can arise, including noise, non-somatic waveforms, multi-unit activity, and drift.
Bombcell employs a multi-step algorithm that first removes noise and non-somatic units based on waveform shape. It then divides units into time chunks to assess stability and remove periods of excessive drift or contamination. Finally, it applies stringent thresholds on isolation metrics to classify units as single or multi-unit activity.
The toolbox offers interactive visualizations and a graphical user interface (GUI) for exploring metrics and adjusting thresholds. It seamlessly integrates with popular manual curation software like Phy, enabling a flexible semi-automated workflow. Bombcell is optimized for efficiency, with rapid runtime and automatic plot generation at each stage. Users can quickly adjust parameters based on their specific needs using summary plots and the GUI. Bombcell's outputs are saved in a simple format, allowing users to easily modify classification criteria without recomputing metrics, promoting unbiased threshold selection.
Conclusion. Bombcell aims to standardize and democratize quality control, improving reproducibility across labs. As high-density recordings continue to evolve, it provides an essential tool for maintaining data integrity and enabling scientific discoveries.

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Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

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Program #/Poster #: PSTR316.07/AA7

Topic: I.07. Data Analysis and Statistics

Title: Real-time AI-based spike sorting at the edge: Closing the loop for Large-scale neural recording

Authors: *A. KELLEY, D. KIPKE, A. GOLABCHI, J. ADAMS, D. R. KIPKE;
NeuroNexus, Ann Arbor, MI

Abstract: Systems neuroscience is advancing, in part, through studies of neural circuits with higher resolution, longer duration, and advanced behavioral and closed-loop experimental paradigms. This progress requires advanced neural probes, high-performance hardware and software, and robust models and algorithms for neural analytics. Fast, precise, and accurate spike sorting is a common issue across most paradigms and workflows. This study aims to create a high-performance spike sorting system that can operate in real-time or near real-time for large-scale neural recordings to support closed-loop experiments.We have developed a machine learning-based solution for unsupervised feature extraction from neural spike snippets extracted from high-bandwidth, multi-channel extracellular recordings. This approach is tailored explicitly for distinct neural probe geometries and site spacing, transforming raw neural signals into an informative feature space. It enables efficient and accurate spikes clustering into distinct neuronal activities, all without prior knowledge of the neuron count or specific waveform characteristics. We have rigorously trained and validated our model using simulated datasets with neurons of varying types and firing rates, ensuring its invariability and scalability to various site architectures and recording environments. The software system is highly concurrent and runs on a multi-core CPU. Results show that our approach meets or exceeds comparable spike sorting algorithms in accuracy and processing speed techniques, especially in complex scenarios typical of modern high-channel recording devices. In simulations running on a quad-core CPU, average latencies remain primarily constant regardless of the number of sites/spikes up to a realistic upper bound of about 1024 sites. In a simulation with 130 neurons with an average firing rate of 13 spikes/sec on a 256-site probe (aggregate average rate of 1,700 spikes/sec across all sites), the average latency/spike is about 85.20 μs for both spike detection and classification and approximately 60.73 μs for just spike classification. This translates to an end-to-end spike sorting efficiency, defined as 1-time_chunk/processing_latency, of about 0.6. These results suggest that this system will support near or real-time spike sorting of large-scale neural recordings for the latest generation of neural probes.

Disclosures: A. Kelley: A. Employment/Salary (full or part-time):; NeuroNexus Technologies Inc. **D. Kipke:** A. Employment/Salary (full or part-time):; NeuroNexus Technologies, Inc. **A. Golabchi:** A. Employment/Salary (full or part-time):; NeuroNexus Technologies Inc. **J. Adams:** A. Employment/Salary (full or part-time):; NeuroNexus Technologies Inc. **D.R. Kipke:** A. Employment/Salary (full or part-time):; NeuroNexus Technologies, Inc.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.08/AA8

Topic: I.07. Data Analysis and Statistics

Title: An open-source, standardized, efficient, and cloud-based analysis pipeline for spike sorting of large-scale electrophysiology data

Authors: *A. P. BUCCINO, D. FENG, S. E. J. DEVRIES, K. SVOBODA, **J. H. SIEGLE**; Allen Inst. for Neural Dynamics, Seattle, WA

Abstract: The rapid adoption of high-density neural probes with thousands of electrodes, such as Neuropixels, is rapidly increasing the yield of extracellular electrophysiology experiments. The resulting massive datasets require automated and efficient processing pipelines. As an example, the Allen Institute for Neural Dynamics (AIND) alone, using acquisition rigs with up to 12 simultaneous Neuropixels probes, generates hundreds of TB of raw electrophysiological data per month.

To meet our demanding requirements in terms of scale and reproducibility, we have developed a standardized and efficient pipeline for spike sorting of large-scale electrophysiology data. The modular pipeline is implemented using the Nextflow scientific workflow system and deployed in the cloud using the Code Ocean computing environment.

We use the SpikeInterface API to: *preprocess* the traces, with filtering, bad-channel removal, denoising, and motion correction; *run spike sorting* using Kilosort2.5; *postprocess* the sorting results to remove duplicated units, perform additional computations (e.g., PCA scores, spike amplitudes, unit locations, correlograms, and more), and calculate over 20 quality metrics for each unit; *curate* the spike sorting output using a combination of quality metrics and a decoder built on previously labeled units; *visualize* the results with web-based technology; and finally collect the results and *export* them into NWB format.

Each step corresponds to a GitHub repo containing both the code and the Docker image to run it, ensuring full reproducibilityThe Nextflow implementation orchestrates the individual components and allows us to specify and tune computational resources for each step. As an example, expensive GPU machines are only requested to run Kilosort2.5, which is the only step that requires GPU capabilities.

In addition to our cloud solution with the Code Ocean platform, Nextflow seamlessly enables deployments on various platforms, such as local workstations and SLURM clusters.

The pipeline and each of its steps are open source and available on GitHub at https://github.com/AllenNeuralDynamics/aind-ephys-pipeline-kilosort25/. The documentation includes detailed step-by-step guides to deploy it on different platforms. So far, we have processed over 1000 sessions at AIND and we hope that this resource will be broadly adopted and used by the neuroscience community.

Disclosures: A.P. Buccino: None. **D. Feng:** None. **S.E.J. DeVries:** None. **K. Svoboda:** None. **J.H. Siegle:** None.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

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Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.09/AA9

Topic: I.07. Data Analysis and Statistics

Support: R01-NS094206
P50-NS123109

Title: Visualizing Stereotactic Navigation of Brain Implants for Large Animal Studies

Authors: *M. LEI[1], A. DAVIS[2], A. SHAHKHAN[2], N. W. PRINS[2], M. D. JOHNSON[2];
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Abstract: Precise implantation of brain recording and stimulation probe technologies requires knowing how rotations and translations within a stereotaxic frame map onto an individual subject's brain anatomy. Such knowledge is especially important for neurosurgical procedures in studies with large animal models. Over the years, Monkey Cicerone, which is based on a Tcl/Tk platform, has been an important software package for many researchers to both plan out neurosurgical instrumentation procedures and track positions of neural probes as they advance through a microdrive. Here, we augmented this software in two important ways: 1) porting the code into python to enable open-source development by the broader neuroscience community, and 2) integrating the functionality of Monkey Cicerone into a module within 3D Slicer, which is a commonly used image computing platform. This platform now has the advantage of seamless integration to co-register subject MRI and CT data, segment and visualize a subject's brain nuclei and axonal pathways, as well as import and transform digital models of hardware associated with the brain implants. This integration now enables researchers to conveniently plan stereotactic neurosurgery within 3D Slicer, enhancing accuracy in chamber placement and subcortical targeting while leveraging its other modules for broader analysis and visualization.

Disclosures: M. Lei: None. **A. Davis:** None. **A. Shahkhan:** None. **N.W. Prins:** None. **M.D. Johnson:** None.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.10/AA10

Topic: I.07. Data Analysis and Statistics

Title: Streamlining in vivo neuroscience experiments: a novel file structure approach

Authors: *E. MARTIANOVA[1], S. LIMA[2], A. LAVOIE[1], Y. DE KONINCK[3], I. YALCIN-CHRISTMANN[4], J.-L. NERON[1];
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Abstract: *In vivo* neural imaging experiments often face a significant challenge: the laborious analysis process can take weeks to yield even preliminary conclusions. While numerous tools are available for data processing, such as converting image stacks into neural signals or calculating ΔF/F signal from raw fiber photometry signals, there is little to no solutions that address the complexity of subsequent analysis steps. There is especially no solution which integrates neural and behavioral data, nor one that considers experimental design. We propose two unique file structures, one for individual neural recordings and one for entire experiments, to greatly simplify data management at each analysis stage. Despite variations in initial processing steps between different data types, such as fiber photometry signals and mini-microscope images, the overall processing pipeline for *in vivo* neural recordings is remarkably similar. Our proposed file structure, utilizing the Hierarchical Data Format (HDF), comprises four main sections: acquisition, behavior, processed data, and analyzed data. Each section corresponds to a key analysis step and includes relevant metadata, such as algorithm names, parameters, linked data, etc. The experiment's file structure, also in HDF, outlines the experimental design, links all associated recordings, and groups analyzed data according to the experimental design. We illustrate the benefits of these file structures using a fiber photometry experiment as an example. The experiment comprises 789 recording sessions of 3 biosensors from 95 animals of 2 strains, 7 different batches and 3 treatments recorded in 15 various behavioral tasks. We demonstrate that our file structures allow us to easily keep track of all these neural recordings, related behavior data and their analysis pipelines, as well as quickly compare results according to the experiment design. This approach streamlines the analysis process and facilitates comprehension of the process. Overall, our file structures facilitate data management, ensure reproducibility, and enhance the efficiency of data analysis in *in vivo* neuroscience experiments.

Disclosures: E. Martianova: A. Employment/Salary (full or part-time):; Doric Lenses Inc.. **S. Lima:** None. **A. Lavoie:** A. Employment/Salary (full or part-time):; Doric Lenses Inc.. **Y. De Koninck:** None. **I. Yalcin-Christmann:** None. **J. Neron:** A. Employment/Salary (full or part-time):; Doric Lenses Inc..

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.11/AA11

Topic: I.07. Data Analysis and Statistics

Title: An open science data platform at the Allen Institute for Neural Dynamics

Authors: *D. FENG, A. P. BUCCINO, M. KAPOOR, C. LAITON, A. LEON, H. LIN, M. MONINGHOFF, S. SESHAMANI, J. H. SIEGLE, J. YOUNG, K. SVOBODA, S. E. DEVRIES; Allen Inst. for Neural Dynamics, Seattle, WA

Abstract: The Allen Institute for Neural Dynamics (AIND) has embarked on the challenge to explore the brain's activity, at the level of individual neurons and the whole brain, to reveal how we interpret our environments to make decisions. Central to this mission is sharing and distributing the data and tools that we are creating to facilitate community collaboration and equip scientists throughout the field to answer these fundamental questions. To this end, we have built a cloud-based data platform that makes our data and tools available to the community as we conduct our science in the open. This infrastructure was designed to support reproducible and scalable analysis and to provide both transparency and access to our data and tools. We have built several data systems within AIND to collect electrophysiological, optophysiological, behavioral, and light sheet imaging data to support diverse research projects. Shortly after data acquisition, data is uploaded to a public S3 bucket in the AWS Open Science Registry, where it is publicly available, along with detailed metadata that documents the source and provenance of the data to support data reuse. Reproducibility and re-use are core to AIND's mission and platform. Data processing and analysis are performed Code Ocean (CO), a cloud-based tool for data science that automatically encapsulates and versions the data, code, and software environment for all analyses and their results. CO leverages open-source data science tools, lowering the barrier of entry to modern cloud computing for scientists and ensuring our tools can be re-used outside the platform. We have developed several automated data pipelines to process electrophysiology, population calcium imaging, light sheet imaging, and behavioral videos. These pipelines build upon existing open-source software and leverage community data standards including NWB and OME. These pipelines are available in public GitHub repositories and can run both on our cloud platform and on-premise computational infrastructure. AIND's data platform for AIND has been built to support doing science in the open, centering reproducible analysis, transparency into data provenance and analysis, community collaboration, and flexible reuse.

Disclosures: D. Feng: None. **A.P. Buccino:** None. **M. Kapoor:** None. **C. Laiton:** None. **A. Leon:** None. **H. Lin:** None. **M. Moninghoff:** None. **S. Seshamani:** None. **J.H. Siegle:** None. **J. Young:** None. **K. Svoboda:** None. **S.E. DeVries:** None.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.12/AA12

Topic: I.07. Data Analysis and Statistics

Support: Funded by the Allen Institute

Title: The AIND metadata schema for describing systems neuroscience experiments

Authors: *S. E. DEVRIES, M. MONINGHOFF, M. KAPOOR, H. LIN, J. YOUNG, K. SVOBODA, D. FENG;
Allen Inst. For Neural Dynamics, Seattle, WA

Abstract: Recent open science efforts have led to an increase in data sharing, yet the re-use of shared data remains stymied by the challenge of understanding exactly what a specific shared data asset is, how it was obtained and packaged. Metadata that documents the features, context, provenance, and reliability of the data are critical to enable the re-use of that data. While this is usually relegated to the methods section of an accompanying paper, this has been shown to often be insufficient as efforts to reproduce notable results from published methods usually fall short (Errington *et al.* 2021). Here, we present the Allen Institute for Neural Dynamics (AIND) metadata schema to describe systems neuroscience experiments. We define a set of classes to document the experimental and analytical provenance of each data asset. These include: (1) a **data description** class that documents high level administrative and funding information; (2) a **subject** class that documents the subject used in the experiment, including species, date of birth, breeding background, sex, and housing information; (3) a **procedure** class that documents any procedures performed to the subject, or tissue removed from the subject, prior to data collection, including surgeries, injections, perfusions, tissue processing, antibody stainings, etc.; (4) an **instrument** class that documents the instrument used to collect data and the devices that are part of it; (5) an **acquisition** class that documents how the data was collected, including device configurations, experimental parameters, and behavior tasks; and (6) a **processing** class that documents how derived data have been processed following collection, including compression, annotation, and other forms of data processing and analysis.This schema has been defined using Pydantic, a Python based data validation library, that we use to generate JSON files to accompany each data asset. This Python integration allows us to develop additional tools for ingesting and visualizing metadata. We have built a GUI to support manual entry of data and have created mappers to automatically ingest metadata from the data acquisition platforms used within AIND.As systems neuroscience is an evolving field where experimental techniques and paradigms are continuously developing, it is crucial that this schema be expandable and flexible. To this end, the schema is version controlled to permit the addition of new concepts and classes

when needed. Maintaining this schema independently from our data collection and processing pipelines further supports this flexibility.

Disclosures: S.E. DeVries: None. **M. Moninghoff:** None. **M. Kapoor:** None. **H. Lin:** None. **J. Young:** None. **K. Svoboda:** None. **D. Feng:** None.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.13/AA13

Topic: I.07. Data Analysis and Statistics

Support: EC Horizon Europe grant #101147319: "EBRAINS-2.0"

Title: In-depth curation of neuroscience data in EBRAINS

Authors: *P. NAJAFI[1], U. SCHLEGEL[2], L. ZEHL[3], A. P. DAVISON[1];
[1]Paris-Saclay Inst. of Neurosci., Ctr. Nationale De La Recherche Scientifique (CNRS), Univ. Paris-Saclay, Saclay, France; [2]Univ. of Oslo, Kolsas, Norway; [3]EBRAINS AISBL, Brussels, Belgium

Abstract: With recent advances in neuroscience data production, the need for robust and efficient data sharing methods is becoming more apparent. The gold standard for data sharing is the FAIR (Findability, Accessibility, Interoperability, and Reusability) guidelines (Wilkinson et al., Scientific Data, 2016). EBRAINS is a digital research infrastructure, developed with European Union funding, to accelerate collaborative brain research between organizations and researchers across neuroscience and brain-related fields. The data sharing service of EBRAINS provides support to researchers in preparing their data and computational models for sharing, and in annotating them with appropriate metadata. This curation service aims to provide the necessary stewardship and workflows for sharing neuroscience data in a FAIR-compliant framework. Having rich and reliable metadata is one of the methods to improve the compliance of the data with FAIR principles. The EBRAINS curation service proposes multiple tiers of metadata richness, with higher tiers being more time-consuming but increasing the FAIRness of the shared data. The highest tier is in-depth curation, which aims to capture detailed, modality-dependent metadata. In this poster, we present the in-depth curation process. At the moment, we support electrophysiology and neuroimaging experiments, and computational workflows. The curation process organizes and captures information about device settings, experimental protocols, specimen preparation, chemicals used, as well as stimulation procedures and the stimuli used. Similarly, for computational workflows, we capture metadata for simulation, data

analysis, and visualization. This aids in tracking the provenance of the computational process and facilitates reproducibility.

Disclosures: P. Najafi: None. **U. Schlegel:** None. **L. Zehl:** None. **A.P. Davison:** None.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

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Program #/Poster #: PSTR316.14/AA14

Topic: I.07. Data Analysis and Statistics

Support: NINDS Grant NS132812
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NINDS Grant NS135851
NIDCD Grant DC020109

Title: Dataset Integrity Done with Git (didg) - A framework for managing neuroscience datasets along processing phases

Authors: *R. FERGER, A. J. BAE, J. L. PENA;
Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Modern neuroscience produces increasing amounts of raw data, e.g. multi-channel electrophysiology or functional imaging. These data often originate from individual experimental sessions, each resulting in one dataset that is self-contained in the sense that it includes all the information for subsequent processing. Thus, these datasets are independent except on the conceptual level imposed by the experiment. Processing in this context refers to each step in the analysis pipeline, automatic or manual, which can be performed on datasets prior to pooling or cross-referencing them. Examples include: spike detection and spike sorting, or extracting downsampled local field potentials in electrophysiology; in functional imaging, regions of interest are defined and fluorescent signals saved as simple time series. In many cases, the extracted relevant data is much smaller than what was originally recorded. Therefore, it is desirable to keep only the smaller and processed datasets for final analysis on a local computer, while the original raw data and intermediate datasets can be stored on suitable server infrastructure. Maintaining the integrity of datasets throughout this process, including the relationship across phases, is crucial for reproducibility of analysis workflows and becomes increasingly challenging with the amout of data. We propose a framework to leverage well-established software tools, namely Git and Git LFS (large file storage) to solve the outlined challenges. Datasets in uniquely named directories are originally added into separate Git branches, following a customizable naming scheme, to allow independent retrieval and

processing of each dataset. Git's history with a common ancestor commit allows pooling of the fully-processed datasets using simple merge stategies, while maintaining a traceable record of each dataset's history. Leveraging Git LFS for storage and transfer of large files ensures binary data integrity and prevents several scenarios of accidental data loss. We also present a new command line tool "didg", under active development, to facilitate the use of this framework, especially for users less familiar with Git. We aim to make didg a helpful and easy-to-use companion for neuroscience labs to manage the processing flow and integrity in a world of increasing data volumes.

Disclosures: R. Ferger: None. **A.J. Bae:** None. **J.L. Pena:** None.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

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Topic: I.07. Data Analysis and Statistics

Support: NIH grant - 5U24EB029005-05
NSF grants – 1935749, 1840218, 2114202
NIH R01DA053028

Title: Enabling Metadata Provenance for Neuroscience Artifacts

Authors: *S. SIVAGNANAM[1,2], K. YOSHIMOTO[1], A. MAJUMDAR[1], W. LYTTON[3]; [1]UCSD, La Jolla, CA; [2]SUNY Downstate Health Sciences University, Brooklyn, NY; [3]SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY

Abstract: When scientific datasets evolve or are reused to create derived datasets, it is crucial to securely preserve the integrity, metadata, and provenance to prevent unintended or malicious alterations during the process. Providing a secure method to efficiently share and verify the data as well as metadata is essential for the reuse of the scientific data. Open Science Chain (OSC) project utilizes consortium blockchain to provide a cyberinfrastructure solution to maintain integrity of the provenance metadata for published datasets and provides a way to perform independent verification of the dataset while promoting reuse and reproducibility. The NSF and NIH funded Neuroscience Gateway (NSG) provides an easy-to-use web portal that allows neuroscience researchers to execute computational and data analysis pipeline on high performance computing resources. Combined, the OSC and NSG platforms form an efficient, integrated framework to preserve and verify the integrity of the artifacts used in research workflows while using the NSG platform. This poster describes the integration of OSC-NSG frameworks to track the provenance of neurophysiological signal data analysis to study brain

network dynamics using the Neuro-Integrative Connectivity (NIC) tool, which is deployed in the NSG platform. Description of methods to identify analogous files that could have been previously used in an experiment is also presented.

Disclosures: S. Sivagnanam: None. **K. Yoshimoto:** None. **A. Majumdar:** None. **W. Lytton:** None.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

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Program #/Poster #: PSTR316.16/AA16

Topic: I.07. Data Analysis and Statistics

Support: NIH grant U19NS107616
LundbeckFoundation Fellow grant

Title: Brainstem: A collaborative electronic lab notebook for experimental neuroscience

Authors: *P. C. PETERSEN[1], A. SURKIS[2], G. BUZSAKI[3];
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Abstract: BrainSTEM (Brain STructured Experimental Metadata) is a collaborative electronic lab notebook for experimental neuroscience. It has a customizable web interface and a standardized yet flexible data model and is designed to capture a range of electrophysiology, imaging, and behavioral data. Granular permissions, including one-click public sharing, promote collaborations and open science. BrainSTEM is designed with ease of adoption and use as a primary consideration and facilitates compliance with NIH and other data-sharing requirements. BrainSTEM provides three key benefits as an electronic notebook solution for experimental neuroscience:

1. **It has a very low barrier to use**
- Data can be entered through web-based intuitive forms and organized in a user-friendly UI.
- Data can be shared with collaborators or publicly with a single click.

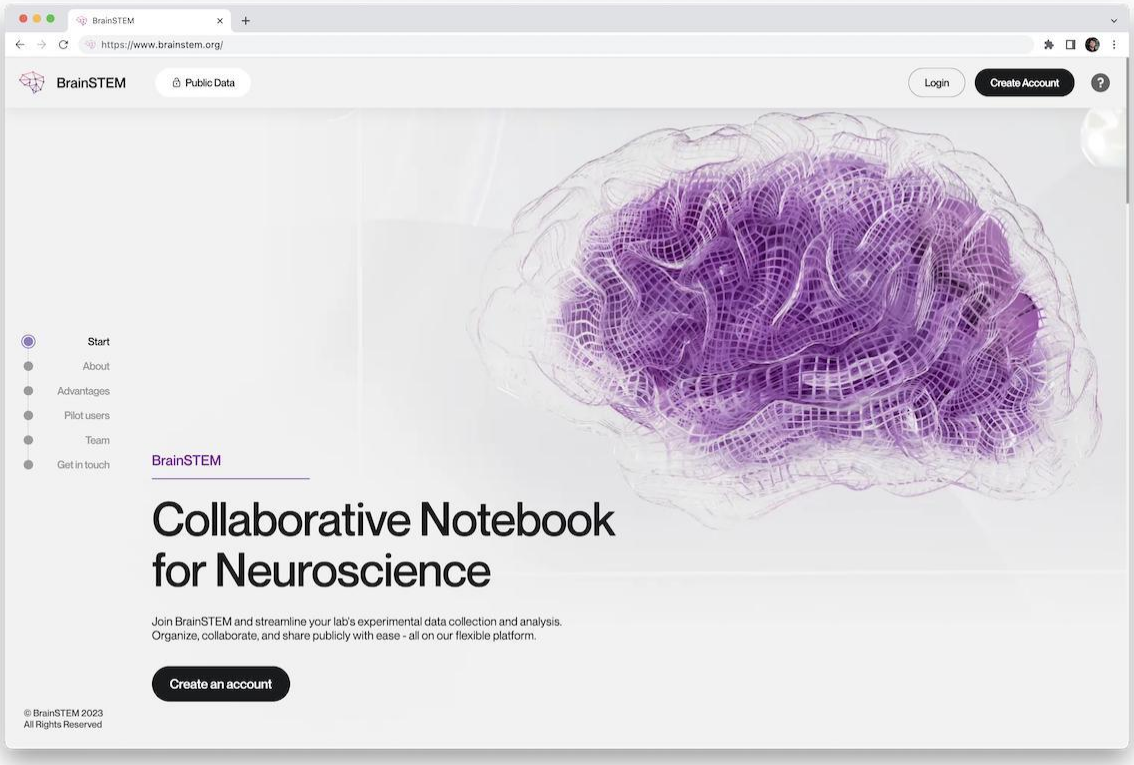
2. **It is centralized**
- It requires no technical knowhow to use or set up.
- Keeps metadata from becoming fragmented across various sources.
- Easily discover and organize data through the relational data structure.

3. **It is standardized**- A standardized yet flexible language applies to current methods and techniques and is ready to accommodate future requirements.
- It promotes a rich level of metadata making experimental data more interpretable.

- Programmable access via an API allows for machine readability and for tools to be built around it.

BrainSTEM has the potential to become the standard metadata model within neurophysiology, make data FAIR, promote standardization, data sharing, and provide better integration across datasets, both within and across collaborative labs and for published datasets.

We are looking for pilot groups - please come by our poster or visit our website www.BrainSTEM.org to learn more.



Disclosures: P.C. Petersen: None. **A. Surkis:** None. **G. Buzsaki:** None.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

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Michael J. Fox Foundation's PPMI – award #8283.03

Title: Embedding Reader-Accessible, Transparent and Thorough Data into Publications - Schol-AR

Authors: *T. ARD[1], M. S. BIENKOWSKI[2], S.-L. LIEW[3], A. W. TOGA[4];
[1]USC Stevens Neuroimaging and Informatics Inst., Altadena, CA; [2]Mark and Mary Stevens Neuroimaging and Informatics Inst., USC, Los Angeles, CA; [3]USC, Los Angeles, CA; [4]USC Stevens Neuroimaging and Informatics Inst., USC, Los Angeles, CA

Abstract: Although neuroscience is a digital field, it is primarily communicated through static text and images in PDF-based articles. Problematically, this format is prone to hand-selected snapshots of the best research results and excludes the majority of quantitative and qualitative data generated in a project. Many attempts have been undertaken to make more study data accessible to readers, such as through the addition of online supplementary materials. However, metrics show that supplementary materials are accessed less than .04% of the time,[1] indicating they are largely ignored by readers. As a result, modern data is absent in standard article communication.[2,3] This widely acknowledged inadequacy limits our ability to effectively and transparently communicate neuroimaging research. Our project, termed 'Schol-AR,' aims to address this gap by directly embedding various forms of scientific data into standard PDF articles as 'augmentations,' seamlessly integrating them as interactive digital entities within a paper. Articles augmented with Schol-AR are widely accessible, journal and platform agnostic, and can display both an article and its associated augmented data simultaneously through a single-click on laptops, computers, tablets, and mobile devices. For readers who prefer printed materials or conference posters, Schol-AR also supports 'point and view' augmented reality (AR) to augment printed works. Critically, all Schol-AR augmentations are automatically compatible with every journal and publisher, as evidenced by augmented articles published across numerous publishers including Elsevier, Nature Publishing Group, Wiley, IOS Press, and IOP Publishing. Here, we demonstrate advances in the Schol-AR framework including the support for additional data formats that authors can directly include as augmentations in their articles. All capabilities are openly accessible to authors and readers. Ultimately, we aim to improve the thoroughness and transparency of scientific articles by providing seamless reader accessibility to research data associated with publications.

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Disclosures: T. Ard: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ardist Inc.. **M.S. Bienkowski:** None. **S. Liew:** None. **A.W. Toga:** None.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

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Program #/Poster #: PSTR316.18/AA18

Topic: I.07. Data Analysis and Statistics

Title: Automatic post-processing and angle calculation for hind-limb clasping: A DeepLabCut extension

Authors: S. JACOBSEN, C. PEDERSEN, K. W. FEJGIN, U. RICHTER, F. GASTAMBIDE, ***T. JANJUA**;
H Lundbeck, Copenhagen, Denmark

Abstract: Hind-limb clasping can be used as a marker of disease progression in several neurodegenerative mouse models. However, the typical subjective 0-3 scoring of the clasping response in rodents presents a challenge for consistent and efficient analysis, as such manual scoring is prone to both human error and biases. Additionally, by reducing the variability observed across a trial to one value on a 4-point scale, one risks losing both inter-trial variability and subtle differences between animals due to its limited scale. To address this, we developed a python-based post-processing tool that automates the analysis of hind-limb clasping data by calculating frame-by-frame angle estimations after utilizing machine learning through DeepLabCut (DLC) on videos. Once the user has utilized DLC to obtain the pose estimation files, the tool restructures the files, applies quality control filters, calculates angles for each video frame, and excludes frames where the animal's movement may distort the analysis. Using DLC, we tracked the left hindlimb, right hindlimb and anus with a respective average accuracy of 80%, 85% and 93%. The model was trained on 300 frames with 400,000 iterations and had a mean pixel error of 2.11 for training and 7.17 for test. Results from our post-processing tool indicate that it streamlines the analysis, although the 2D limitation necessitates careful interpretation of the data. The development of this tool fills a practical gap in the field by providing an automated solution for hind-limb clasping analysis, thereby increasing efficiency, consistency, and the level of overall variability. Future work will aim to enhance the tool's capabilities by incorporating inputs from multiple cameras to mitigate the current 2D limitation. This advancement will further improve the accuracy of the solution, reinforcing its utility in neurodegenerative disease research.

Disclosures: S. Jacobsen: A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **C. Pedersen:** A. Employment/Salary (full or part-time):; H Lundbeck. **K.W. Fejgin:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **U. Richter:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **F. Gastambide:** A. Employment/Salary (full or part-

time):; H. Lundbeck A/S. **T. Janjua:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

Location: MCP Hall A

Time: Tuesday, October 8, 2024, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.19/AA19

Topic: I.07. Data Analysis and Statistics

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R01 DC 014701

Title: Advanced computer vision models enable Annolid to automatically segment and track multiple animals from single labeled video frames

Authors: *C. YANG, T. A. CLELAND;
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Abstract: Accurate analysis of animal behavior relies on innovative methodologies to overcome challenges in segmenting and tracking multiple individual animals, particularly in visually complex environments. To address this, we present a novel approach that integrates three cutting-edge computer vision models into Annolid, a deep learning-based software package designed for the segmentation, labeling, and tracking of research targets within video files, with a primary focus on animal behavior analysis. Our strategy combines the following elements: First, we utilize Grounding DINO for text-based object detection, enabling users to specify keywords or phrases to automatically detect animals or other objects of interest in a video frame. The candidate bounding boxes then are input into High Quality Segment Anything (HQ-SAM), based on Meta AI's Segment Anything Model. HQ-SAM generates masks based on the bounding box prompts of visually discrete objects via zero-shot generalization. Finally, we employ Cutie, a state-of-the-art video object segmentation model, to predict and segment multiple instances across video frames. This process can be based either on the initial frame labels generated with Grounding DINO and HQ-SAM or on a frame labeled manually with polygons in the Annolid GUI.
The addition of these new tools to Annolid enables end users to easily specify and track animals and objects of interest, significantly reducing manual annotation efforts while achieving accurate results in multiple animal tracking experiments. We demonstrate the efficacy of our approach on selected idTracker.ai datasets with videos containing multiple mice, zebrafish, or other animals, including a video of 80 interacting fruit flies, as well as on diverse naturalistic videos comprising our own Multiple Animal Tracking & Behavior (MATB) dataset. These examples showcase Annolid’s robustness in tracking individual animals amidst complex interactions and temporary

occlusions in rich environments. Annolid is open-source research software, freely available at: https://cplab.science/annolid.

Disclosures: C. Yang: None. **T.A. Cleland:** None.

Poster

PSTR316: Software Tools: Neurophysiology, Behavior, and Tools for Data Integration and Curation

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Title: Markerless dexterous hand function analysis system: evaluating hand functions in spinal cord injury

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Abstract: Spinal cord injuries, particularly at the cervical level, substantially impair daily functions, emphasizing the need for advanced rehabilitation methods and precise evaluations. While motion capture has been utilized to assess locomotor functions, accurately evaluating hand dexterity remains a complex challenge in experimental animals. Our study introduces a markerless hand function analysis system that incorporates two high-speed cameras and innovative Deeplabcut 2.3.9 to accurately evaluate critical aspects such as finger separation, speed, and dexterity. This detailed kinematic analysis enables us to reveal significant disparities in motor control between individuals with spinal cord injuries and healthy controls. We validate the system's capacity to track changes in hand function during a pellet retrieval task in both intact and injured mice models. Through quantifying dynamic hand movements, we believe that the system and methods developed in this study can assess therapeutic effectiveness and refine rehabilitation strategies in terms of hand function following spinal cord injuries.

Disclosures: A. Shon: None. **J. Vernam:** None. **X. Du:** None. **W. Wu:** None.

Poster

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Topic: I.07. Data Analysis and Statistics

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Title: Benchmarks and Calibrated Neural Networks for Localizing Vocalizations in Social Interactions

Authors: *A. TANELUS[1], R. E. PETERSON[2], V. IVAN[3], D. M. SCHNEIDER[2], D. H. SANES[2], A. H. WILLIAMS[4];
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Abstract: Social animals congregate in groups and communicate with vocalizations. To study the dynamics of natural vocal communication and their neural basis, one must reliably determine the sender and receiver of the vocal signal. Existing approaches to address this problem rely on estimating source positions using time delays between microphones in an array (e.g. beamforming), or by surgically affixing miniature microphones to the animal. Although effective in some contexts, these approaches are not robust to reverberant environments (beamforming) or not scalable to large social groups (mini microphones). Thus, there is considerable interest in developing non-invasive sound source localization and vocal call attribution methods that work off-the-shelf in typical laboratory settings. To this end, we developed (1) a supervised deep learning framework with calibrated uncertainty estimates that achieves state-of-the-art sound source localization performance in reverberant environments, (2) novel hardware solutions to generate benchmark datasets for training/evaluating sound source localization models across labs, and (3) curated and released the first large-scale benchmark datasets for vocal call localization in social rodents. In addition, we detail a procedure to generate synthetic training data with acoustic simulations for pre-training sound source localization models.

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Poster

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Topic: I.07. Data Analysis and Statistics

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Title: Development of a rodent virtual reality system for measuring reward

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Abstract: The goal of the current project was to develop innovative tools for investigating effects of drugs of abuse on motivation related circuitry (via optical functional imaging) while measuring behavioral indices of reward, reinforcement and aversion in a highly versatile virtual environment (VR). Thus, hardware and software VR tools were developed for administering and analyzing behavioral tasks, and imaging tools made for performing multiphoton microscopy experiments in behaving mice. The VR software includes tools for creating simple linear mazes, more complex mazes for measuring learned regional associations, regional assignments for operant conditioning, whisker-based haptic feedback for simulating physical structures (walls, virtual objects, etc), external device control for triggering stimuli (e.g. TTL pumps, stimulators, etc) and measuring activity (e.g. optical imaging tools), audio control for pairing tones with VR regions, analyses tools for measuring global and regional defined locomotor activity and preference performed with single files or in a batch file analysis. The imaging tools include a novel form of multiphoton microscopy that uses interferometric optical backscatter (double barreled oblique back scatter microscopy) for locating endoscopic lenses, measuring cell/tissue morphology and movement (e.g. microglia motility). Hardware tools include a 360 degree mouse treadmill, a headfixed apparatus with optical shielding for multiphoton recordings with the long working distance cousa objective on a customize multiphoton microscope. We demonstrate use of the developed tools for measuring psychostimulant effects in behavioral tasks while performing functional imaging.

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Poster

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Title: High-resolution volumetric 3d pose tracking: a novel approach for 3d selectivity of neural coding of egocentric boundary cells

Authors: *M. PATEL[1], S. MALMBERG[2], Y. GU[1], B. PLUMMER[1], M. BETKE[1], M. E. HASSELMO[2];
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Abstract: Pose estimation has become a well-established research problem in cross-disciplinary computer vision. 2D and 3D pose estimation techniques are integral in a range of neuroscience research domains, such as neuro-inspired robotics, behavioral or clinical neuroscience, human-computer interaction, and neural circuits. Although the accuracy of recent works on benchmark datasets has improved significantly, this improvement rarely translates to real-world applications where quality 3D ground-truth data are scarce. To address this issue, the recent research trend in pose estimation has shifted toward developing markerless self-supervised or weakly-supervised methods that do not rely on 3D ground truth data. We use a novel end-to-end high-resolution volumetric architecture that utilizes a modified multi-view constraint to provide weak supervision for 3D pose reconstruction. Pairing this with calcium imaging of retrosplenial neurons as mouse forages in an open field environment, we present our analysis of 3D tracking of behaviors and egocentric boundary cells (EBCs). Using 3D tracking, we detect the selectivity of neural coding in EBCs for different levels of head elevation. By tracing the 3D head direction vector to barriers, we examine EBCs with varying amounts of selectivity for the top versus the bottom of the barrier as the mouse interacts with the barrier. Furthermore, we present our analysis for correlations between several quantified behaviors and the firing of cells.

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Poster

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European Research Council
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DFG RTG 2413 SynAGE

Title: Advancements in EthoML/VAME, an unsupervised machine learning pipeline for analyzing behavior in spontaneously behaving mice

Authors: S. R. MILLER[1], ***P. NAMBIAR**[1], S. BANGERA[1], K. LY[1], E. BRADY[1], J. SHIN[1], R. THOMAS[2], S. REMY[3], E. D. ROBERSON[4], P. BAUER[5], J. J. PALOP[1];
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Abstract: Recent advancements in machine learning (ML) and computer vision have led to the emergence of several unsupervised algorithms for automated analysis of spontaneous animal behavior (VAME, MoSeq, etc.). These algorithms can leverage a variety of supervised pose-estimation tools (e.g. DeepLabCut, SLEAP, LightningPose) to identify behavioral patterns across time. These methods can empower experimenters by analyzing full frame-by-frame sequences of spontaneous behavior and assessing sex, genotype, and treatments interactions. We have developed EthoML/VAME (www.github.io/EthoML/VAME), which has been validated in transgenic and knock-in mouse models of Alzheimer's disease (Miller, 2024). Here, we provide further validation of the approach by comparing variations on the EthoML/VAME methods, specifically exploring the effectiveness of different egocentric alignment strategies, hierarchical clustering cost functions, and correlation clustering methods. We also describe new summary measures of ML behavioral features capturing intersubject variability in motif usage, motif speed, and motif-motif transitions. To evaluate the performance of our method, EthoML/VAME outcomes are benchmarked against standard measures using a logistic regression classifier. We conclude that s EthoML/VAME outcomes of spontaneous behavior effectively captures age-, sex-, and disease-dependent manifestations with highest sensitivity and specificity than standard behavioral approaches.

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Poster

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Title: A Standardized 3D Ethological Observatory for high throughput Behavioral Analysis

Authors: *J. M. ROACH[1], S. S. LIM[2], J. WU[1], A. SABATH[2], J. R. RAVENEL[3], A. CHOUDHURY[2], S. SINGH[2], B. C. SHIELDS[1], T. DUNN[1], M. R. TADROSS[1];
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Abstract: Unique imaging conditions necessitate specific ground truth datasets, hindering data comparability across labs. Efforts for data transparency, such as the NIH Data Commons, aim to address these challenges but require careful oversight. The Standardized 3D Ethological Open-field-arena (ST3DEO) is an effort to standardize the acquisition of 3D kinematic behavioral data, enabling high-throughput behavioral analysis with enhanced accuracy and reproducibility. Through precise optimization of lighting conditions and mirror utilization, the ST3DEO facilitates standardized behavioral context and imaging quality allowing for high throughput behavioral studies to be run at scale. By standardizing the arena design, orchestration of data analysis and automation of pose estimation using the DANNCE AI system were facilitated, minimizing dependencies on imaging backgrounds. Key adaptations were made to the DANNCE Center of Mass network (COM), including leveraging mirrors and exploiting symmetrical imaging environments to enhance network convergence and augment training datasets effectively. Fine-tuning of network weights reduced errors, quantified through metrics such as mean per joint position error (MPJPE), percentage of correct keypoints (PCK), and standard deviation of bone lengths. An ensemble model, averaging predictions from multiple models, showcased potential to outperform human annotators in prediction variance. Furthermore, models finetuned on one arena demonstrated maintained performance across others, ensuring stability and reproducibility of results. The development of a user-friendly graphical interface (GUI) simplified recording initiation, ensuring proper data formatting without requiring programming experience. Data organization and standardization, facilitated through structured schemas ensured transparency for researchers and met standards for orchestration engineers. Efficient data pipeline management, including advanced video compression techniques, facilitated real-time processing without sacrificing accuracy. High-frequency camera acquisition, coupled with calibration using a checkerboard pattern, ensured accurate data capture and calibration application. Overall, this project represents a meticulous integration of advanced AI techniques with careful hardware and software considerations, culminating in a robust markerless pose estimation system for murine subjects.

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Poster

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Title: Deciphering spontaneous behavioral alterations and treatment effectiveness in Alzheimer's disease mouse models with machine learning

Authors: *S. R. MILLER[1], K. LAUDERDALE[2], P. NAMBIAR[3], P. HONMA[4], K. LY[5], S. BANGERA[6], J. SHIN[7], C. CAI[8], K. SHEN[1], Z. YAN[6], A. MENDIOLA[6], T. SAITO[9], T. SAIDO[10], R. THOMAS[6], E. D. ROBERSON[11], K. AKASSOGLOU[12], S. REMY[13], J. J. PALOP[14];
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Abstract: Computer vision and machine learning (ML) approaches are being developed to provide scalable, unbiased and sensitive methods for assessing mouse behavior. Here, we used DeepLabCut and the ML-based VAME segmentation platform to assess spontaneous behavior in knock-in and transgenic models of Alzheimer's disease (AD) and to test the role of AD-related neuroinflammation in these behavioral manifestations. We found that the organization of

behavioral sequences is markedly altered in *App*$^{NL\text{-}G\text{-}F}$ and 5xFAD mice, including age-dependent changes in motif utilization and increases in transitions and randomness. Notably, blocking fibrinogen-microglia interactions in 5xFAD-*Fgg*$^{\gamma 390\text{-}396A}$ mice largely prevented disorganized behavioral sequences, indicating that AD-related spontaneous alterations are amenable to therapeutic interventions and driven by neuroinflammation. Classifier analyses revealed that VAME outcomes had higher specificity and sensitivity than standard behavioral outcomes. We conclude that spontaneous behavior effectively captures age- and sex-dependent disease manifestations and treatment efficacy in AD models.

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