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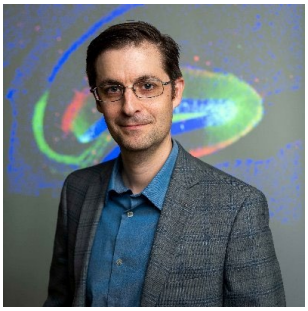
THURSDAY, JUNE 6th 2024

KEYNOTE SPEAKER



Daniel Dombeck, Ph.D.

"Microscopy, Methods, and Memories."

Dr. Dombeck is a Professor at Northwestern University. His laboratory studies the mechanisms underlying memory formation, storage, and recall. Dr. Dombeck has developed virtual reality systems and high-resolution imaging techniques to image the brains of mice while learning virtual mazes. Dr. Dombeck has a Ph.D. in Physics from Cornell University and numerous high-impact publications.

Website: http://www.dombecklab.org/

INVITED SPEAKERS

Andrew Saykin, Ph.D.

Director of IU Center for Neuroimaging, Director of Indiana Alzheimer's Disease Research Center. Professor of Radiology & Imaging Sciences

"New frontiers in Alzheimer's research and therapy: Harnessing data science and systems biology on the way to precision medicine."

Website: https://medicine.iu.edu/faculty/6962/saykin-andrew

Krishna Jayant, Ph.D.

Assistant Professor, Weldon School of Biomedical Engineering at Purdue University

"Spacetime Codes: Traveling Waves in sensory processing and motor control."

Website: https://nanoneurotech.com/research/

BRUKER INVITED TALKS

Savannah Snyder, Ph.D.

Field Sales Representative, Bruker Corp.

Website: www.bruker.com

Daniel Wahl, MD, Ph.D.

Associate Professor of Radiation Oncology and Associate Professor of Neurosurgery

“Rewiring of cortical glucose metabolism fuels human brain cancer growth.”

Website: https://medicine.umich.edu/dept/cancer-biology/daniel-wahl-md-phd

DATA BLITZ

Brandon Coventry, Ph.D., Postdoc, University of Wisconsin-Madison
Laboratory of Kip Ludwig, Ph.D.
“A closed-loop stimulation and recording implantable pulse generator platform for chronic preclinical neuromodulation studies.”

Yanru Ji, Graduate Student, Purdue University
Laboratory of Ranjie Xu, Ph.D.
“Developing a Vascularized Neuroimmune Organoid Model for Studying Sporadic Alzheimer’s Disease and Drug Screening.”

Sára Nemes, Graduate Student, Indiana University School of Medicine
Laboratory of Liana Apostolova Ph.D.
“Sex-associated differences in plasma and cerebrospinal fluid biomarkers in Early-onset Alzheimer’s disease.”

Apostolia Topaloudi, Ph.D., Postdoc, Purdue University
Laboratory of Peristera Paschou, Ph.D.
“Mendelian randomization analysis of the largest Tourette Syndrome GWAS to date identifies causal association with novel genes.”

POSTER SESSION DAY 1

Poster 1: Vasculogenic Direct Cell Reprogramming Therapies Induce Memory Improvement and Reduce Disease Burden in a Mouse Model of Alzheimer's Disease

Authors: Alzate-Correa D, Stranan J, Rincon-Benavides MA, Lawrence WR, Ripsky S, Hagan M, Nguyen T, Higuita-Castro N, Gallego-Perez D.

The Ohio State University

Abstract: Alzheimer's Disease (AD) and is characterized by cognitive decline, loss of synaptic connections and neurons, and the formation of two main neuropathological lesions, amyloid plaques and neurofibrillary tangles. Recent studies indicate that AD patients also present reduction in cerebral blood flow (CBF) and Blood-Brain-Barrier (BBB) dysfunction, suggesting that cerebrovascular impairment plays a key role in the onset of AD. Consequently, therapeutic interventions aiming to reestablish the cerebrovascular function may attenuate AD development. To implement cell therapies for AD, we used electroporation to deliver 3 pro-vasculogenic reprogramming factors Etv2, Foxc2, Fli1(EFF) to directly reprogram mouse primary embryonic fibroblasts (pMEFs) into induced endothelial cells (iECs) and use these cells as therapeutic agents in the transgenic model of AD (3xTg-AD). To evaluate their therapeutic potential, iECs were delivered with 3 intracranial injections into the lateral ventricles (LV) of 3xTg-AD or Wild-Type Control females. Our results indicate that transfection of pMEFs with the EFF factors leads to an angiogenic transcriptional program. Furthermore, iECs induce an increase in global CBF and significantly reduce the spatial memory deficits in the Barnes Maze. In addition, injected cells labeled were able to survive for at least 4 weeks, migrate to the brain vasculature, and induce an increase in the total vascular area in the 3xTg-AD mice. Injection of EFF transfected also seems to correlate with a reduction in cortical amyloid-beta load. Finally, transcriptome analysis led to the identification of potential gene expression programs that may explain the effects of EFF-transfected cells in the AD brain.

Poster 2: Study of Brain Metal Distributions and Interactions in Relation to Alzheimer's Pathogenesis using Synchrotron X-ray Techniques

Authors: Pavani Devabathini, Alexis Webb, Wei Zheng, Yansheng Du, and Linda H. Nie

Purdue University

Abstract: Metals such as mercury, lead, and iron have been implicated as potential environmental factors that might contribute to the onset or progression of Alzheimer's disease (AD). However, comprehensive understanding of the complex metal-induced neurodegenerative mechanisms remains elusive. To study these mechanisms, it is necessary to determine spatial metal distribution in the brain, explore their molecular interactions, and establish their correlation with neuropathology. We employed synchrotron micro-X-ray fluorescence (S-μXRF) imaging to investigate the microscopic distribution of these metals in brain. Our results showed the existence of mercury and lead aggregates in addition to other metals. Selenium appeared to colocalize with mercury to a greater extent and to a relatively lesser extent with lead. In contrast to the smaller Hg/Pb aggregates observed in healthy brain, the significantly larger Hg/Pb aggregates from AD brain appear

to be colocalized with other metals. The presence of detectable amounts of mercury and lead in the brains of both AD patients and healthy individuals underscores the ubiquitous presence of these metals in our environment. Although the data indicate that selenium plays a role in the sequestration of mercury and lead in brain tissue, whether the interaction is beneficial or detrimental warrant further investigation. In addition, further exploration is needed to understand the distinct distribution and aggregation patterns of Hg/Pb and other metals in AD and healthy brains. Overall, this line of research has the potential to unveil new insights into the roles metals play in neurodegeneration, leveraging innovative synchrotron x-ray techniques.

Poster 3: Systems approaches to identify neuron-specific metabolic signatures in Alzheimer's Disease

Authors: Boyu Jiang, Roshni Manikandan, Anke Tukker, Hyunjin Kim, Aaron Bowman, Priyanka Baloni

Purdue University

Abstract: Neurodegenerative diseases like Alzheimer's Disease (AD) have increasingly been implicated as metabolic disorders. Recent studies have indicated a change in the metabolic trajectory of central and peripheral systems leading to the pathophysiological condition. In order to predict the complex metabolic changes at cell-type level in the brain, we employed systems biology approaches such as the genome-scale metabolic models (GEMs). Our present study focuses on generating cell-type-specific in silico metabolic model of human neurons. We analyzed single nucleus RNA-sequencing data of neurons from two different AD studies: the Religious Orders Study and Memory and Aging Project (ROSMAP) and Seattle Alzheimer's Disease Brain Cell Atlas (SEA-AD) that had AD and cognitively normal samples included. Using the generic human metabolic reconstruction, Recon3D, as template and applying CORDA method along with the snRNA-seq data, we generated the neuron-specific metabolic model. We also made our model cell-type-specific by adding a new compartment for 'synapse' to simulate metabolic activities in the synaptic space between neurons. To assess both comprehensiveness and specificity of our model, we tested it against 200 known metabolic tasks important for mammalian cells and neurons. Our current iNeuron metabolic reconstruction of the human neuron contains 2597 metabolites, 4178 metabolic reactions, and 1668 genes. Our model can achieve essential metabolic functions of neurons, including the synthesis of several important neurotransmitters and the utilization of glucose, lactate, and ketone bodies for ATP generation. Our objective is to integrate multi-omics data with the iNeuron metabolic reconstruction and identify cell-type-specific metabolic signatures associated with the Alzheimer's disease.

Poster 4: Identification of R-loop modulators in Alzheimer's disease neuronal cells through a proximity-based labeling system.

Authors: Geethma Lirushie, Bryce Colón, Jean-Christophe Rochet and Hana Hall

Purdue University

Abstract: R-loops are co-transcriptional, three-stranded nucleic acid structures consisting of DNA: RNA hybrids and a displaced single-stranded DNA. We have previously shown that persistent R-

loops accumulate in the genomes of aging neurons in the retina of the fruit fly, Drosophila melanogaster. Notably, our recent preliminary data show an accumulation of R-loops in a Drosophila Alzheimer's disease (AD) model and in iPSC-derived neurons from an AD patient. Persistent R-loops can cause genomic instability and lead to the accumulation of DNA: RNA hybrids in the cytoplasm, triggering immune responses. Cells possess mechanisms to regulate R-loop homeostasis, and thus, to identify and characterize these R-loop binding proteins, we will use a proximity-based labeling system with a highly efficient mutant form of biotin ligase, termed "TurboID", tethered to a catalytically inactive form of ribonuclease H1 (RH1) enzyme. The catalytically inactive RH1, which binds DNA: RNA hybrids without resolving R-loops, will direct TurboID ligase to biotinylate adjacent proteins, effectively tagging the R-loop-associated proteins. These proteins will then be purified using streptavidin affinity and identified using mass spectrometry. We will identify AD-specific R-loop modulators that facilitate R-loop-dependent DNA damage using rat cortical neuronal cell cultures seeded with human tau protein as a model system. This study aims to enhance our understanding of the link between R-loops and DNA damage in AD.

Poster 5: A human induced pluripotent stem cell model of Alzheimer's Disease-associated fractalkine receptor polymorphism to assess AD-related microglial dysfunction

Authors: Tutrow KT, Harkin J, Gomes C, Hernandez M, Huang KC, Bissel S, Puntambekar S, Lamb BT, Meyer JS.

Indiana University School of Medicine

Abstract: Dysfunctional microglial activity has been identified as a potential mechanism leading to accumulation of amyloid beta and subsequent neurodegeneration in Alzheimer's Disease. The CX3CR1/fractalkine axis serves as a mechanism for bi-directional communication between microglia and neurons, respectively, to promote a resting, anti-inflammatory state in microglia. Previous studies have demonstrated that deficiency in CX3CR1 signaling leads microglia to a more pro-inflammatory phenotype, phagocytosis deficits, and microglia-mediated susceptibility of neurons to cell death. The CX3CR1-V249I polymorphism was recently identified as a potential risk allele for Alzheimer's Disease with worsened Braak staging in post-mortem Alzheimer's patients. However, the role of fractalkine dysfunction in human cells and the mechanisms by which microglia with the CX3CR1-V249I SNP contribute to neurodegeneration remain unclear. To address this shortcoming, we utilized human induced pluripotent stem cells and CRISPR/Cas9 technology to elucidate the effects of the V249I polymorphism on microglia-like cells compared to an isogenic control cell line. We demonstrate effective differentiation from isogenic control and CX3CR1-V249I backgrounds into human microglia-like cells, which express characteristic microglial markers and are functionally phagocytic. Homozygous V249I microglia demonstrated decreased phagocytosis of amyloid beta in vitro compared to isogenic controls. Additionally, homozygous V249I microglia demonstrate increased stress-induced cell death and altered proliferation. These findings suggest that the CX3CR1-V249I polymorphism may cause a dysfunctional microglia phenotype that subsequently contributes to neuronal dysfunction and death. Ongoing work will investigate transcriptomic differences found in CX3CR1-V24I microglia and utilize 2D and 3D co-culture model systems to elucidate how the CX3CR1-V249I polymorphism contributes to Alzheimer's Disease-related neurodegeneration.

Poster 6: Impact Induces Phagocytic Defect in Reactive Microglia

Authors: Ruilin Yu, Edmond A. Rogers, Palak Manchanda, Connor H. Beveridge, Caitlin E. Randolph, T. Brock Beauclair, Krupal P. Jethava, Riyi Shi, Gaurav Chopra

Purdue University

Abstract: Traumatic brain injury (TBI) significantly increases the lifetime risk of neurodegenerative diseases, such as Alzheimer's Disease (AD). TBI patients' brains have shown increased load of amyloid β (Aβ) plaques, a hallmark for AD. Our hypothesis is that microglia lose their ability to engulf Aβ leading to TBI related AD. While the association of TBI and AD is increasingly strong in both clinical and preclinical investigations, there is a lack TBI models to relate cellular response as a platform for drug screening. We have developed an in vitro impact model for primary cells using to represent impact injury on a chip (Sci. Rep. 12, 11838, 2022). This "TBI-on-a-chip" model was used to characterize the molecular and cellular changes following impact. We discovered that impact reduced the phagocytosis of Aβ in primary murine microglia, along with increased inflammatory markers at 7-days post impact (DPI), which was surprising because inflammation is usually correlated with increased microglial phagocytosis. The "TBI-on-a-chip" model can also do neuronal recording and we found decreased neuronal firing frequency at 7 DPI. Given that lipid metabolism is increasingly implicated in brain trauma and neurodegeneration, we used mass spectrometry to evaluate the changes in lipids secreted by microglia and histiotypical networks at 7 DPI. We found many sphingomyelins, glycerophospholipids, and phosphatidylserines were significantly affected by impact, which are known to play important roles in the resolution of neuroinflammation and the pathogenesis of neurodegeneration. We believe the "TBI-on-a-chip" platform will be a useful tool for developing immunomodulatory or neuroprotective drug candidates.

Poster 7: Transcriptomic Analysis of Neuronal Response to Sub-acute and Chronic Manganese Exposure

Authors: Xueqi Tang, Priyanka Baloni, Anke M Tukker, Hyunjin Kim, Michael Aschner, and Aaron B. Bowman

Purdue University

Abstract: Manganese (Mn)-induced neurotoxicity is broadly acknowledged, yet not fully understood. One of the key obstacles is the gap between human neurotoxicity development via chronic exposure to low levels of insults, and laboratory accessible acute high concentration settings. Aiming to overcome this gap, we evaluated transcriptomic changes under sub-acute and chronic conditions, and compared canonical pathways affected. 0, 0.05, 0.5, 5, and 50 μM Mn were exposed to differentiated SH-SY5Y neuroblastoma cells for 40 days and to mature human induced pluripotent stem cell (hiPSC)-derived cortical neurons for 9 days. Total RNA was extracted by the end of the exposure to prepare libraries for bulk RNA sequencing analysis. The results displayed a Mn concentration-dependent increase in the number of differentially expressed genes (DEGs) in SH-SY5Y. Pathway analysis indicated that insulin/PI3K/AKT signaling were significantly affected in all exposed groups. This extends our previous findings that insulin and downstream pathways are highly sensitive to sub -cytotoxic Mn. Insulin related signaling alterations in response to Mn exposure were validated in hiPSC-derived neurons. Comparisons between the two models revealed more pathways

significantly affected in hiPSC-derived neurons than SH-SY5Y although the latter was exposed for longer duration. Furthermore, hiPSC-derived neurons displayed higher complexity as more genes were affected in each pathway. Identifying comparable pathways across different exposure durations suggests that alterations in these pathways may drive the transition from acute response to neurodegeneration in chronic Mn exposures. This further emphasized the significance of investigating these signaling with near-threshold Mn overload.

Poster 8: Investigating PFOS Induced Neurobehavioral Hyperactivity and Motor Behavior

Authors: Hurshal Pol, Josephine M. Brown-Leung, Fatema Currim, and Jason R. Cannon

Purdue University

Abstract: PFAS are a group of persistent organic pollutants that accumulate in the human brain. Perfluorooctanesulfonic acid (PFOS), the most prevalent PFAS pollutant, targets dopaminergic pathways which may lead to motor and neuropsychiatric disorders, including Parkinson's Disease, attention deficit/hyperactivity disorder (ADHD). We hypothesized that chronic exposure to PFOS causes motor deficits in mice. Mice were treated with drinking water containing 1.0 mg/kg/d PFOS or 0.5% tween-20 vehicle. They performed neuro behavioral tests after 16 months of dosing, cognitive and motor function were assessed. Locomotor activity measured exploratory activity levels in a box over one hour. Male PFOS-treated mice showed significantly elevated activity levels compared to controls. There was no effect on female mice. Nigrostriatal motor function was assessed via challenging beam traversal in which mice traversed a wire-mesh beam. The results demonstrated male PFOS-treated mice took significantly less time to traverse the beam whereas female PFOS-treated mice took significantly more time to traverse the beam. Male mice had significantly increased errors/step and total errors compared to female mice, with no effects of PFOS treatment. This study demonstrates that chronic exposure to PFOS causes sex-specific hyperactivity in male mice and potential decreased motor function in PFOS-treated female mice. Absent PFOS-related changes in errors/step indicate changes in motor function are likely independent of the nigrostriatal pathway affected in Parkinson's disease. Changes in activity level may suggest PFAS affect dopaminergic pathways (e.g. mesolimbic pathway) involved in neuropsychiatric disorders such as ADHD. Further experiments will be conducted to assess PFOS's feminizing activity towards male mouse models

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Poster 9: Chronic perfluorooctanesulfonic acid (PFOS) exposure alters motor neurobehavior in male mice thereby eliminating behavioral sex effects

Authors: Josephine M. Brown-Leung, Hurshal Pol, Fatema Currim, Tauqeer Syeda, and Jason R. Cannon

Abstract: Per- and polyfluoroalkyl substances (PFAS) are a group of "forever chemicals" that pollute the environment and are found in all humans. Our previous studies determined out of multiple PFAS, only perfluorooctanesulfonic acid (PFOS) was selectively toxic to dopaminergic neurons in C. elegans. A pilot study demonstrated PFOS-treated mice had decreased serotonin and metabolites of dopamine and serotonin in the ventral striatum and ventral midbrain. Since changes in neurotransmitters are implicated in altered brain function, we hypothesized chronic PFOS exposure alters neurotransmitter metabolism, resulting in altered neurobehavioral function. We dosed male

and female mice with 1.0 mg/kg/d PFOS or 0.5% tween-20 (vehicle) in drinking water for 16 months (n=9-18/treatment-sex). Neurobehavioral experiments included open-field locomotor activity, challenging beam traversal (CBT), and gait. Brains were dissected for neurochemical analysis. For locomotor, PFOS-treated males demonstrated increased rearing and total activity (p-values≤0.017), and decreased habituation and grooming (p-values≤0.017) compared to vehicle. CBT results suggest that PFOS-treated males had decreased errors/step, total errors, and latency (p-values≤0.008) compared to vehicle. PFOS-treated female mice demonstrated decreased steps (p-value=0.013). Interestingly, for total activity, grooming, errors/step, total errors, and latency to traverse the beam; PFOS-treated male behavior became equivalent of female-vehicle-treated mice. These results demonstrate that chronic PFOS causes hyperactivity and decreased non-associative memory in male, but not female mice. Importantly, this suggests exposure to PFOS may alter neurobehavior in male mice that results in elimination of sex effects. Future analysis will assess additional neurobehavioral endpoints and analyze neurochemistry (including dopamine and serotonin), especially in the mesolimbic pathway.

Poster 10: Exosomal miRNAs in biofluids of the rotenone-induced rat model of Parkinson's Disease induce dopaminergic neuron cell death in primary midbrain neuronsExosomal miRNAs in biofluids of the rotenone-induced rat model of Parkinson's Disease induce dopaminergic neuron cell death in primary midbrain neurons

Authors: F. Currim, J.M. Brown-Leung, T. Syeda, JC. Rochet, R. Singh, J.R. Cannon

Purdue University

Abstract: Parkinson's Disease (PD) is a progressive and chronic neurodegenerative condition with the hallmark pathology being degeneration of nigrostriatal dopamine neurons. PD development is typically through a combination of genetic and environmental factors that converge on common pathophysiological processes. Rotenone, a naturally occurring substance widely employed as an insecticide and pesticide, is a potent inhibitor of mitochondrial complex I, a pivotal pathway associated with PD pathogenesis. The concept of PD pathology spread underscores the critical role of intercellular communication, which is facilitated through a specific group of extracellular vesicles known as exosomes. Various studies have highlighted that the cargo, particularly exosomal miRNAs, carried by exosomes has a discernible impact on the physiological responses in recipient cells, thus influencing the progression of PD pathology. Due to their presence in different biofluids, such as serum and cerebrospinal fluid (CSF), exosomal miRNAs not only hold promise as potential biomarkers for early PD diagnosis but also offer insights into the molecular mechanisms involved in PD pathogenesis. Our preliminary findings suggest patterns of miRNA alterations observed, a notable finding is the specific modification of miR-181c-5p, in CSF and in serum of rats acutely exposed to rotenone. To address these objectives, our study tested the hypothesis that exosomal miRNAs present in the CSF and serum of rats are responsible for the functional alterations in recipient cells. Exosomes were extracted from serum of rotenone rat models of PD and were quantified and its impact on primary neurons was investigated. Mitochondrial function and cell viability was analysed by various techniques. Further, specific cell loss of dopaminergic neurons was investigated. Primary midbrain neurons treated with serum exosomes from rotenone exposed rats exhibited an increase in the total cellular as well as mitochondrial ROS levels relative to control conditions. Additionally, a significant reduction in cell viability was observed in primary midbrain

neurons, particularly affecting dopaminergic neurons when treated with serum exosomes from rats administered with rotenone versus vehicle treated rats. Further, primary midbrain neurons treated with rotenone exhibited increased cell loss when exposed to serum exosomes from rotenone treated rats. The study offers promise not only for the early detection of PD through exosomal miRNA biomarkers but also for the modulation of cellular functions and viability, which may be valuable in predicting disease prognosis and assessing appropriate treatment responses

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Poster 11: THE IMPACT OF CHRONIC MANGANESE ON GLUTAMATE EXCITOTOXICITY IN HUMAN IPSC-DERIVED CORTICAL MODEL OF ALZHEIMER'S DISEASE

Authors: Hyunjin Kim, Anke M. Tukker, David Yi, Fiona E. Harrison, Aaron B. Bowman

Purdue University

Abstract: Manganese (Mn) is an essential metal widespread in the environment, but in excess can cause neurotoxicity. Alzheimer's disease (AD) is a chronic multifactorial neurodegenerative disorder. Most AD cases cannot solely be attributed to familial inheritance and involve contributions from environmental risk factors. Perturbed glutamate neurobiology is an important overlapping pathology shared by Mn neurotoxicity and AD. Neuronal hyperexcitability has been reported in AD patients. Studies have shown that acute exposure to high Mn levels inhibits synaptic glutamate uptake. However, the effects of chronic exposure to pathophysiologically relevant Mn levels and its implication in AD etiology remains unknown. Hence, we hypothesized that chronic Mn exposure increases susceptibility to glutamate excitotoxicity depending on AD genetic risk. We utilize cortical neurons and astrocytes generated from induced pluripotent stem cells derived from neurotypical and AD patients. Cells were cultured for ~100 days and subsequently exposed to Mn (vehicle, 0.5 or 5μM) for up to 40 days. Alterations in glutamate uptake were quantified using 14C-glutamate. We observed a significant 30~40% decrease in uptake in AD neurons/astrocytes, an effect not seen in neurotypical controls. Gene expression and immunocytochemical analyses indicated an absence of inflammatory mediators and astrocyte reactivity, suggesting impaired glutamate uptake is likely a direct effect of Mn rather than a secondary effect caused by neuroinflammation. scRNA-sequencing revealed alterations in biochemical pathways implicated in Mn toxicity, glutamate neurotransmission, and/or AD. In summary, we provide insight into discerning transcriptomic/functional alterations caused by chronic Mn and how an individual's genetic predisposition to AD may alter this pathophysiology.

Poster 12: Assessment of Pon1(-/-) and chlorpyrifos exposure as risk factors for Parkinson's disease in a rat model.

Authors: Reeya Tanwar, Fatema Currim, Josephine M. Brown-Leung, Sofia Schuman, Mathew Thomas Corson, Dia Jhaveri, Tauqeer Syeda, Shreesh Sammi, Jason R Cannon

Purdue University

Abstract: Background and Purpose: Chlorpyrifos, an organophosphate pesticide used globally in agriculture, has been linked to detrimental human health effects due to acetylcholinesterase inhibition, causing cholinergic symptoms. In August 2021, the US EPA announced plans to ban chlorpyrifos in food production due to health risks, but the ban's reversal has left populations vulnerable to exposure. Epidemiological and laboratory studies have correlated environmental

exposures to Parkinson's Disease (PD). Our lab's preliminary data showed a dose-dependent loss of functional mitochondria in C. elegans and inhibition of dopamine and acetylcholine function with a 2-day chlorpyrifos treatment. Recent studies indicate that carriers of the MM PON1-55 genotype exposed to organophosphates have over a 2-fold increased PD risk compared to those with wildtype or heterozygous genotypes. The interaction between genetic factors and environmental exposures in PD risk remains underexplored, necessitating a comprehensive understanding. Methods: To evaluate PON1 knockout (KO) impact on PD susceptibility, we conducted a pilot study on 9–11-month-old PON-1 KO Sprague Dawley rats, exposing them to chlorpyrifos in a dose-dependent manner to identify the optimal dose. A follow-up study used the highest dose from this initial study. Neurobehavioral assessments included Postural Instability (PI), Vibrissae Elicited Forelimb Placing (VEBT), and Cylinder tests. Brains were dissected for molecular assays and neurochemical analysis. Results and Conclusion: Our dose-dependent study showed a decline in rearing behavior of PON-1 KO rats of both sexes and increased PI distance, though no difference in VEBT was observed. In the follow-up study, the highest dose resulted in significant declines in rearing behavior, VEBT, and PI assays. These findings align with our preliminary C. elegans data, indicating chlorpyrifos exposure increases PD vulnerability. Future experiments will include molecular and neurochemical analyses and a comparative study with wildtype Sprague Dawley rats.

Poster 13: Investigating Metabolic Alterations in PFOS-Exposed Mice: Implications for Neurotoxicity and Potential Link to Neurodegenerative Diseases

Authors: Esraa Gabal, Claire Wolfer-Jenkins, Josephine Brown, Fatema Currim, Jason Cannon, Priyanka Baloni

Purdue University

Abstract: Perfluroroctane Sulfonate (PFOS) belongs to a class of chemicals known as Per- and polyfluoroalkyl substances (PFAS) which are highly abundant in the environment due to their prevalence in various industrial products. Studies have reported numerous lipid metabolites such as glycerophospholipids, fatty acids and carnitines associated with PFAS exposure. In this study we hypothesize that cholesterol and bile acid metabolism is affected by PFAS exposure and we will investigate changes at transcriptome and metabolite level. We will employ computational approaches to generate genome-scale metabolic networks of mice brain, liver and plasma to investigate tissue-specific metabolic signatures associated with the exposure to PFOS. We have collected transcriptomic data for Mus musculus from GEO public database, including datasets of bulk-RNA, total-RNA, and microarray and used it for metabolic network construction. Our analysis approach includes use of Shambhala-2 for cross-platform normalization of expression datasets and use of iMM1865 genome-scale model of mice to generate tissue-specific metabolic networks. Using experimental data from mice, we will have the ability to investigate gene and metabolite changes in plasma, brain, and liver in control and PFOS-exposed conditions. Transcriptomics and metabolomics data will be integrated with the metabolic networks to identify changes in levels of cholesterol, primary and secondary bile acids associated with PFOS-exposure and linking to neurotoxicity. We aim to analyze the genes and metabolites in cholesterol and bile acid pathways from our study, with the intention of translating our findings to human. We will use the data from AD Knowledge portal and correlate our findings in cognitively normal and Alzheimer's disease (AD)

patients' data. This translational aspect of our study will help in connecting PFOS exposure to neurotoxicity and identify provide potential links to neurodegeneration.

Poster 14: Effects of NGLY1 and NFE2L1 expression on the propagation of PD neuropathology

Authors: Bryce Colón, Aswathy Chandran, Jennifer Hensel, Selim Boudoukha, Yuhua Ji, Kevin Lee, Chris Rochet

Purdue University

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by a selective loss of midbrain dopaminergic (DA) neurons. There are currently no disease-modifying treatments for PD. Lewy bodies, a pathological hallmark of the disease, are intracellular inclusions composed of aggregated forms of the presynaptic protein alpha-synuclein (aSyn). Mechanisms for maintaining proteostasis and processing misfolded proteins are dysregulated in PD as a result of environmental insults or genetic perturbations. In turn, this dysregulation results in aSyn aggregation, leading to further proteasomal dysfunction and oxidative stress as part of a vicious cycle that results in the selective death of DA neurons. Consequently, strategies to enhance proteasomal activity and increase the antioxidant response have the potential to reduce aSyn aggregation and slow PD progression. NFE2L1 is a master transcription factor expressed in the endoplasmic reticulum and targeted for proteasomal clearance by the ER-associated protein degradation (ERAD) pathway under basal conditions. Proteasome dysfunction leads to an accumulation of NFE2L1, allowing for the protein's translocation (after several processing steps) to the nucleus, where it can induce the expression of genes involved in proteostasis and antioxidant signaling. One of these processing steps involves NFE2L1 de-glycosylation by N-glycanase 1 (NGLY1), resulting in an unconventional asparagine-to-aspartate post-translational modification. How NGLY1 regulates and alters NFE2L1 function has yet to be fully elucidated. Evidence of parkinsonian symptoms associated with NGLY1 deficiency and of NFE2L1 down-regulation in post mortem PD brains suggests that disruption of the NGLY1-NFE2L1 axis can cause oxidative stress and protein aggregation in the brain, in turn leading to neurodegeneration. We hypothesize that NGLY1-deficient neurons have a proteostasis defect that should be reflected by increased sensitivity to proteasome inhibitors. Consistent with this hypothesis, we found that NGLY1-deficient, iPSC-derived cortical neurons are unable to upregulate proteasome subunit gene expression when treated with the proteasome inhibitor epoxomicin. Current efforts are focused on examining the efficiency of aSyn seeded aggregation in iPSC-derived neurons from NGLY1-deficient patients. The results of these studies will yield insights into molecular mechanisms underlying neurotoxicity associated with defects along the NGLY1-NFE2L1 axis, setting the stage for developing disease-modifying therapies.

Poster 15: Effects of NFE2L1 expression on Nurr1 stability in a rat rAAV-alpha-synuclein model

Authors: Hensel JA, Gogusoglu SA, Chandran A, Cannon JR, Rochet JC.

Purdue University

Abstract: Two neuropathological hallmarks of Parkinson's disease (PD) and other synucleinopathy disorders are the loss of nigral dopaminergic (DA) neurons and the aggregation of the presynaptic

protein alpha-synuclein (aSyn). Evidence suggests that aSyn aggregation disrupts cellular proteostasis, including protein clearance by the ubiquitin proteasome system (UPS) or lysosomal autophagy. The transcription factor Nfe2L1 induces the expression of proteasome subunits and autophagic proteins and has been linked to PD, with published findings showing that Nfe2L1 is downregulated in DA neurons in post-mortem PD brains. We hypothesized that Nfe2L1 alleviates aSyn-mediated neurodegeneration by inducing the expression of neuroprotective proteins such as proteasome subunits. To address this hypothesis, we compared rats injected unilaterally in the substantia nigra with rAAV-aSyn ± rAAV-Nfe2L1 in terms of motor function, nigral DA cell viability, and striatal DA terminal density. Contrary to our hypothesis, rats injected with viruses encoding aSyn and Nfe2L1, but not aSyn virus alone, showed evidence of motor asymmetry, a unilateral loss of neurons expressing tyrosine hydroxylase (TH), and a decrease in striatal TH immunoreactivity. Because rats co-expressing aSyn and Nfe2L1 showed no change in the number of nigral neurons expressing the general neuron marker HuC, we inferred that the apparent loss of DA neuron phenotype reflected a decrease in TH expression rather than overt DA cell death. One potential explanation for this phenomenon is that the combined effect of aSyn and Nfe2L1 overexpression, known to cause an increase in GSK-3β activity and proteasome activity, respectively, could result in the destabilization of Nurr1, a transcription factor responsible for the expression of TH and other factors associated with the DA phenotype of nigral DA neurons. This idea is supported by evidence that GSK-3β phosphorylates Nurr1, marking it for UPS-mediated degradation. Current efforts are focused on validating this model in cultured neurons expressing a Nurr1-GFP biosensor ± different combinations of Nfe2L1 and aSyn. Additional studies are aimed at scoring individual nigral neurons in our rAAV rat model for levels of aSyn, Nfe2L1, and Nurr1, determined via immunofluorescence staining. The results of these studies will yield insight into molecular mechanisms underlying altered proteostasis in the brains of PD patients, laying a foundation for developing disease-modifying therapies.

Poster 16: Effects of alpha-synuclein pS129 on the phosphorylation of neighboring residues Y125 and Y136

Authors: Wenzhu Qi, Jennifer Hensel, Jean-Christophe Rochet

Purdue University

Abstract: Parkinson's disease (PD) is a neurologic disorder defined pathologically by the degeneration of dopaminergic neurons and the accumulation of Lewy bodies, which are enriched with aggregated forms of the presynaptic protein alpha-synuclein (aSyn). Two high priorities in the synucleinopathy field are to understand how different combinations of aSyn post-translational modifications (PTMs) affect the protein's aggregation, and how the binding properties of antibodies specific for modified forms of aSyn are influenced by PTMs located near the target epitope. pS129-aSyn is widely used as a marker for aSyn inclusion formation and has been shown to be increased in both cellular and animal models of PD. pY125-aSyn is reported to be present at a higher level in the brains of synucleinopathy patients, but it has been found in different studies to inhibit aSyn oligomer formation or to have no effect. Therefore, the role of Y125 phosphorylation in modulating aSyn aggregation is unclear. Moreover, little is known about the impact of neighboring pS129 on Y125 phosphorylation. Here, we examined the tyrosine phosphorylation of recombinant human WT aSyn and a semi-synthetic pS129-aSyn variant incubated with increasing amounts of Syk tyrosine kinase. pY125- and pY136-aSyn were detected via Western blotting using antibodies specific for each

phosphorylation site. The yield of pY125-aSyn increased with increasing Syk concentration for both the WT and pS129-aSyn variants, whereas the yield of pY136-aSyn only increased systematically for the WT protein, but not pS129-aSyn. The efficiency of Syk-mediated phosphorylation was identical for Y125 and Y136 in the context of WT aSyn, whereas phosphorylation occurred preferentially at Y136 or equally at both sites when pS129-aSyn was incubated with low or high amounts of kinase, respectively. With a fixed amount of Syk, the presence of pS129 in the semi-synthetic variant was found to increase Syk-mediated phosphorylation of both Y125 and Y136 compared to WT aSyn. From these data, we infer that S129 phosphorylation alters the conformation of the aSyn C-terminal domain, making Y125 and, to an even greater degree, Y136 more accessible for Syk phosphorylation. Moreover, S129 phosphorylation may favor electrostatic interactions between Syk and aSyn, given the presence of acidic residues in the Syk recognition sequence. These results advance our understanding of the role of PTMs in modulating aSyn conformational properties, potentially impacting the formation of neurotoxic aggregates in the brains of PD patients.

Poster 17: Development of a sensor-based cranial sensorimotor assay to detect swallowing changes in a mouse model of Parkinson's Disease

Authors: Rodgers B, Hernandez B, Nawrocki R, and Schaser A

Purdue University

Abstract: Swallowing deficits are common in Parkinson’s disease (PD) and significantly impact quality of life. However, the underlying cause of these swallowing deficits is currently unknown. One possible mechanism responsible for the complex swallowing deficits that exist in PD is aggregation of the protein alpha-synuclein in the cranial sensorimotor system. To determine the effect of early synuclein-specific aggregation on swallowing function, we focused on developing a sensor-based assay to examine swallowing function in our transgenic mouse line. Our preliminary results depict the initial development of a sensor-based swallowing assay. In our preliminary testing, we set out to confirm the feasibility of using our flexible sensor to measure respiratory surface electromyographic (sEMG) activity in our mouse model while under anesthesia. The fabricated sEMG electrode consists of a commercial glass slide used as a sacrificial substrate, a thick layer (>1µm) of polytetrafluoroethylene (Teflon), a thick layer (>1µm) of polyvinyl alcohol (PVA), a thin layer of parylene-C (1µm thick), and a thin layer of gold (50nm thick) deposited through a shadowmask laser cut with the desired sensor geometry. We compared the fabricated sensors to commercially available sensors and have determined that we are able to collect sEMG activity using our fabricated sensors. Additional testing to minimize signal attenuation and signal to noise ratio is currently underway. Analysis of additional mice with early alpha-synuclein pathology in the cranial sensorimotor system, as well as optimization for sEMG during an awake swallow task, is ongoing.

Poster 18: A role for the bacterial amyloid protein CsgA in cross-seeding alpha-synuclein aggregation

Authors: Dem Santiago Santiago, Jean-Christophe Rochet

Purdue University

Abstract: Curli is a bacterial protein secreted in fibrillar form under strenuous conditions and is mostly known for its role in biofilm formation. The idea that this protein may be involved in the

pathogenesis of Parkinson's disease (PD) has led to the hypothesis that curli can interact with alpha-synuclein (aSyn), a presynaptic protein that forms aggregates in the brains of PD patients. Evidence suggests that aSyn aggregates originate in the enteric nervous system and then spread into the midbrain and cortex via a mechanism involving retrograde transport through the dorsal motor nucleus of the vagus in the brainstem. Curli secretion, a process influenced by gut microbiome-altering factors such as diet, health status, and xenobiotic consumption, could potentially facilitate aSyn aggregation and propagation via a cross-seeding mechanism in the extracellular space or in enteric neurons. Studies have revealed that curli-producing bacteria in the gut microbiome can induce aSyn aggregation in the gut and brain of synuclein transgenic mice, thereby exacerbating gastrointestinal dysfunction and motor impairment. Additionally, it has been demonstrated that CsgA, the main component of curli, can induce aSyn fibrillization in recombinant protein solutions. However, the effects of CsgA pre-formed fibrils (PFFs) on aSyn aggregation have not been defined despite the fact that fibrillar CsgA is the physiologically relevant form encountered by aSyn in vivo. This study aims to fill that knowledge gap by investigating the interaction between CsgA PFFs and aSyn. Our approach involves producing fibrils from recombinant, histidine-tagged E. coli CsgA purified via metal affinity chromatography, as well as isolating native curli fibrils from E. coli and Salmonella sp. strains that secrete copious amounts of the fibrillar protein. These isolates will be incubated with recombinant, monomeric aSyn to assess aSyn aggregate formation via cross-seeding. Furthermore, CsgA PFFs and curli fibrils will be examined for the ability to be internalized in cultured neurons and to induce intracellular seeded aSyn aggregation. Stemming from the known effects of CsgA monomers on aSyn, we hypothesize that CsgA PFFs and native curli fibrils will accelerate aSyn aggregation. The results of this study will serve to advance our understanding of the role of curli (and more generally, the gut microbiome) in PD pathology, setting the stage for developing new therapeutic strategies.

Poster 19: A role for casein kinase-2 dysregulation in cortical synaptic dysfunction associated with synucleinopathy disorders

Authors: Alicia Scott, Sayan Dutta, Hammad Khan, Rodrigo M. Ferreira, Uma K. Aryal, Leigh-Ana Rossitto, Krishna Jayant, Xu Chen, Jean-Christophe Rochet

Purdue University

Abstract: Cortical dysfunction is thought to contribute to the non-motor symptoms associated with Parkinson's disease (PD) and other synucleinopathies. Recent studies have reported functional changes in cortical circuitry in pre-clinical models of PD, but with limited mechanistic insight. We hypothesized that 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 aggregation. We showed via immunohistochemical staining that PFF injection in either region leads to the presence of aSyn aggregates that stain positive for a form of the protein phosphorylated on serine residue 129 (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. The results of gene ontology analysis of the phosphoproteomic changes suggested that aSyn PFF administration led to perturbations of

synaptic signaling. Further, motif enrichment analysis coupled with kinase prediction indicated that the majority of differentially abundant motifs 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 analysis revealed that the regulatory beta and catalytic alpha subunits of CK2 exhibited colocalization 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.

Poster 20: Effects of endosulfine-alpha expression on alpha-synuclein pathology and neuronal activity

Authors: Gideon Drafor, Chandnee Chandrasekaran, Ranjie Xu, Wen-Hung Wang, Jean-Christophe Rochet

Purdue University

Abstract: A key pathological feature of the brains of individuals with Parkinson's disease (PD) and other synucleinopathy disorders is the presence of aggregates enriched with fibrillar forms of the presynaptic protein alpha-synuclein (aSyn). aSyn undergoes accelerated aggregation in the presence of phospholipid membranes by adopting an exposed alpha-helical structure, a state that favors membrane-induced self-assembly. Endosulfine-alpha (ENSA), a highly conserved member of the cyclic AMP-regulated phosphoprotein family expressed in the CNS, is a regulator of the ATP-sensitive potassium (KATP) channel as well a binding partner of membrane -associated aSyn. We hypothesized that ENSA could inhibit seeded aSyn aggregation in neurons exposed to aSyn preformed fibrils (PFFs). To address this hypothesis, rat cortical cultures treated with aSyn PFFs were untransduced or transduced with adenovirus encoding ENSA-WT or the control protein LacZ downstream of the neuron-specific synapsin promoter. After 5 or 6 days, the cells were fixed, stained for pS129-aSyn (a variant enriched in pathological aSyn inclusions), and imaged via confocal microscopy. A subset of cultures were incubated during the fixation step with 1% (v/v) triton X-100 (TX100), a treatment that enables the visualization of detergent-resistant pS129-aSyn puncta thought to consist of bona fide amyloid-like fibrils while eliminating soluble proteins that contribute to the background. PFF-treated cultures transduced with ENSA virus exhibited a reduced number of pS129-aSyn puncta compared to untransduced cultures or cells transduced with LacZ virus. Conversely, aSyn aggregation was enhanced in rat cortical neurons depleted of ENSA via shRNA-mediated knockdown, and in human cortical neurons derived from iPSCs with an ENSA gene disruption. From these results, we infer that ENSA plays a role in mitigating seeded aSyn aggregation in neurons. Current efforts are focused on determining the impact of ENSA down-regulation (previously observed in the brains of individuals with synucleinopathy disorders, Alzheimer's disease, or Down syndrome) on neuron activity, based on the hypothesis that ENSA stimulates neurotransmitter release by inhibiting KATP channel function. To this end, we are using assays designed to monitor the firing properties of neurons obtained from embryonic rat brains or generated

from human iPSCs via multielectrode array (MEA) recordings and calcium transient imaging. These studies will provide valuable insights into the molecular mechanisms underlying ENSA-mediated neuroprotection and the role of ENSA in regulating neuronal activity, ultimately setting the stage for developing new therapies.

Poster 21: Lymph nodes are de novo sources of estrogen, and its decreased level in middle-aged male mice causes a non-remitting progressive EAE disease phenotype

Authors: Shehata Anwar, CheMyog Jay Ko, Makoto Inoue

University of Illinois Urbana-Champaign

Abstract: Estrogen is a steroid hormone that regulates immunity produced by gonads and extragonadal sites. It helps reduce the frequency of relapses of relapsing-remitting multiple sclerosis (MS). Previously, we reported that extragonadal estrogen modulates the phenotype of experimental autoimmune encephalomyelitis (EAE), a mouse model of MS. Here, we report that lymph nodes (LNs) are de novo estrogen synthesis sites. LNs highly express aromatase, an essential estrogen synthesis enzyme, and 17β-estradiol unaffected by ovariectomy. Interestingly, the expression level of LN estrogen is reduced in middle-aged male, not female mice, who developed non-remitting severe EAE disease. Moreover, middle-aged female EAE mice were responsive to IFNβ treatment, a first-line MS treatment, while middle-aged male EAE mice were IFNβ-resistant. Our findings indicate that LNs are de novo sources of estrogen, which modulates EAE disease phenotype, and its decreased level in middle-aged male mice causes a non-remitting progressive EAE disease characterized by severe neurodegeneration.

Poster 22: The Role of Microglia in Blast Traumatic Brain Injury: Polarization State After Exposure to Secondary Injury Factor Acrolein

Authors: Krishnan N, Adam CE, Martinez JE, Beauclair TB, Mufti SJ, Bowers AN, Shi R.

Purdue University

Abstract: Blast traumatic brain injury (bTBI) can result in debilitating pathologies like neurodegenerative disease years after the injury is sustained, making the increasing prevalence of bTBI concerning. The key to preventing the long-term consequences of bTBI likely lies in targeting secondary injury, which is damage due to chemical factors like acrolein, a lipid peroxidation product. Even after mild injury, secondary injury can promote pathological consequences for years, making untangling these mechanisms a necessity. One cell type that can attenuate secondary injury is microglia. Microglia activate after injury and can assume one of two polarization states: M1, which promotes inflammation, or M2, which reduces inflammation. Understanding how secondary factors like acrolein affect polarization could help us reduce neuroinflammation to mitigate long-term neurodegeneration. To this end, BV2 mouse microglia were exposed to a clinically-relevant concentration of 50 μM of acrolein for 4 hours, then incubated in acrolein-free medium for 24 hours. Microglial polarization state was evaluated via immunocytochemistry. Acrolein treatment resulted in a 131.75% increase in levels of inducible nitric oxide synthase (iNOS), indicating increased M1 polarization relative to untreated controls. Interestingly, acrolein treatment did not result in a notable change in levels of M2 polarization marker CD206, as those in acrolein-treated cultures were only 1.32% lower than those in untreated controls. This suggests that acrolein promotes

inflammatory microglial phenotypes and this could be an important post-injury mechanism by which chronic neuroinflammation develops. Moreover, tracking and targeting this mechanism over time could be critical to treating long-term post-bTBI neurodegeneration.

Poster 23: Neuronal Network Changes in Blast-Induced Mild TBI: Novel Insights from a 'bTBI-on-a-Chip' Model

Authors: Jhon Martinez, Casey Adams, Shatha J. Mufti, Timothy Beauclair, Nikita Krishnan, and Riyi Shi

Purdue University

Abstract: Traumatic brain injury (TBI), a leading global cause of death and disability, includes Blast-induced TBI (bTBI), which is common in war zones and presents significant challenges due to the limited understanding of its mechanisms, particularly mild Blast-Induced TBI (mbTBI), often characterized by minimal initial symptoms and frequent underdiagnosis. Studies have shown that secondary pathological biochemical processes in mTBI can result in long-term neurodegeneration. Nevertheless, most models lack the spatial and temporal resolution needed for detailed studies. This study utilized an in vitro blast model, 'bTBI-on-a-chip,' to study neuronal network changes following a blast, featuring neuronal networks grown on MicroElectrode Arrays (MEAs) and a shock tube-based device to generate the blast shockwave. Analog electrical signals from extracellular spike activity were amplified, digitized, and manually discriminated using waveshape templates. Real-time network activity was monitored using the raster display before, during, and after injury. The signals were converted to timestamps for storage and analysis using the Multichannel Acquisition Processor System from Plexon Inc. Using this model, we observed an immediate decrease in network activity following a mild blast, indicated by reductions in spike rate, burst rate, burst duration, spikes in bursts, and burst amplitude, a measure of burst synchronization. This revealed an immediate network abnormality following a mild blast. Overall, this in vitro mbTBI model offers a unique platform to study post-blast neural network activity dysfunction with unprecedented temporal resolution, providing insights into the dynamics and mechanisms of functional deficits and aiding in the development of effective interventions for blast-induced neurotrauma recovery. (The authors acknowledge the support of the state of Indiana and Plexon Inc. in this research.)

Poster 24: Diagnosis of Isocitrate Dehydrogenase (IDH) mutant Glioma by Desorption Electrospray Ionization Mass Spectrometry

Authors: Mahdiyeh Shahi, Hannah Marie Brown, Rong Chen, Diogo Garcia, Erik Middlebrooks, Kaisorn Lee Chaichana

Purdue University

Abstract: Glioma is the most common neoplasm in the central nervous system (CNS), including brain and spinal cord. According to the World Health Organization (WHO) classification of tumors of CNS, adult-type glioma is classified into IDH mutant (IDH-mut) and IDH wildtype (IDH-wt). IDH mutation status plays an important role in the diagnosis and prognosis as patients with IDH-mutant glioma exhibit higher survival rates. Tumor resection through craniotomy surgery is the most important prognostic modulator in glioma patients. However, the optimal tumor resection is difficult to achieve with the diffuse nature of gliomas, which demands sensitive and rapid tools for

intraoperative diagnosis. The commonly utilized methods for molecular diagnosis don't satisfy the requirements for intraoperative evaluations. IDH-mut enzyme converts α-ketoglutaric acid to 2-hydroxyglutarate (2HG), which is a unique oncometabolite in IDH-mut glioma. Cystathionine, a key intermediate in the transsulfuration pathway, has been found in high concentrations in glioma tumors. Desorption electrospray ionization mass spectrometry (DESI-MS) as a rapid and sensitive tool enables detection of IDH mutation in brain tissue with minimal sample preparation. Here, DESI-MS was used for intraoperative diagnosis of IDH mutation in glioma biopsies. The intraoperative results from 129 core biopsies of 34 patients showed sensitivity, specificity, and accuracy of all at 100%. Initial offline studies of cystathionine revealed high concentrations in IDH-mutant and IDH-wildtype tumors comparing to the normal mouse brain. The results show the value of DESI-MS as a rapid and sensitive tool for intraoperative evaluation of glioma with potential for achieving better patient outcomes.

Poster 25: Neuronal injury in the brain induced by T cells in murine cryptococcus-associated IRIS

Authors: Jinyan Zhou, Shehata Anwar, Makoto Inoue

University of Illinois Urbana-Champaign

Abstract: Cryptococcus-associated immune reconstitution inflammatory syndrome (C-IRIS) often occurs in immunocompromised patients who are receiving reconstitution therapy and the immune system becomes overreactive. Patients with C-IRIS exhibit many central nervous system complications, including headache, fever, cranial neuropathy, and visual disturbance, potentially complicating the progression and recovery processes. Pulmonary disease has also been reported in patients with C-IRIS, however, little is understood about its etiology and pathogenesis, making clinical diagnosis and treatment highly inefficient. Previously, we have developed a mouse model of C-IRIS using immunocompromised mice, with intranasal infection of Cn serotype A H99 (CnH99) and intravenous transfer of CD4+ T cells after CnH99 infection. This mouse model showed weight loss, high mortality, systemic upregulation of pro-inflammatory cytokines, elevated levels of CD4+ T cells in the lungs, infiltration of CD4+ T cells into the brain, and cerebral edema. Here, utilizing our established mouse model of unmasking C-IRIS, we investigated how pulmonary dysfunctions are mediated and found that pulmonary dysfunctions in C-IRIS mice were significantly suppressed by inhibition of CD4+ T cells to the brain. In addition, intracerebroventricular injection of CD4+ T cells isolated from the lung of C-IRIS mice induced C-IRIS disease. Further, brain CD4+ T cells elicited neuronal damage in the nucleus tractus solitarius, a region located in the hindbrain and known for processing information related to respiration. Our findings provide unique insight into the mechanism behind pulmonary dysfunctions in C-IRIS and nominate potential therapeutic targets for treatment.

Poster 26: Dynamic fluorescent cyclic AMP biosensors to study recombinant and endogenous adenylyl cyclase 1 (AC1) signaling in HEKΔAC3/6 KO and SH-SY5Y neuroblastoma cells

Authors: Sabbir Alam, Emily K Davidson, Kayla Johnson, Camryn J Fulton, Amanda H Klein, Val J Watts

Purdue University

Abstract: Adenylyl cyclases (ACs) mediate the production of cyclic AMP (cAMP) and play a pivotal role in regulating physiological processes. Adenylyl cyclase 1 (AC1), activated by Ca2+/calmodulin (CaM), is highly expressed in the central nervous system and is implicated in chronic pain modulation. However, the lack of a robust neuronal cell model and technological limitations for measuring cAMP have hindered the study of AC1 and the identification of potential therapeutic interventions. Specifically, most cell-based models of AC activity lack essential neuronal properties necessary for mimicking native cellular conditions, which has hindered our ability to assess endogenous AC activity and inhibitor responses in a neuronal model. To address this gap, we aim to establish a neuronal model utilizing SH-SY5Y neuroblastoma cells expressing genetically encoded cAMP biosensors to study endogenous adenyl cyclase activity and evaluate potential inhibitors of AC1 signaling. The initial studies use our novel HEKΔAC3/6 KO cells stably expressing AC1 (ACΔ3/6KO-AC1) to assess cAMP signaling with several dynamic cAMP biosensors called cAMP Difference Detector in Situ (cADDis). We transduce and express intracellular, raft membrane, non-raft membrane, nuclear, and A-kinase anchor protein (AKAP) targeted cADDis in HEK 293 ACΔ3/6 cells stably expressing AC1. We observed that direct stimulation of AC1 by Forskolin or stimulation by the calcium ionophore, A23187, resulted in rapid and sustained cAMP kinetics, characterized by initial spikes followed by stabilization after 10-12 minutes. Distinct AC1 activity is observed using subcellularly targeted cAMP biosensors, with differing responses in raft versus non-raft membrane regions. A second series of experiments uses SH-SY5Y neuroblastoma cells to investigate the regulation of endogenous AC1 activity and explore potential μ-opioid receptor (MOR)-AC1 interactions. While SH-SY5Y cells endogenously express AC1 mRNA, significant Ca2+/CaM-stimulated cAMP accumulation is not readily observed in endpoint cAMP assays. Using intracellular targeted cADDis, we confirm A23187-stimulated endogenous AC1 activity and chronic μOR agonist DAMGO-mediated sensitization of AC1. We also report naloxone-mediated inhibition of the overshoot in SH-SY5Y cells, focusing on endogenous MOR and AC1. In conclusion, this study presents promising advancements towards establishing a robust neuronal model expressing genetically encoded cAMP biosensors to study endogenous AC1 activity and explore potential AC inhibitor responses related to chronic pain treatment.

Poster 27: Effect of adolescent binge-like ethanol consumption on cognition-like behaviors in crossed high alcohol-preferring mice

Authors: Aroor A, Chester J.A

Abstract: Binge drinking of alcoholic beverages is common among adolescents, making them more susceptible to alcohol-induced neuronal damage and cognitive impairment. Studies have shown that alcohol exposure during adolescence impairs cognition-like behaviors in rodent models. This study investigated the impact of adolescent binge-like ethanol consumption on prepulse inhibition (PPI), a measure of cognitive processing and novel object recognition (NOR), a measure of recognition memory, in adult crossed high alcohol-preferring (cHAP) mice, which are selectively bred for high alcohol preference. Adolescent (PND 34-36) male and female cHAP mice were exposed to either 20% ethanol (EtOH) in water or water alone (H2O) in a 4-day binge-like drinking procedure for 4 weeks. PPI was conducted 1 day (T1) and 1 week (T2) and NOR was conducted 15 days after the last day of consumption. Ethanol intake increased from Week 1 to 4, with females consuming significantly more ethanol than males at Week 3. For PPI, at T1, there were no effects of ethanol

exposure on %PPI. However, at T2, EtOH mice displayed increased %PPI compared to H2O mice. For NOR, EtOH males showed a trend toward lower object recognition than H2O males, indicating memory impairment, while there was no difference between EtOH and H2O females. Our findings suggest binge-like alcohol consumption during adolescence alters cognitive processing, assessed by PPI and NOR, the latter which may be a sex-dependent effect.

Poster 28: Escalation of intravenous fentanyl self-administration and assessment of withdrawal behavior in male and female mice

Authors: Chen Y, Xiao T, Kimbrough A

Purdue University

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.

Poster 29: Social isolation increases reward-directed motivation in high alcohol-preferring female mice

Authors: Enkh-Amgalan S, Mukadam AA, Karth MM, Koss WA, Chester JA

Purdue University

Abstract: Heavy alcohol consumption is a significant public health concern, directly contributing to alcohol use disorder (AUD) and various neuropsychiatric conditions. Negative affect following stress may increase reward-seeking behaviors that lead to heavy drinking. We investigated the effects of social isolation stress on reward-directed motivation and stress hormone response [corticosterone (CORT)] in mice selectively bred for high alcohol preference (HAP). Male and female HAP mice were first (Pre-isolation) assessed for binge-like alcohol drinking and baseline and 30-minute CORT response to restraint stress. Mice then received progressive ratio (PR) training with Nesquick strawberry milk in touchscreen chambers. Based on their response breakpoint, mice were then counterbalanced into two groups (Post-isolation): socially isolated for a month or group-housed as

controls. There was a trend where group-housed females' post-isolation 30-minute CORT response was higher than their pre-isolation 30-minute CORT response. Isolated female HAPs exhibited significantly higher breakpoints and target-to-blank touch ratios in PR testing compared to group-housed females. No significant differences in PR performance were observed in male HAPs. Prior binge-like alcohol drinking levels did not correlate with PR performance. The results suggest that social isolation increases reward-directed motivation in female HAP mice. Ongoing studies are examining whether impulsivity following social isolation stress contributes to heightened reward-seeking and other affective behaviors.

Poster 30: Alcohol exposure in aged mice: Effects on recognition memory and compulsive-like behaviors.

Authors: Roma Kamat, Tori Burke, Parker Davis, Soyol Enkh-Amgalan, Julia Chester

Purdue University

Abstract: Alcohol consumption in older-aged populations may worsen memory and anxiety. We examined whether alcohol consumption in aged mice affects memory and anxiety-related compulsive behaviors. Mice selectively bred for low alcohol preference (LAP) (14 – 15-months-old, 10M, 8F) underwent a three-week Drinking-in-the-Dark (DID) procedure receiving either tap water or a 20% alcohol solution four days/week. After DID, anxiety-related compulsive behavior was tested with marble-burying tasks. Then, LAPs underwent an Object Recognition Memory task where familiar over novel object recognition was assessed. LAPs displayed low but physiologically relevant alcohol intake across the 3 weeks. Alcohol exposure did not affect marble burying. LAPs displayed low levels of object exploration, hindering reliable memory assessment. Interestingly, alcohol-exposed females took longer to explore objects compared to control females, while LAP males did not show this effect. This suggests aged LAPs have reduced motivation for exploration, and alcohol may increase anxiety or neophobia toward objects in LAP females.

Poster 31: Inhibition of the Cortical Amygdala reduces alcohol dependent drinking behavior in female mice

Authors: Tiange Xiao, Alyssa Boisvert, Yueyi Chen, Xiaoling Cheng, Jingliang Zhang, Xi Cheng, Zhefu Que, Danielle McAuliffe, Yang Yang, Alexander Chubykin, Adam Kimbrough

Purdue University

Abstract: Alcohol Use Disorder (AUD) is a significant public health concern, marked by chronic and excessive drinking leading to cycles of intoxication, withdrawal, and craving. While extensive research has characterized the importance of key brain regions in AUD, the cortical amygdala (CoA) has recently been identified as a critical brain region involved in AUD. Prior data has shown that inhibiting activity in the CoA reduced alcohol consumption in alcohol-dependent male mice, but its impact on withdrawal behavior and excessive drinking in females remains unexplored. Thus, we aimed to determine the impact of inhibiting CoA signaling on alcohol dependent drinking and withdrawal behavior in alcohol-dependent female mice. Using a chemogenomic approach, we injected the CoA of mice with either pAAV8-hSyn-mCherry (sham) or pAAV8-hSyn-hM4D(Gi)-mCherry (Gi). To establish alcohol dependence, C57BL/6J female mice underwent two-bottle choice (2BC) / chronic intermittent ethanol vapor exposure (CIE) for 6 cycles or remained Air exposed during

CIE weeks for nondependent controls. During the final week of 2BC mice were given injections of saline or CNO during drinking. Mice were then given a final CIE session and then tested during withdrawal the following week for behavioral signs of withdrawal with saline or CNO injections. Behavioral tests included the open field test, von Frey test, and tail suspension test. The average alcohol intake, prior to CNO testing, during the final week of drinking was 0.97 ±0.20 g/kg in non-dependent and 2.36 ±0.25 g/kg in alcohol-dependent mice. Following CNO injection to inhibit CoA signaling, the alcohol-dependent::Gi groups exhibited alcohol consumption of 2.07 ±0.36 g/kg with saline injection and 1.08 ±0.23 g/kg with CNO injection, demonstrating a significant reduction in alcohol intake, an effect that was not shown in alcohol-dependent mice with sham virus. Intriguingly, although alcohol-dependent mice showed significant signs of withdrawal behavior, inhibition of the CoA did not influence these behaviors, suggesting that the CoA may be key for alcohol dependent drinking independent of effect on withdrawal symptoms. Brain tissue was collected during withdrawal to examine the impact of inhibiting the CoA in alcohol-dependent mice on neural network structure and function and this data is currently being processed/analyzed.

Poster 32: Individual Subjective and Physiologic Response to Sham and Real Intravenous Endotoxaemia

Authors: Awadzi UJ., Ward M., and Steinhubl S.

Purdue University

Abstract: The intravenous endotoxaemia model has been employed for the study of inflammation in humans for decades. The model involves the intravenous administration, typically as a single bolus, of lipopolysaccharides (LPS) into human volunteers. The majority of volunteers subsequently experience flu-like symptoms and variable changes in their physiologic parameters, serum biomarkers, as well as alterations in responses like mood and anxiety. While multiple studies have looked at various measures of response to an LPS injection, this study seeks to investigate the nocebo-effects of informing volunteers of the potential side-effects that accompany LPS injection but are randomly assigned to receive a placebo saline injection instead. We hypothesize that the informed placebo-receiving volunteers will exhibit almost similar LPS-induced changes in sickness and mood behaviors. To compare the differences between subjective and physiologic response to placebo and LPS, volunteers will be randomized to receive 1 ng/kg LPS or saline placebo. During the study, continuous physiologic responses using multivariable ECG-based torso patch and wrist wearable sensor, serum biomarkers (cytokine levels of IL-6, IL-1, TNF-α, cortisol), and subjective assessment (sickness, state anxiety and mood questionnaires) will be collected. Then, we will compare changes from baseline measurements using ANOVA techniques, and perform other analyses with Spearman's rank correlation coefficient and regression. Overall, we seek to understand the nocebo effect of anticipating inflammatory responses after intravenous injection. The value of this research lies in the multi-modal exploration of the nocebo effect, built off knowledge of each individual’s unique variability in their multi-day pre-exposure baseline of continuous physiologic parameters.

Poster 33: Comorbidity and Genomic Networks for Depression Reveal Causal Relationships and Pleiotropy between Neuropsychiatric and Non-psychiatric Disease

Authors: Guangxin Chen, Qingyi Zhong, Zhiyu Yang, Pritesh Jain, Petros Drineas , Peristera Paschou

Purdue University

Abstract: Depression is one of the most prevalent psychiatric disorders and is a leading cause of health ailment worldwide. It is highly heritable and is frequently comorbid with other mental and physical traits. This observation motivated us to look deeper into the genetic and phenotypic connections between depression and other traits in order to identify correlations as well as potentially causal connections between them. We analyzed data from the UK biobank to systematically evaluate relationships between depression and other heritable traits from both a phenotypic and a genetic aspect. We constructed a comorbidity network connecting depression and other disorders on over 300,000 participants of European ancestry. We investigated the genetic correlation for each connection in the network. We also looked into potentially causal relationships using Mendelian randomization for all pairs of significantly correlated disorders and uncovered horizontal pleiotropic genetic variants and genes contributing to disease etiologies. We found diseases like gastro-oesophageal reflux disease (GORD), body mass index, and osteoarthritis to be direct causes for depression. We highlights the broad connections between depression and diverse traits, indicating a complex etiology and possible existence of subtypes for depression. Our findings highlight the value of cross-trait analysis in better understanding the neurobiology of complex psychiatric disease.

Poster 34: Shared genetic basis of physical activity and brain health demonstrated by magnetic resonance images

Authors: Yuxin Guo, Eddie Yang, Zirui Fan, Juan Shu, Tengfei Li, Peristera Paschou, Hongtu Zhu, Bingxin Zhao

Purdue University

Abstract: Physical activity (PA) is essential for mental health. Apart from environmental and social factors, PA engagement can be determined by personal motivation and cognition performance. Understanding the biological intercorrelation between PA and brain health and the underlying genetic pathways will help develop PA as an early behavioral indication for potential mental conditions and design personalized, efficient behavior interventions. In this study, we collected genome-wide association study data for self-reported and device-measured PA and brain magnetic resonance imaging (MRI)-derived traits from over 40,000 individuals in the UK Biobank cohort. We identified a widespread overlapping genetic basis of PA and total brain volume, average cortical thickness, grey matter connectivity involving the somatomotor network as well as white matter microstructure. Additionally, using disease genetics data collected from independent samples, we observed that less active and sedentary behaviors might be a causal factor of neurological disorders like major depression disorder. Moreover, the association between PA and brain structure can be mediated by conditions like hypertension and type 2 diabetes. Our results indicated the complexity of PA-brain relationships which might involve multiple organs and systems.

Poster 35: Multi-ancestry Genome-wide Association Study (GWAS) for Neuroticism

Authors: Kaka MO, Topaloudi A, Chen G, Paschou P

Purdue University

Abstract: Neuroticism is a heritable personality trait characterized by negative emotionality and is genetically correlated with psychiatric disorders such as schizophrenia. However, studies of this trait have largely been Eurocentric to date. Given the differences in genetic structure among populations, it is imperative to investigate whether non-European populations can already start benefiting from the results uncovered in large-scale European studies. Here, we report a multi-ancestry GWAS for the trait, consisting of Africans, South, and East Asians from the UK Biobank, as well as summary statistics from the largest European Neuroticism GWAS. Using the meta-regression model in MR-MEGA and the random and fixed effect models in METASOFT, we report a total of 93 genomic risk loci. Five of these were in novel regions that mapped to genes TENM3, CDH12, and LRFN5. Leveraging the linkage disequilibrium diversity in non-European populations, we performed a multi-ancestry fine-mapping using MESuSiE. An overall improvement in the credible set sizes was observed as compared to the single-ancestry fine-mapping conducted using SuSiE across all 93 regions. We then identified a total of 16 causal SNPs out of which 9 were ancestry-specific, and 7 were shared between pairs of ancestries. This suggests a basis of a shared genetic architecture for Neuroticism. We further prioritized all genes from this study and performed tissue and cell-type enrichment analyses as well as transcriptome-wide association studies. In all, this study successfully reports the importance of increasing diversity in genetic studies to discover new insights into the etiology of mental health traits.

Poster 36: Better Characterizing Learning Deficits in FX Mice To A Delayed Working Memory Paradigm

Authors: S. NAREDDULA, R. MOFFITT, V. SALDARRIAGA, R. RUDNICKI, A. A. CHUBYKIN

Purdue University

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder that widely affects information processing in the brain resulting in deficits in learning and memory. One of the most prevalent forms of ASD is Fragile X Syndrome (FXS), which results from a mutation in the FMR1 protein. Previous studies have shown alterations in cell morphology, synaptic connections, and neural circuits pertaining to sensory perception in FXS model systems. Consistent with this, our lab has identified significant differences in the visual response of FX mice to a passive visual perceptual experience paradigm, specifically regarding evoked low frequency theta (4-8 Hz) oscillations in the primary visual cortex (V1). These oscillations are a possible mechanism for visual working memory, and their impairment in FX mice leads to a learning disability. However, currently, there is no widely accepted working memory behavior paradigm in mice. Here, we describe a new modified working memory paradigm based on a classical go/no-go visual discrimination task wherein mice are required to wait for a period of time following a visual stimulus before responding. We validated this new method and characterized the learning disability in FX mice compared to WT mice. We found that the FX mice consistently required more training days and reached lower overall training scores compared to the WT. Additionally, we discovered that FX mice demonstrated distinct movement patterns consistent with impaired memory during freely moving behavior. Our findings highlight the efficacy of this novel method for studying working memory in mice, shedding light on the underlying mechanisms and potential avenues for therapeutic targets.

Poster 37: Integrative analysis of Transcriptome and Proteome-Wide Association Study identifies novel genes implicated in Tourette's Syndrome

Authors: Shekhar S, Topaloudi A, Yu D, Jain P, TS GWAS3 Working group, Scharf J, Mathews C, Paschou P

Purdue University

Abstract: Tourette Syndrome (TS) is a neurodevelopmental disorder characterized by involuntary motor and phonic tics. Genome-wide association studies (GWAS) have advanced the genetic understanding of TS by identifying genome-wide loci significantly associated with TS. However, determining the biological mechanisms and pathways of GWAS signals remains challenging. To characterize the effect of genetic variation-mediated gene expression in TS and to understand the biological underpinnings of the disorder, we performed a global and unbiased transcriptome-wide and proteome-wide association study (TWAS and PWAS, respectively) in the largest cohort of TS patient samples of general European ancestry consisting of 13,247 TS cases and 536,217 healthy controls. Single tissue and cross-tissue transcriptomic imputation was performed using FUSION and Joint Tissue Imputation (JTI) tool to predict tissue-specific gene expression based on the latest GWAS summary statistics of TS (N total = 549,464; N effective = 41,204). Precomputed gene expression weights from 49 GTEx tissues (version 8) were used to estimate a gene's association with TS. As transcriptome expression sparingly correlates with proteome expression, we performed PWAS to complement TWAS analysis. The FUSION pipeline was used to perform PWAS analysis based on protein expression weights from Dorso-Lateral Prefrontal Cortex (DLPFC) tissue. Gene associations were considered significant if the TWAS or PWAS p-value was less than 0.05/number of genes per tissue. Additionally, colocalization analysis was computed for genes with a p-value less than 1x10E-5 using the COLOC function. Genes with a posterior probability value equal to or greater than 0.75 were considered evidence for the expression quantitative trait loci (eQTL)-GWAS pair influencing both the expression and the GWAS trait in a particular region. TWAS analysis using individual tissue-based prediction matrix of gene expression identified 60 unique genes whose transcript expression was significantly associated with TS. Notably, cross-tissue TWAS identified 16 genes and additional loci that are not significant at the genome-wide levels. PWAS analysis, based on protein expression from DLPFC tissue, identified two unique genes. Interestingly, both genes identified by PWAS were also implicated using the TWAS method suggesting them to be linked with TS, making them key findings of the study. For these two genes, genetic variant-mediated change in transcript and protein expression is significantly associated with TS etiology. In conclusion, results from our TWAS and PWAS analysis allow us to identify novel genes associated with TS disorder and identify biological pathways that can be validated via biological experimentation to strengthen our analysis.

Poster 38: Evaluating Cognitive Performance in Isolation with VR for Improved Spaceflight Readiness

Authors: Zoss PA, Amin S, Steinhubl SR, Goergen CJ, and Ward MP

Purdue University

Abstract: Long-term spaceflight will see humans face challenges and stressors that must be overcome with limited access to Earth-based assistance. One pressing requirement is to maintain functional cognitive performance levels in such high-stakes tasks where failure can result in the loss of entire crewed missions. The stress of living in isolation for extended periods of time can negatively affect mental health and adversely alter performance. The current effects of spaceflight stress on performance and the ability to accurately quantify performance on an individual basis are not fully understood. Earth-independent strategies are needed to help monitor and maintain performance levels in the stressful environments of long-term spaceflight missions. We propose that a combination of wearable sensors (e.g., heart rate, respiration, blood pressure, electrodermal activity, and skin temperature) and Virtual Reality (eye tracking) technology can be used to track contextualized changes in autonomic physiological activity corresponding to mental health and cognitive functionality. The degree of difficulty in quantifying performance is a challenge that has not yet been fully overcome. We expect our analytical methods and data collection systems will be able to find patterns in autonomic biomarkers from individuals to track cognitive functionality without the dependency on Earth-bound communications. The contextual information added from VR systems allows for a more reliable perspective on collected data and performance metrics. This technology will allow for better monitoring and timely interventions during long-term spaceflight missions to maintain cognitive performance levels and hence spaceflight readiness.

Poster 39: Repairing Age-Related Memory Updating Decline by Increasing Excitability of Neurons

Authors: Derek Baldwin, Chad Brunswick, Janine Kwapis

Pennsylvania State University

Abstract: Previously formed memories can be updated to reflect new or changed information. Notably, memory updating deficits are common in Alzheimer's disease and related dementias. Using the memory updating paradigm Objects in Updated Locations, we have observed that aging mice suffer impairments in their ability to update memories. We hypothesize that this reduced updating capability is the result of decreased allocation, or overlap, between neurons encoding the memory. Here we use two methods to target highly excitable neurons with update information. In theory, this will increase allocation between the original memory and the update memory, creating a functional link between the two. By presenting a memory update 75 minutes after the original memory has formed, we can use residual excitability to bias update information into being incorporated into the original memory. We can also use DREADD's to manually increase neuron excitability during updating. We have shown success restoring memory updating in old mice using both methods. The range of diseases capable of being addressed by these treatments would be much larger if the mechanism targeted by this treatment was similarly affected in old and young mice. To test this, we are repeating these experiments in young mice while using a subthreshold update. Subthreshold updates are shorter and therefore harder to learn, providing a target to rescue through treatment. Around 50 million people across the world struggle with dementias such as Alzheimer's. Successfully restoring memory updating in aging mice is a vital step in understanding the mechanisms dysregulated by these diseases.

Poster 40: Identifying the impact of glycine-N-methyltransferase on rhythmic gene expression in the Drosophila melanogaster eye

Authors: Lammert SD, Weake VM

Purdue University

Abstract: In Drosophila melanogaster, the circadian clock governs physiological cycles over a 24-hour period through the regulation of rhythmic gene expression. H3K4me3, an active transcription mark that is important for rhythmic gene expression and maintaining photoreceptor health in aging, is decreased at the global level in aging Drosophila photoreceptors. Histone methyltransferase activity relies on the ratio of the methyl donor S-adenosyl-methionine (SAM) and S-adenosyl-homocysteine (SAH), a potent inhibitor of methyltransferases. This ratio is regulated primarily by glycine-N-methyltransferase (Gnmt). In aging vs. young Drosophila eyes, there is a significant increase in abundance of both Gnmt and SAH. Our research shows that increased expression of Gnmt has a significant impact on rhythmic gene expression in the Drosophila eye. By profiling the nuclear transcriptome of young photoreceptor cells over a 24-hour day, we observed that overexpression of Gnmt is sufficient to alter rhythmicity in 26% of all significantly expressed genes in the eye. Of these genes, 34% lose rhythmicity when Gnmt is overexpressed, many of which are enriched in aerobic respiration and oxidative phosphorylation. We also observe decreased amplitude of multiple core clock genes when Gnmt is overexpressed. These data support our hypothesis that an age-dependent increase of Gnmt contributes to altered rhythmic gene expression in photoreceptors. Our future work will further elucidate the impact of Gnmt on histone methylation in the aging eye.

Poster 41: Characterizing the epigenetic landscape in the aging eye

Authors: Makayla Marlin, Dr. Vikki Weake

Purdue University

Abstract: As organisms get older, changes in gene expression occur, which correlate to age-related ocular diseases. Histones play a critical role in the regulation of gene expression and chromatin structure, influencing a range of cellular processes, including age-related changes in the eyes of Drosophila melanogaster. Histone proteins can be post-translationally modified by acetylation, methylation and many other marks. These histone modifications contribute to chromatin regulation processes such as transcription. While it is known that epigenetic modifications are a hallmark of aging and change with age, it is unknown how these histone modifications are changing with age in the eye. Previous studies in the Weake lab show a global decrease in histone 3 lysine 4 trimethylation (H3K4me3) and histone 3 lysine 36 trimethylation (H3K36me3) across expressed genes in fruit fly eyes as they age. However, many other modifications have not been studied in the aging eye. This study aims to elucidate how histone marks change in aging photoreceptors by examining D10 and D50 flies. By employing Nuclei Immuno-Enrichment (NIE) to affinity bind photoreceptor nuclei, coupled with Cleavage Under Targets and Release Using Nuclease (CUT&RUN), a chromatin profiling strategy, we will investigate how histone mark distribution and levels change as the flies age. This study could provide valuable insight into the molecular mechanisms responsible for age-related gene expression, along with ocular diseases that result from aging.

Poster 42: The role of the circadian transcriptome in aging photoreceptors

Authors: McGovern SE, Meng G, Marlin MN, Weake VM.

Purdue University

Abstract: The molecular circadian clock governs 24-hour rhythmic processes throughout the body and is conserved across diverse organisms. In humans, circadian clock dysfunction contributes to negative physiological consequences, including the development of neurodegenerative and ocular diseases. Our lab studies Drosophila melanogaster photoreceptor neurons to understand the molecular basis for age-dependent retinal degeneration. Previous work from our lab demonstrates that the circadian clock is necessary for photoreceptor health, as disruption of core clock components leads to premature retinal degeneration, changes in gene expression and chromatin accessibility. Rhythmic gene expression is a critical output of the circadian clock, so we performed nuclear RNA-seq of photoreceptors collected every 4 hours in light:dark conditions to capture rhythmic patterns of transcription throughout the day. We found that nearly three-quarters of transcripts are expressed rhythmically, and half exhibit a change in rhythmicity during aging. Despite sweeping changes in rhythmic transcript levels, CUT&RUN targeting circadian clock transcriptional activators, Clock and Cycle, shows that their occupancy on chromatin is only minimally altered with aging. We therefore sought to identify additional epigenetic factors that contribute to changes in rhythmic transcription with aging. The histone H3 lysine 4 trimethylation (H3K4me3) mark is associated with active chromatin and decreases genome-wide during aging in photoreceptors. Knockdown of the three methyltransferases responsible for depositing H3K4me3 results in many rhythmic gene expression changes that recapitulate what is seen during aging. Our current work continues to examine the intersection of the circadian rhythm and epigenetics to understand the mechanisms underlying age-dependent retinal degeneration.

Poster 43: Identifying the impact of H3K4 methylation on the circadian clock in the D. melanogaster eye

Authors: Meng G, McGovern SE, Marlin MN, Weake VW

Purdue University

Abstract: To synchronize with environmental stimuli, predominantly light, the circadian clock directs the physiological and behavioral cycles with a periodicity of approximately 24 hours. During aging, the circadian clock is progressively dysregulated, correlating with the development of various neurodegenerative diseases. In the eye, circadian disruption affects retinal development and accelerates age-dependent photoreceptor degeneration, suggesting that a functional circadian clock has a protective effect on photoreceptor neurons. On the molecular level, in aging Drosophila photoreceptor cells, we observed approximately half of the expressed genes exhibit altered rhythmicity at the transcript level. However, the mechanisms underlying these changes in rhythmicity gene expression during aging remain poorly explored. H3K4me3 is a ubiquitous chromatin modification that is present at actively transcribed gene promoters. In mammals, H3K4 methyltransferases MLL1 and TRX have been reported to interact with the core circadian regulator CLOCK and regulate circadian gene expression. Additionally, H3K4me3 levels decrease in the aging Drosophila photoreceptors. Our research shows that knocking down of any of the three H3K4

methyltransferases (Set1, trr and trx) in Drosophila photoreceptors drastically alters the rhythmic transcriptome. Collectively, knockdown of H3K4 methyltransferases causes 67% of genes to show different rhythmic transcription, including loss of rhythm, gain of rhythm and change of rhythm. Moreover, a substantial proportion of genes shows similar changes in rhythmic expression patterns in both aged and H3K4 methyltransferase knockdown flies, including core circadian regulators. Our data indicate that age-dependent changes in H3K4 methylation contribute to changes of the rhythmic transcriptome in aging photoreceptors.

Poster 44: The essential metabolic enzyme, S-Adenosylhomocysteinase (AHCY), is oxidatively modified under oxidative stress conditions in the Drosophila eye resulting in the perturbed one-carbon metabolism.

Authors: Stanhope SC, Singhal K, Feng Y, Doud E, Jannasch A, Mosley A, and Weake V.

Purdue University

Abstract: Oxidative stress increases in the aging eye and contributes to the development of ocular diseases including age-related macular degeneration and diabetic retinopathy. In Drosophila melanogaster, blue light exposure is a model to induce oxidative stress and premature retinal degeneration through excessive calcium influx and increased production of reactive oxygen species (ROS). In this study, we used a blue light model to induce stress followed by redox proteomic profiling of the eye to identify proteins susceptible to oxidation. Drosophila is an excellent model system for proteomic studies because their proteome is considerably smaller than that of vertebrates while showing substantial homology to humans. We identified oxidation on a cysteine residue of the enzyme S-adenosylhomocysteinase (AHCY), which plays a crucial role in one-carbon metabolism. One-carbon metabolism influences response to oxidative stress through antioxidant biosynthesis and transcriptional status of the cell via epigenetic mechanisms via the production of the universal methyl donor S-adenosylmethionine (SAM), which generates the methyltransferase inhibitor, S-adenosylhomocysteine (SAH). AHCY is highly conserved across eukaryotes and is the sole enzyme to catabolize SAH. Interestingly, oxidation of AHCY under stress conditions in the eye correlates with increased SAH suggesting a decrease in enzymatic activity. Methyltransferases are highly sensitive to the SAM:SAH ratio, and disruption to this index may inhibit methylation reactions, alter the epigenome and subsequent gene expression. We are investigating a novel redox-regulation mechanism of AHCY in which oxidative modifications alter AHCY enzyme activity to dampen the transcriptional response to oxidative stress.

Poster 45: Mapping the Cerebral Cortex of the Developing Long Evans Rat in Stereotaxic Coordinates

Authors: Nanos A, Grobengieser A, and Dooley JC

Purdue University

Abstract: Rats are increasingly being used in developmental neuroscience research due to their larger size and richer behavioral repertoire compared to mice. This creates a need for atlases of the developing rat brain to serve as navigational aids for electrophysiological recordings and targeted injections of drugs and viruses. However, the existing atlases show coronal sections through the

brain, which pose a challenge for manipulations targeting the cerebral cortex, as it runs orthogonal to this plane. To address this issue, we have created cortical maps of the Long Evans rat at postnatal days 8, 12, 16, and 20. These maps relate primary cortical areas to standard stereotaxic coordinates and were created with a stereotaxic apparatus. By using a fluorophore-tipped syringe, we created a 1 by 1 mm grid projected from the dorsal plane onto the cortical surface, using bregma as a stereotaxic landmark. Next, we dissected the cortex from the rest of the brain, flattened it, and sectioned it tangentially to its surface. Finally, after using cytochrome oxidase staining to reveal the primary sensory areas, the spatial extent of these areas is digitally reconstructed. By using our grids as a reference, these maps will allow researchers with access to stereotaxic instruments to precisely target areas of interest in the cerebral cortex

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Poster 46: The role of transcription factor Meis2 in the development of GABAergic amacrine cells in the mammalian retina.

Authors: Kerstein PC, Alqahtani S

Purdue University

Abstract: Of the major classes of retinal neurons, amacrine cells (ACs) exhibit the greatest diversity, with more than sixty molecularly distinct subtypes. Each AC subtype is thought to carry out a specific function necessary for the detection of a single visual feature. The genetic factors that control AC diversity during retinal development are unknown, but are important for understanding the genetic basis of AC function, morphology, and their contribution to visual behaviors. Identifying these genetic factors has been difficult, however recent single-cell transcriptomics studies have unveiled genetic distinctions between the two major groups of ACs—the GABAergic and Glycinergic ACs. One of the most distinct genetic differences between GABAergic and Glycinergic ACs is the expression of the transcription factors Meis2 and Tcf4, respectively. In this study, we focused on the role of Meis2 in GABAergic AC development. Based on the expression of Meis2 and its known roles in the nervous system, we hypothesized that Meis2 is necessary for the neuronal specification, survival, and morphology of GABAergic ACs. To test this, we used Meis2 conditional knockout mice, Meis2Flox/Flox (Meis2CKO) crossed with Six3Cre or Ptf1aCre mice to selectively delete Meis2 from the whole developing retina or AC precursor cells, respectively. In both Meis2CKO mouse lines, we observed a reduction in both the total number of ACs and the inner plexiform layer (IPL) thickness. Furthermore, both Meis2CKO mouse lines had a 60% reduction in the total number of GABAergic ACs. Finally, in both Meis2CKO mouse lines, we observed AC subtype differences in IPL stratification. We found thinning of the dopaminergic (TH+) AC layer of the IPL, but no changes in cholinergic (ChAT+) layers of the IPL in both Meis2CKO mouse lines. These results suggest that Meis2 is necessary for both the survival and dendritic stratification of at least some of the GABAergic ACs in the mammalian retina.

FRIDAY, JUNE 7th 2024

KEYNOTE SPEAKER

Gary Lynch, Ph.D.

"Diverse, sexually dimorphic encoding across the primary hippocampal circuit."

Dr. Lynch is a Professor at the University of California, Irvine. His laboratory studies the physiological and biochemical processes responsible for rapidly producing stable changes in synapses, and the anatomy and physiology of circuitries responsible for encoding certain types of memory. Dr. Lynch is a pioneer in the study of the neural circuits involved in memory and is one of the most cited authors in neuroscience.

Website: https://cnlm.uci.edu/lynch/

INVITED SPEAKERS

Bengi Baran, Ph.D.

Assistant Professor, Psychological and Brain Sciences at University of Iowa

"Sleep as a potential biomarker and treatment target for schizophrenia"

Website: https://psychology.uiowa.edu/people/bengi-baran

Anke Tukker Ph.D.

Post Doctoral Research Associate

"Persistent effects of developmental methylmercury exposure."

Website: https://hhs.purdue.edu/directory/anke-tukker/

DATA BLITZ

Baylen Ravenscraft, Graduate Student, Indiana School of Medicine
Laboratory of Naikui Liu, MD, Ph.D.
"Rescuing the Powerhouse by Blocking the Kiss of Death: Mitochondrial Therapeutics for Heme Coordination, Membrane Electrostatics, and Next-Generation Blueprints for Neuroprotection."

Chunqi Qian, Ph.D., Assistant Professor, Michigan State University
"Wireless Integrated Sensing Detector for simultaneous EEG and MRI"

David Gauthier, Graduate Student, University of Illinois Urbana-Champaign
Laboratory of Benjamin Auerbach, Ph.D.
"Altered auditory discrimination in a rat model of autism."

WORKSHOP

"Communicating your research in a world of misinformation."

Sahir Rizk, Ph.D., Assistant Professor of Biochemistry at Indiana University South Bend
Maggie Fink, Graduate Student, University of Notre Dame

An interactive workshop engages participants in activities designed to on science help the participants identify their strengths and expertise with a focus on how to address the rising public distrust in science. The workshop uses games and group activities to train scientists at all levels on how to engage the public in meaningful conversations about research. Dr. Rizk and Maggie Fink provide techniques on how to effectively communicate ideas in-person, or through writing (blogs, op-eds, etc), and how to use art and storytelling to create interest and engage a wider audience.

POSTER SESSION DAY 2

Poster 1: Identifying Early Digital Biomarkers of Vaccine-Induced Inflammation through Wearables: Integrating Circadian Rhythm-Adjusted Baseline

Authors: Amin S, Drager L, Wilson D, Anderson J, Sekaric J, Wegerich S, Goergen CJ, Steinhubl S, Ward MP

Purdue University

Abstract: Wearables track physiological signals like heartrate (HR), physical activity, respiration (RR), galvanic skin resistance, and temperature, enabling examination of daily patterns and circadian variations specific to a person. Heart rate variability (HRV), also circadian in nature, correlates with inflammatory biomarkers. This feature of HRV and other biosignals may therefore have utility in the development of a personalized approach to assess vaccine-triggered inflammation via continuous analysis of disruptions in cardiac circadian patterns. The electrocardiogram (ECG), tri-axial accelerometry, and skin temperature data from healthy adults receiving first, second, or booster doses of SARS-CoV-2 vaccines (Moderna and Pfizer) were captured by Vitalpatch® RTM. A set of 22 metrics (from N = 35 participants who received the second dose) that included hourly means of biosignals (e.g. HR, RR, skin temperature) and features derived from them (e.g., frequency domain metrics like high and low-frequency power, Poincare metrics, and entropy metrics) was computed. A polar coordinate system based on a 24-hour clock was employed to map combinatorial or singular metrics, enabling visualization and simultaneous quantitative analysis of personalized vaccine response when post-vaccine data deviates from pre-vaccine trends. Circadian-adjusted baseline was computed by grouping individual metrics by clock time and calculating mean and standard deviation from each group. Our analysis revealed that 21 of 22 metrics changed significantly post-vaccination ($p<0.05$), with HR, temperature, low-frequency power, and Poincaré area being the earliest indicators [mean[95% Confidence Interval] = 17.63[13.05-22.21], 16.96[11.90-21.55], 16.73[12.66-21.26], and 15.57[10.38-20.77] hours post-vaccine, respectively) as determined by Wilcoxon signed rank-sum test and circadian-adjusted baseline. Study credits-PhysIQ Inc., Purdue-CVIRL.

Poster 2: Towards Unlocking Structural and Functional Heterogeneity of the Vagus Nerve using High Resolution Imaging and a Heuristic Action Potential Interpreter for Precise Neuromodulation

Authors: Beshay Y, Biscola N, Bartmeyer P, Havton L, Ward M

Purdue University

Abstract: Neuromodulation via electrical stimulation can be a promising therapy for refractory conditions such as epilepsy, chronic pain, and gastroparesis. The vagal nerve is of interest due to its diverging anatomy and parasympathetic control of many organs between the brain and the colon. Because of its diverse involvements, however, untargeted vagal nerve stimulation (VNS) can lead to unwanted side effects. Presently, anatomical mapping of the vagal nerve is lacking. With the advancement of imaging techniques such as light microscopy (LM) and transmission electron microscopy (TEM), studies have revealed the anatomy of the vagal nerve to be comprised of fascicular and sub-fascicular structures. We further hypothesize that those structures compose

functional modules that can be precisely harnessed to produce targeted therapy. We developed an accessible and easy to use MATLAB-based Heuristic Action Potential Interpreter (HAPI) that capitalizes on the underlying relationship between a fiber's diameter and its action potential shape to predict the compound action potential (CNAP) upon recruitment of one or more myelinated fibers or unmyelinated axons in annotated nerve segment images. Predictions from three nerve cross sections of a rat vagus nerve were validated against observed CNAPs in experimental settings suggesting that animal use can be minimized with computational tools. Moreover, we postulate that HAPI-predicted CNAPs can be used as biologic feedback in closed-loop VNS for precise neuromodulation. Finally, we provide a computational tool that can supplement many feats in neuroscience research.

Poster 3: Implantation of Flexible Electrodes for Simultaneous in-vivo Extracellular Recording and Two-Photon Imaging

Authors: Alec Booth, Hammad Khan, Om Kolhe, Krishna Jayant

Indiana University School of Medicine

Abstract: Rigid silicon electrodes have been used in human and animal brain models to understand the dynamics of neural computation. When implanted chronically, however, glial proliferation can rapidly disrupt the interaction between neurons and electrodes, drastically reducing recording fidelity. Flexible electrodes have the potential to minimize tissue damage and inflammation, allowing for long-term recordings over several months. In line with this objective, the Nano-neurotechnology Lab has developed a 6-μm thick, flexible, and biocompatible Parylene probe to facilitate chronic recordings in awake mice. However, flexible electrodes present a unique engineering challenge as the force required to insert into the brain causes the probe to buckle and fail during insertion. Methods and Results: A shuttle was designed using a glass micropipette and a custom, 3D-printed insertion system which provided reproducible probe implantation into the cortex. The procedure was developed on brain phantoms made of 0.6% agarose with a comparable Young's modulus to mouse brain tissue. Utilizing 3D-printed pieces and the surface tension of diluted poly-vinyl-acrylate adhesive to align the probe, insertion of the electrode and retraction of the shuttle was accomplished in awake mice. Conclusion: The implications of flexible electrodes are extensive. Long-term implantation opens the door for understanding behavioral dynamics over time. Moreover, the flexibility of these probes allows for the combination of 2-photon optical microscopy, thus enabling multi-modal investigation of neuronal physiology. A low-cost, consistent procedure is the first step in the implementation of these flexible probes for further advancements in fundamental neuroscience research.

Poster 4: Focal cooling modulates cortical coding via compartmentalized changes in the electrical structure of L5 pyramidal neurons

Authors: Meisam Habibimatin, Shulan Xiao, Krishna Jayant

Purdue University

Abstract: Focal cooling is a neuromodulatory technique that alters neural dynamics and influences behavior. However, the biophysical mechanisms explaining the impact of cooling on cortical circuits are still poorly understood. In this study, we present a biophysically based account

of how L5 pyramidal neurons are impacted by focal cooling from a perspective of neural coding and input-output transformations. Using plasticity protocols, somato-dendritic patch clamping, targeted focal cooling, calcium imaging, and two-photon transmitter uncaging as a way of encoding input information streams across the distal tuft and basal dendrites, we show that moderate focal cooling with a temperature drop of ~5°C amplifies plasticity in distal apical tuft but not basal dendrites in an N-methyl-D-aspartate (NMDA) and Kv4.2-dependent manner. This triggers a compartmentalized temperature modulation of dendritic excitability in the apical tuft. Given the sensitivity of tuft dendrites to temperature compared to basal dendrites, we show that moderate focal cooling potently modulates top-down integration and somato-dendritic coupling through altering the electrical structure of dendrites across the basal-distal tuft axis, evidenced by increased amplitude of backpropagating action potentials (bAPs) and Ca2+ plateau potentials. We then demonstrate a unique biophysical effect wherein although the calcium plateau is amplified by cooling, the rate of recovery of Na+ in the apical dendrite is slowed down, ensuring a reduction in axo-somatic output. Critically, our results reveal a previously overlooked effect wherein the Kv4.2 channel's sensitivity to temperature could be differentially regulated across dendritic regions to impact coding.

Poster 5: Spinophilin and Neurabin: Biochemical and Functional Divergence in Homologous Synaptic Scaffolding Proteins

Authors: Nikhil Shah, Wesley Corey, A.J. Baucum II

Indiana University School of Medicine

Abstract: Protein complexes within the post-synaptic density (PSD) of striatal medium spiny neurons (MSNs) regulate MSN activity and striatal circuit function. Spinophilin and neurabin are homologous, PSD-enriched actin binding proteins that target protein phosphatase 1 (PP1). Despite considerable structural and functional overlap, global knockout (KO) studies demonstrate unique roles for these two proteins. Our previous studies found that spinophilin KO (Spino-/-) mice had decreased rotarod motor learning in a 5-day, 3-trial per day paradigm. In contrast, neurabin KO (Nrb-/-) mice outperformed the WT comparison group in both a 5-day and 8-day rotarod paradigm. Both Spino-/- and Nrb-/- mice had a trend for increased rotarod reversal behavior, a previously reported index of impaired action selection. Preliminary data suggest that mice lacking neurabin specifically in direct pathway medium spiny neurons (dMSNs; NrbΔdMSN) outperform WT controls in a 5-day rotarod paradigm while also demonstrating increased reversal behavior. Using immunoblotting, we found that rotarod motor learning impacts striatal synaptic protein expression. Specifically, we found increased neurabin, but not spinophilin, expression as well as increased expression of PP1, and known spinophilin/neurabin interacting proteins Homer1, GluA1, and GluA2 . While our in-situ hybridization studies found overlapping distributions of spinophilin and neurabin mRNA within the striatum, spinophilin mRNA was enriched in puncta within the neuropil compared to peri-nuclear localization of neurabin mRNA, consistent with the known local, dendritic spine translation of spinophilin compared with cell-body translation of neurabin. Collectively, these studies delineate unique contributions of spinophilin and neurabin to striatal circuit function and PSD biochemistry, while suggesting unique regulation or actions by which they may do so.

Poster 6: Probing Multiplexed Dendritic Computations Using Two-photon 3D Holographic Uncaging

Authors: Shulan Xiao, Saumitra Yadav, and Krishna Jayant

Purdue University

Abstract: Cortical layer 5 pyramidal neurons (L5 PNs) receive a myriad of feedforward and feedback synaptic inputs with unique spatiotemporal patterns across their basal and tuft dendritic arbors. However, how these synaptic inputs integrate and multiplex amidst noisy backgrounds remains poorly understood. Here, using two-photon holographic uncaging, dynamic somato-dendritic clamp from L5 neurons in acute brain slices, and detailed modeling, we first reveal distinct rules underlying feedforward multiplexing, classification, and binding across basal synaptic input streams. We then probe how top-down feedback aids in recognizing these input sequences in a cell-type-specific fashion. We find that dendritic NMDA and Na+ nonlinearities aid in the classification and barcoding of basal dendritic input streams by regulating spike timing and gain, including amidst background noise. Strikingly, we found that these barcoded somatic spikes interact dynamically with coincident apical dendritic inputs, generating global bursts, hallmarks of integration between feedforward and feedback input streams. Notably, this integration differed across different subtypes of L5 PNs with non-overlapping anatomical projections and electrophysiological properties. Our findings reveal a crucial multibranch integration framework, demonstrating the essential nature of diverse dendritic nonlinearities and their distinct channel dependencies for multiplexing feedforward and feedback spatial-temporal synaptic input patterns.

Poster 7: Ensemble dynamics across L2/3 reveal a flexible coding framework

Authors: Saumitra Yadav, Hayagreev V. S Keri, Hammad F. Khan, Shulan Xiao, Scott R. Pluta, and Krishna Jayant

Purdue University

Abstract: Sparse coding is a hallmark of L2/3 under sensory input, yet the structure of this sparsity remains poorly mapped. In our study, we develop and employ a custom video-rate volumetric Bessel-beam microscope with submicron resolution and axial depth of 120um to dissect the functional dynamics across L2 and L3 during active whisker touch. In preliminary data, we test whether there is a difference in functional representation across L2/3. Our findings are significant- we discover that while L3 is more robust upon repeated sensory drive, L2 is more variable, revealing distinct coding regimes across this layer. We highlight a key metric – ensemble variance and show that it captures a specific L2/3 structure. We describe ongoing work and future directions focused on this effort, specifically during active touch discrimination.

Poster 8: Human-Machine Natural Language Processing Documentation Translation Technique

Authors: Bryant Beeler, Larissa Makasi

Purdue University

Abstract: There is a strong pull for user-manuals and training documentation to be converted and translated into a format that can instantly integrate into existing data structures, tables, and digital formats for streamlined analysis, engagement, computation, and throughput. The synthesis, utilization, and application of information is imperative to the modern digital world. For instance, in facing life decisions such as whether to pursue self-study, university classes, or online tutorials, it was revealed that the failure to identify, organize, and engage bodies of information can lead to numerous failures and misallocation of time. There exists a need to analyze and sort information in real-time due to the excess and constant bombardment of information. In short, information overload was rampant and extremely costly. This paper describes a Digital Training Documentation Classification Technique that aims to minimize interface and handoff losses and to standardize training information into Bloom's Taxonomy for classification of educational learning objectives. In the effort to leverage modern technologies to navigate and engage a body of information, the authors streamlined a learning process that declares broad labels and classifiers. A classification model was developed to curate and deliver information customized for the end-users' individual training needs. The model seamlessly translates documentation and other source instructions and reading materials into a format that can readily be manipulated and engaged by software and ultimately human factors in the form of training material. The authors applied metrics and dimensions to the body of information that make it more navigable, user friendly, and software friendly.

Poster 9: Decoding Electrical Signals Between Neurons: An Approach to Analyze Voltages from Neuronal Firing

Authors: Casey Adam, Jhon Martinez, Shatha Mufti, Edmond Rogers, Nikita Krishnan, Timothy Beauclair, Riyi Shi

Purdue University

Abstract: Neuron signaling is crucial to health, involves voltage spikes propagating through and between cells, and is altered by drugs or conditions (i.e. injury, disease). Quantifying signal relations could improve understanding, treatment, and detection of diseases. However, such analysis is often qualitative, time consuming, or correlative, limiting information gained. For example, crosscorrelation uses relative spike timings to construct histograms called correlograms whose shape is dictated by signal relationships. Correlograms should be created for each signal pair and correlogram shape properties quantified. However, analyses are often by hand, so correlograms are rarely quantitative. To overcome this limitation, we developed an algorithm to analyze signals from neuronal populations with minimal user effort and output statistics on: (i) signal spike counts, (ii) intervals between spikes, (iii) and signal relationships via correlograms. Specifically (iii), the algorithm: tests for independent signals by testing for uniform correlograms, determines whether one signal follows/leads another by calculating correlogram area left of zero, and quantifies firing patterns by counting correlogram peaks. Outputs were validated on microelectrode array recordings from cells given Bicuculline, a drug that synchronizes firing. The uniformity metric captured synchronization caused by bicuculline. Peak count decreased after bicuculline, suggesting less diverse firing patterns. Area left of zero showed that bicuculline imposes strict firing order on cells. In the future, this algorithm could be employed to analyze signals in other conditions to better understand neuronal communication and gain insight into

disease treatment, detection, and management. The authors acknowledge the state of Indiana and Plexon Inc. for their support.

Poster 10: Influence of Cuff Electrode Contact Symmetry on Low Frequency Alternating Current Induced Activation

Authors: Awadh Alhawwash, M. Ryne Horn, Nathaniel Lazorchak, Ken Yoshida

Purdue University

Abstract: The aim of this work is to identify where and how peripheral motor axons are activated using a low frequency sinusoidal current (LFAC) applied to implanted cuff electrodes. In-silico models and in-vivo experiments were conducted to explore the effect of contact asymmetry within a cuff electrode on activation of peripheral nerve fibers. The in-silico study used a volume conductor model coupled to the MRG active nerve models of different calibers. The model simulated unipolar and bipolar electrodes of varying contact symmetry and pitch. Acute in-vivo experiments were conducted on six rats using an asymmetrical tripolar cuff on the tibial branch of the sciatic nerve. The LFAC was applied in a bipolar configuration with six frequencies while measuring the induced EMG and twitch force. The in-silico findings indicated that the spatial potential distribution of bipolar electrodes could approximate those of unipolar configurations as the symmetry ratio of the cuff contacts-to-edges decreased. Also, membrane depolarization was influenced by the dominant cathodic pole and showed a rapid response to the virtual cathode forming outside the cuff. In-vivo findings indicated two modes of nerve activation: a burst mode seen as several EMG spikes, and a unitary mode with a single EMG spike. Both types of activation were evident in the in-silico experiments indicating that burst activation occurred with the virtual-cathode, while unitary activation was due to direct cathodic-activation. The asymmetry of cuff electrode contacts significantly alters the induced extracellular potential and, consequently, the nerve response, highlighting the dominance of either cathodic or virtual-cathode activation. These findings suggest that any asymmetry in cuff designs may lead to unbalanced polarization effects, which are critical for optimal peripheral nerve stimulation using LFAC.

Poster 11: Achieving Sub-15-nm Axial Resolution in 3D Imaging of Dendritic Spines in Mouse Brain Tissue Using 4Pi Super-Resolution Microscopy with In-Situ PSF Retrieval

Authors: Hao-Cheng Gao, Fan Xu, Xi Cheng, Alexander A Chubykin, Fang Huang

Purdue University

Abstract: Super-resolution microscopy offers an enhanced view of subcellular organelles and molecular distributions at the nanoscale. Utilizing interferometric techniques, 4Pi single-molecule localization microscopy (4Pi-SMLM) achieves sub-15-nm 3D localization precision throughout whole cells. However, extending 4Pi-SMLM to tissue imaging encounters challenges due to optical complexity in tissue. Tissue environment distorts and scatters single-molecule emissions, causing discrepancies between the detected emission pattern and the conventional bead-based 4Pi-PSF model, resulting in imaging artifacts and increased localization uncertainties. Here, we introduced a novel 4Pi-SMLM technique that retrieves the 4Pi-PSF directly from raw blinking datasets, addressing the inaccuracies of the traditional in-vitro method. This approach leverages in-situ PSF retrieval and coherent pupil interference, naturally incorporating tissue-induced aberrations.

Additionally, our method utilizes dynamic model updating to counteract time-dependent systematic misalignments. This enables the 3D visualization of dendrites and dendritic spines with an axial localization precision below 3 nm within 50-μm thick mouse brain tissues, approaching the theoretical limit of CRLB estimation. We also introduce a tailored analysis technique, providing details of spine organization and arrangement for nanoscale morphological studies. Collectively, these advancements equip researchers with the tools to explore the ultrastructure of the nervous system inside brain tissue context, revealing key mechanisms of synaptic plasticity that influence brain development and neurological disorders.

Poster 12: Modeling of Human Arm Kinematics and Cardiovascular Dynamics: Construction of a Humanoid Arm for Enhancing Wearable Technology Accuracy in Nervous System Response Studies

Authors: Hailey Haglid, Craig Goergen, PhD, Steven Steinhubl, MD, and Matthew P Ward, PhD

Purdue University

Abstract: Understanding how to effectively measure autonomic nervous system (ANS) mediated changes in physiology is essential for the management of many chronic conditions using wearable digital health technologies (DHTs). Photoplethysmography (PPG) is a common and popular method of non-invasively monitoring physiological changes mediated by the ANS. PPG sensors measure subdermal capillary blood flow, which is modulated by the mechanical action of the heart and respiratory system, along with changes in vascular tone. Algorithmic analysis of PPG data can provide estimates of heart rate, breathing rate, blood pressure, and oxygen saturation levels. Currently, wearable technologies with PPG sensors can only reliably collect data 24.0%-57.6% of the time they are worn due to motion artifacts which can obscure data and render the sensor-derived data unreliable for monitoring important health conditions over short (e.g., seconds to minutes) to long (weeks to months) timescales. This project aims to develop a humanoid arm capable of replicating human motion and an artificial circulatory system with modular and measurable pulse and blood pressure. In addition, a motion recording sleeve for collecting real-time motion data from volunteers will be utilized to project human motion onto the arm. Through this device, wearable technologies can be tested for their reliability while undergoing various motions. If there are shown to be large discrepancies between the known vitals and the vitals recorded by the PPG sensors, algorithms can be developed to refine PPG sensor data and create more accurate data collection during motion.

Poster 13: Investigating deficits in neuronal population activity associated with social behavior in a Scn2a-deficient mice.

Authors: Shivam Kant, John Min, Brody Alan Deming, Xiaoling Chen, Jingliang Zhang, Edward Nieh, Yang Yang

Purdue University

Abstract: Mutations in the SCN2A gene which encodes the alpha subunit of voltage-gated sodium channel NaV1.2 are one of the leading monogenic cause of Autism Spectrum Disorder (ASD), which is characterized by deficits in social communication and expression of emotions. NaV1.2 is essential for proper neuronal function, and compromised SCN2A expression can not only hinder

dynamics of single neurons, but also the dynamics of a neural network. Our lab has characterized a novel mouse model of Scn2a-defiency which recapitulates several behavioral phenotypes seen in autistic individuals such as social deficits. A major brain region affected in ASD is the striatum. In our study, we are investigating deficits in the dynamics of a neuronal population in the striatum associated with social behavior of Scn2a-deficient mice. To achieve this, we use in-vivo calcium imaging in freely moving and socially interacting mice. We have quantified several parameters related to neuronal activity associated with behavior. Furthermore, we are using data from hundreds of neurons and map it to a lower dimension representation of the brain states using a dimension reduction technique called MIND (Manifold inference from neuronal dynamics) defined by the activity of the neuronal population. These manifolds are labelled by behavioral states to underscore the functional state differences in different behavioral conditions. Our preliminary findings suggest that there is lower correlation of neuronal activity with social behavior in the Scn2a-deficient mice. Collectively, we aim to understand the deficits in neuronal population activity associated with social behavior in Scn2a deficient mice, and further evaluate whether the population activity specifically encodes social behavior-specific information. The framework of this study can be used to evaluate the efficacy of therapies at not only a phenotypic, but also at an intermediate region-specific neuronal population level.

Poster 14: Optimizing Prime Editing in human iPSCs for generating and correcting SCN2A genetic variations related to epilepsy and autism

Authors: Kyle Wettschurack, Bill Skarnes, Yang Yang

Purdue University

Abstract: The transformative CRISPR-based technologies greatly facilitate the arrival of the genetic medicine era. Unlike traditional CRISPR, which generates a double-strand DNA break for genome editing, prime editing (PE) only creates a nick (single-strand DNA break). Thus, PE is considered to have an enhanced safety profile and holds enormous potential for precision genome editing to correct disease-causing genetic variants eventually in patients. Human induced pluripotent stem cells (hiPSCs) have emerged as a preferred system for disease modeling and testing for precision genetic interventions in human genetic background. Although PE can perform genome editing in hiPSCs, the efficiency is far from ideal. Indeed, using PE to create or correct disease-causing genetic variants related to neurological disorders in hiPSCs-based models is still in its infancy. Here, we developed a fluorescent assay to monitor prime editing efficiency in hiPSCs. We nucleofected PE reagents that introduce the H67Y (C->T) change in iPSCs expressing blue fluorescent protein (BFP), converting BFP into green fluorescent protein (GFP). Edited vs unedited iPSCs can be easily quantified by flow cytometry. Following the co-delivery of epegRNA and plasmid-based prime editors, we demonstrated successful conversion of BFP to GFP. PE efficiency was improved 10-fold with the addition of nicking gRNA (ngRNA) from 4% to 40% of cells. We plan to use the BFP->GFP assay to test new reagents and accessory factors that may improve the efficiency of PE in iPSCs to obtain an optimal editing condition. We will then apply our optimized conditions to edit single nucleotide variants to create or correct disease-causing mutations in the voltage-gated sodium channel Nav1.2 (encoded by the SCN2A gene), which has recently been identified as the leading cause of monogenic autism and epilepsy. In conclusion, we demonstrated

the successful use of prime editing in human iPSCs and provided a valuable assay for researchers to optimize the efficiency of prime editing and make precision modifications in iPSCs.

Poster 15: Unraveling the phenotypic consequences of SCN2A splice-site genetic variant identified from a child with autism using CRISPR/Cas9 engineered iPSC derived-cortical neurons

Authors: Manasi Halurkar, Kyle Wettschurack, Morgan Robinson, Erik Rossi, Maria Olivero-Acosta, Nadia Lanman, William Skarnes, Yang Yang

Purdue University

Abstract: SCN2A encodes the alpha subunit of the voltage-gated sodium channel NaV1.2, crucial for neuronal function. Mutations in SCN2A are linked to neurodevelopmental disorders, including autism spectrum disorder (ASD) and epilepsy. This study investigates a specific splice-site variation (SCN2A c.3973-1G>A) using induced pluripotent stem cells (iPSCs). Identified in a child with ASD, this variation occurs at the conserved splice site before Exon 22. We employed CRISPR/Cas9 to engineer the c.3973-1G>A variation into a reference iPSC line, integrating a GCaMP6f biosensor at the AAVS1 safe harbor site. These iPSCs were then differentiated into cortical neurons that are known to express NaV1.2. mRNA/cDNA analysis from these neurons suggests that the c.3973-1G>A variation causes Exon 22 skipping. Further molecular analysis indicates this variant shortens SCN2A mRNA around the mutation site without affecting upstream or downstream SCN2A mRNA levels. Notably, the variation reduces NaV1.2 protein expression, suggesting protein degradation. Ongoing research is assessing various protein degradation pathways and the variant's impact on neuronal excitability. Additionally, we are working on patient-derived iPSCs and 3D cortical organoid models to study this variant's effects in more physiologically relevant systems. Our findings will elucidate the transcriptional and phenotypic changes in neurons caused by the c.3973-1G>A variation, aiding in identifying key disease phenotypes for personalized therapeutic interventions.

Poster 16: hiPSC-derived cortical neuron and organoid models carrying the SCN2A Epilepsy-Associated Mutation L1342P reveal cortical development impairments and hyperexcitability

Authors: Maria I. Olivero-Acosta, Zhefu Que, Morgan Robinson, Hope Elizabeth Harlow, Vinayak Shankar, Seoyung Hong, Shivam Kant, Muhan Wang, C. Max Otterbacher, Purba Mandal, Trang Nguyen, Manasi Halurkar, Kyle Wettschurack, Benjamin Zirkle, Layan Yunis, Ningren Cui, Xiaoling Chen, Jingliang Zhang, Jiaxiang Wu, Brody Deming, Yuanrui Zhao, William C. Skarnes and Yang Yang

Purdue University

Abstract: The SCN2A gene encodes for the neuronal sodium channel NaV1.2, which mediates action potential initiation and propagation (Sanders et al., 2018). This protein is expressed mainly in the proximal axonal initial segment (AIS) and soma of glutamatergic excitatory cortical neurons (Kruth, Grisolano, Ahern, & Williams, 2020). SCN2A pathogenic mutations have been associated with epilepsy. An example is the recurrent Nav1.2-L1342P mutation identified in five patients worldwide presenting an early-onset severe seizure phenotype that remains hard to treat with current medications (Que et al., 2021). It is one of the very few rare SCN2A mutations able to

impact brain structure. Given that no disease-modifying treatment exists, there is an urgent need to generate novel tools to probe mutation-specific disease mechanisms, evaluate therapeutic interventions, and study interactions with other cell types. Here, we use CRISPR/Cas9 edited human induced pluripotent stem cell-derived cortical neuron monolayers and, for the first time, generated cortical organoids models carrying the SCN2A epilepsy-associated mutation Nav1.2-L1342P. Additionally, we used an isogenic control cell line, which was further CRISPR-engineered to remove the mutation and explore the use of genetic engineering to restore neuronal function. In both Nav1.2-L1342P cortical neuron models, we observed enhanced electrical activity hallmarks and probed the effect of the mutation on neuron development. We observed that the presence of the Nav1.2-L1342P variant decreases neuronal complexity in a 2D model, and using a 3D cortical model, we observed enhanced synapse formation and altered neuronal content. Our findings suggest that Nav1.2-L1342P epilepsy-associated variant influences neuron development. This may predispose cortical organoids carrying the Nav1.2-L1342P mutant to cellular and functional maturity, impairing the formation of proper networks and leading to the hyperexcitability described in patients.

Poster 17: Identifying altered cell type composition and molecular pathways in adult Scn2a-deficient mice through Single Nucleus RNA Sequencing

Authors: Purba Mandal, Ye-Eun Yoo, Jingliang Zhang, Xiaoling Chen, Muriel Eaton, Hongyu Gao, Nadia Lanman, Yang Yang

Purdue University

Abstract: The SCN2A gene encodes a voltage-gated sodium channel crucial for action potential initiation and propagation. Mutations in the SCN2A gene have been identified as one of the leading monogenic causes of autism spectrum disorders. The Yang lab has established a Scn2a-deficient mouse model, which displays severe neuronal and behavioral deficits. However, the cellular and molecular mechanisms leading to the deficits observed in this autism-like mouse model at a single-cell resolution remain elusive. To address this, single nucleus RNA sequencing (snRNA-seq) has been employed to investigate the medial prefrontal cortex (mPFC) of wild-type (WT) and homozygous Scn2a-deficient (HOM) mice. The mPFC is responsible for higher-order cognitive functions, including decision-making and social behavior, which are often impacted in autism. Our analysis unexpectedly uncovered altered cell type proportions in the mPFC between WT and HOM groups. Specifically, we observed an increased number of GABAergic neuron clusters in HOM mice compared to WT mice. Ongoing analysis aims to investigate cell-type-specific gene expression differences and enriched pathways. These findings will reveal the impact of Scn2a deficiency on gene expression and the composition of different cell populations in the mPFC of Scn2a-deficient mice. Ultimately, these insights may advance our understanding of the underlying pathology and pave the way for targeted therapeutic strategies directed at novel molecular targets in a cell-type-specific manner.

Poster 18: Investigating neural circuits involved in psilocybin's prosocial effects in Scn2a-deficient mice

Authors: Brody Deming, Jingliang Zhang, Yang Yang

Purdue University

Abstract: The CDC estimates that 1 in 36 children in the United States has Autism Spectrum Disorder (ASD). A major characteristic of ASD is social abnormalities. Genetic variants in SCN2A, a gene encoding the voltage-gated sodium channel NaV1.2, have been identified as a leading monogenic cause of ASD. However, there are few FDA-approved drugs to assist with the social impairments seen in SCN2A-related ASD patients. Recent research on psychedelics has revealed potential therapeutic benefits in treating multiple psychiatric disorders. Few studies have evaluated the use of psychedelics as potential therapeutics in individuals with ASD, and no study has evaluated their use in SCN2A-related ASD. In this study, we examined psilocybin's influence on social deficits seen in Scn2a-deficient mice. Our results reveal that a 0.3 mg/kg intraperitoneal injection of psilocybin increases the sociability of Scn2a-deficient mice during the 3-chamber assay. Recent evidence has highlighted that psilocybin acts on brain regions such as the lateral habenula (LHb), a small epithalamic brain region that regulates many social behaviors, and the dorsal raphe nucleus (DRN), the main serotonergic hub of the brain. Additionally, we have found that chemogenetic activation of the LHb and DRN->LHb neurons also increases sociability in Scn2a-deficient mice. We are currently investigating the role of the DRN->LHb circuit in psilocybin's prosocial effects. Our results shed light on the importance of neuromodulation in SCN2A-related ASD. With recent advocacy to reschedule these compounds, our study will potentially expand the utility of psychedelics as a promising therapeutic to alleviate disease phenotypes of SCN2A-related ASD.

Poster 19: Unraveling SCN2A: exploring the effects of premature stop codons in mouse model behavior

Authors: Maria Fernanda Hermosillo Arrieta, Katelin E J Scott, Ahmad Al Saneh, Lionel Gissot, Karli Benson, Karina Kruth, Carly van der Heide, Christopher Ahern, Aislinn Williams

University of Iowa

Abstract: The gene SCN2A encodes for the voltage-gated sodium channel, NaV1.2, which plays a vital role in the propagation and backpropagation of action potentials. Premature termination codon (PTC) mutations in SCN2A have been linked to autism spectrum disorder and intellectual disability, which are associated with sociability and learning differences. PTCs are hypothesized to reduce protein by 50%, and are expected to cause similar behavioral phenotypes regardless of where they occur in the gene. Here, we have characterized the behavioral phenotypes of two SCN2A mouse models with patient PTC mutations, Y84X and R1626X. Preliminary data show that Scn2aY84X/+ males have deficits in motor learning in the rotarod assay compared to their wild-type (WT) littermates despite normal locomotor function. This is not observed in the female Scn2aY84X/+ mice or in mice carrying the R1626X mutation. Additionally, female Scn2aY84X/+ mice show lower levels of anxiety-like behavior in the elevated zero maze compared to their WT counterparts. This trend has not been observed in male Scn2aY84X/+ mice or the R1626x line. Finally, we have not observed any impairments in either line associated with gait adaptation and associative learning on the Erasmus Ladder. These data suggest that behavioral phenotypes differ between these two mouse models of SCN2A PTCs. Future work will involve expanding our data set to be fully powered to detect sex differences and exploration of other behaviors, such as tail

chasing and rearing, seizure activity, and sleep dysregulation. We aim to use these mouse models to understand the disease mechanisms of SCN2A PTCs.

Poster 20: The severity of absence-like seizures in Nav1.2 deficiency related disorder is associated with strain difference in mice.

Authors: Zaiyang Zhang; Jingliang Zhang; Brody Deming; Shivam Kant; Riyi Shi, Yang Yang

Purdue University

Abstract: Voltage-gated sodium channel 1.2 (NaV1.2) related disorder is a monogenic disease that results in neurodevelopmental disorders. Patients with loss-of-function (LoF) NaV1.2 mutations display diverse phenotypes including autism spectrum disorder (ASD) symptoms and late onset seizures. These seizures vary in severity and oftentimes are resist to antiepileptic drugs. The individual differences observed in disease manifestation and drug response suggest that genetic background might play a role in the etiology of NaV1.2 LoF epilepsy. To study human genetic diseases, mouse models have provided numerous insights. It has been suggested that certain stains (e.g., DBA/2J) of mice are more susceptible to seizures than others (e.g., C57BL/6) due to their differentially expressed genes. Interestingly, we found a significant difference in absence seizure severity between transgenic mice of these two strains, despite their same NaV1.2 LoF genetic construct. This suggests that the genetic background of these two mouse strains may have influenced neurodevelopmental events in response to NaV1.2 LoF, which greatly impact the resulting seizure phenotype. We are currently studying studying the transcription and translational differences in an effort to elucidate the etiology of NaV1.2 LoF related seizures. By examining the underlying mechanisms, the study can provide valuable insights into the individual differences in human epilepsy patients and foster precision medicine in future clinical treatments.

Poster 21: Dissecting the Mechanisms of Seizure Phenotypes in Scn2a-deficient Mice Using Neuropixels and Two-Photon Imaging

Authors: Yuanrui Zhao, Jingliang Zhang, Zongyue Chen, Xiaoling Chen, Jiaxiang Wu, Ye-Eun Yoo, Maria I. Olivero-Acosta, Brody A. Deming, Kyle Wettschurack, Manasi S. Halurkar, Shivam Kant, Purba Mandal, and Yang Yang

Purdue University

Abstract: Mutations in the SCN2A gene, which encodes the NaV1.2 sodium channel, are linked to severe neurological disorders, including epilepsy, autism spectrum disorder, or intellectual disability (ASD/ID). The SCN2A loss-of-function (LoF) mutations particularly result in ASD/ID and late-onset epileptic encephalopathy. This study aims to elucidate the underlying mechanisms associated with epilepsy linked to SCN2A deficiency using high-resolution Neuropixels recording and two-photon calcium imaging in a Scn2a-deficient mouse model. We used PTZ (pentylenetetrazole)to induce seizures in these Scn2a-deficient mice. We are studying neural activity during baseline and PTZ-induced seizure states by recording with Neuropixels, which will provide comprehensive, multi-region electrophysiological data longitudinally across the brain. Notably, we are observing elevated synchronization patterns, which are strongly enhanced across multiple brain regions during induced seizure episodes in Scn2a-deficient mice. Conversely, two-photon imaging was conducted in motor cortical layers to visualize calcium transients reflective of

neuronal activity at the cellular level and hypersynchronizations at the network level. Furthermore, we are performing experiments to test the efficacy of the available sodium channel blockers like phenytoin and GABA enhancing anti-epileptic medication (e.g., clonazepam) on these identified neuronal and circuitry phenotypes. This study will highlight the utility of high-throughput Neuropixels recording and advanced neuroimaging techniques to uncover the complex neural basis of epilepsy associated with SCN2A deficiency. Our study would also provide basis for evaluating the effectiveness of potential therapeutic interventions.

Poster 22: Effects of neonatal gonadal hormones on Autism Relevant Phenotypes

Authors: Emily Hagan, Pravda Quinones-Labernik, Danielle Preuschl, Charlotte Tesar, Sarah L Ferri

University of Iowa

Abstract: Social behavior is seen across almost all living species and disruptions to such behaviors can result in decreased chances of survival, reproduction, and quality of life. Social deficits are exhibited in neuropsychiatric and neurodevelopmental disorders, and are a core symptom of autism spectrum disorder (ASD). ASD affects 1 in 36 children and 4 times as many males than females. Within an ASD diagnosis, males are more likely to exhibit social impairments. The mechanism of this robust sex bias is not well understood. Here, we used neonatal injections of gonadal hormones as a novel experimental system to disrupt sex-specific developmental pathways in mice to determine their effects on behaviors relevant to ASD. We found that testosterone administration on the day of birth, which is equivalent to late gestation in humans, induces male-specific deficits in social approach and fear memory. However, these deficits only occurred when injected on the day of birth. Furthermore, while testosterone injected on the day of birth did cause social and contextual fear conditioning deficits, it did not affect anxiety-like behavior. Administration of D-cycloserine, a NMDAR partial agonist, which has been shown to ameliorate social deficits preclinically, alleviated the testosterone-induced social and fear deficits. Surprisingly, estradiol given on the day of birth did not lead to social deficits. Currently we are investigating the mechanisms of these sex specific vulnerabilities to social and fear deficits. These findings will aid in advancing the current understanding of how the brain is susceptible to social impairments and help identify novel treatment targets.

Poster 23: Ribbon synapse assembly and refinement in human inner ear organoids

Authors: V.Shweta Reddy and Dr. Eri Hashino

Indiana University-Purdue University Indianapolis

Abstract: Mechanosensitive hair cells in the inner ear form specialized ribbon synapses with afferent sensory neurons that transmit electrical signals to the brain. Little is known how these ribbon synapses are assembled and refined during human inner ear development. Using human pluripotent stem cell-derived inner ear organoids as a model system, we tested if temporal changes in expression of pre- and post-synaptic proteins recapitulate those in the mouse inner ear. Human embryonic stem cells were differentiated into inner ear organoids, and at d80, 120, 160 or 200, samples were fixed and subjected to wholemount immunofluorescence followed by tissue clearing. We found that the number, size and shape of CTBP2+ puncta, representing pre-synaptic

ribbons, undergo temporal changes during hair cell maturation. Quantitative immunofluorescence analyses of Z-stack images of cleared samples revealed a temporal increase in the number of CTBP2+ puncta per hair cell and a steady increase of CTBP2+ area between d80 and 200. These results suggest that the number of ribbon synapses increases during hair cell differentiation, followed by a decrease due to refinement. Additionally, the size and shape of ribbons change during hair cell maturation. These results are consistent with the temporal morphological changes during ribbon synapse maturation in mouse cochlear and vestibular hair cells. Investigation is currently underway to assess temporal changes in the recruitment of ribbons to the active zone.

Poster 24: Developmental Exposure of Human Induced Pluripotent Stem Cell-derived Cortical Cultures to Methylmercury Induces Persistent Functional Effects

Authors: Madeleine M. Strom, Anke M. Tukker, Michael Aschner, Aaron B. Bowman

Purdue University

Abstract: Methylmercury (MeHg) exposure during early neurodevelopment has long been associated with neurological impairments, yet the underlying mechanisms and long-term consequences remain elusive. This study investigates the hypothesis that developmental MeHg exposures cause persistent changes in spontaneous neuronal network activity in human-induced pluripotent stem cell (hiPSC)-derived both male and female cortical cultures. This study modeled early developmental exposures utilizing male and female hiPSC-derived cortical glutamatergic neuron models. Developing cultures were exposed to environmentally relevant MeHg concentrations of 0.1 μM and 1.0 μM for a six day period. Once functionally mature, cells were plated onto microelectrode arrays (MEAs) at day 110 of differentiation, and spontaneous neuronal network activity was assessed. Mean burst and network burst (MBR and MNBR) MEA activity showed significant and persistent changes in the exposed cultures. MBR was increased in the 0.1 μM cultures and decreased in the 1.0 μM group. The spontaneous MNBR was significantly decreased (p<;0.05) in 0.1 μM and 1.0 μM MeHg-exposed groups. Findings indicate that developmental exposure to MeHg of differentiating hiPSC-derived cortical cultures results in altered electrical and physiological activity in fully matured cultures. To fully understand sex differences in baseline MEA activity, more tests would need to be run; it is too early to understand whether effects are cell line or sex dependent. Understanding these persistent electrophysiological effects holds significant implications for prenatal and neurodevelopmental exposure to MeHg. This research was supported by NIH R01 ES07331 (ABB/MA) and R01 AG080917 (ABB).

Poster 25: Without a Trace: Investigating Residual Effects of Methylmercury on Neuronal Development

Authors: Serena Shughoury, Mia S. Fleisher-de Kozan, David S. Yi, Anke M. Tukker, Aaron B. Bowman

Purdue University

Abstract: Methylmercury (MeHg), a heavy metal notorious for its developmental neurotoxicity, poses a significant threat to the developing brain. Chronic (low-dose) exposure to MeHg during brain development has been linked to neurological and developmental deficits, potentially increasing susceptibility to neurodegenerative diseases. Even after the cessation of exposure,

persistent developmental changes occur in developing neurons. Prior experiments suggest a link between early exposure and lasting neurotoxic effects; however, mercury analysis 1-2 weeks after exposure displayed detectable quantities of MeHg in the cultures, which we suspected was due to MeHg being bound to the culture dishes. We hypothesized that following the cessation of MeHg exposure, the differentiated neuron cell cultures will rapidly eliminate MeHg, resulting in undetectable levels within a few days post-exposure. In this study, a human induced pluripotent stem cell (hiPSC)-derived cortical glutamatergic culture was exposed to non-cytotoxic levels of MeHg (1 μM) during a critical developmental window (days 4–10 in vitro). Aliquots of daily media formulations and samples were collected daily, starting at the initiation of MeHg exposure. Additionally, cells were collected and lysed on days 11, 14, 17, and 21. The concentration of MeHg in the media and cell samples was quantified using a direct mercury analyzer. Following exposure cessation, MeHg concentrations significantly decreased in both cell media and within cells, indicating potential detoxification mechanisms. The calculated half-life of MeHg within cells suggests efficient removal of MeHg within a few days post-exposure, highlighting the importance of understanding the elimination kinetics of MeHg in neuronal cells for neurotoxicity research.

Poster 26: Investigating genetic drivers of tumor recurrence in patient-derived pilocytic astrocytoma cell lines

Authors: Koenig JK, Dershem V , Burket NJ, Cooper S, Tailor JK

Indiana University School of Medicine

Abstract: Pilocytic astrocytoma (PA) is the most common brain tumor in children and considered benign, as surgical resection is often curative. However, recurrence of PA has been reported even after gross total resection, leading to increased morbidity and lower overall survival compared to less aggressive PA. Maintaining stable pLGG cell cultures has traditionally been challenging, but has been improved through enrichment of stem cell populations in patient-derived cells. We have established 4 cell lines from primary and recurrent pediatric PA patient tumors and have cultured them in neural stem cell media to enrich for stem-like populations. We aim to characterize stem-like features and the mutational landscape of these lines with the objective of genetically manipulating proposed drivers of recurrence, such as CDKN2A. Deletion of the tumor suppressor CDKN2A has been associated with malignant transformation and aggressive phenotypes in gliomas, including pLGG. Among these cultures, differences in molecular landscape and growth rate have been observed. Examination of neural stem-like markers by RT-qPCR and immunocytochemical staining has shown expression of markers such as nestin and SOX2 in these lines. Future studies will further characterize features of stemness in these lines and will employ CRISPR-Cas9 technology to induce genetic knockout of CDKN2A in these lines and investigate phenotypes of aggression and recurrence through assays in vitro and in vivo. These studies have the potential to experimentally identify molecular risk factors for recurrence in PA which could impact molecular diagnostics and provide rationale for therapeutic targeting of these molecular alterations to prevent recurrence.

Poster 27: Investigating the Functional Consequences of NF2 Gene Loss in Neural Progenitors and Developmental Origins of Spinal Ependymoma

Authors: Noah Burket, Sidrah Ahmed, Jia Wang, Scott Cooper, Victoria Dershem, Hongyu Gao, Rob Bell, Chi Zhang, Jignesh Tailor

Indiana University School of Medicine

Abstract: NF2-related schwannomatosis (NF2) is caused by mutations in the NF2 gene resulting in patients developing multiple central nervous system (CNS) tumors, one of which is spinal ependymoma (SP-EPN). SP-EPNs are intramedullary tumors that disrupt critical nerve tracts as they grow; however, there are no medical therapies for this tumor. Potential therapeutic targets may be discovered through study of the developmental origins of SP-EPN, which are currently unknown. Prior studies revealed that SP-EPNs may arise from radial glia (RG) progenitor cells and are likely driven by NF2 mutations. Therefore, we hypothesize that NF2 gene loss may prevent normal RG cell development and the tumorigenesis of SP-EPNs. CRISPR-Cas9 was used to create NF2 knockdowns in human neuroepithelial stem (NES) cells. These cells were cloned and validated through western blot, RT-qPCR, and microscopy. Spatial transcriptomics was performed on a SP-EPN from a patient with NF2. Preliminary studies with NES cells with knocked-down NF2 show that these cells retain an early neural progenitor phenotype and form clusters when allowed to differentiate. Cells taken from these clusters continue to proliferate and form more clusters. Our spatial data shows diffuse expression of astrocyte and ependymal cell genes with rare populations of cells that express RG cell genes. Our in vitro findings suggest that NF2 loss may be preventing normal NES cell differentiation, forming clusters of cells that maintain a persistent progenitor-like phenotype. The spatial data suggests a developmental hierarchy exists within SP-EPN which may be initiated by aberrant RG-like cells.

Poster 28: State-dependent activity in the infant rat dorsal raphe nucleus

Authors: Yasko AP, Dooley JC

Purdue University

Abstract: Throughout early infancy, the brain develops neural circuitry that processes sensory information. Traditionally, experiences during wake are heavily credited as a driver of sensory processing development. However, infants spend more time asleep than awake, and infant brains are more active during sleep. Rodent studies reveal that during the first 10 postnatal days (P10), sensory-driven neural activity is actively inhibited during periods of wake. This inhibition subsides by P12, but the underlying mechanism behind this state-dependent modulation of sensory processing remains unclear. One possible explanation for the state-dependent modulation of sensory responses is the expression of transient serotonin receptors (5HTr), which are present in the primary cortical areas until around P12. In early infancy, when serotonin activates these transient 5HTr, excitatory neurotransmission of sensory information is suppressed. In adults, 5HTr are no longer present, so serotonin release does not suppress sensory responses. While we know that in infants, serotonin inhibits neural activity, we have yet to understand how serotonin levels vary in infants across wake and sleep. The dorsal raphe nucleus (DRN) is home to many of the brain's serotonergic neurons. Thus, to assess state-dependent serotonin activity in infants, we recorded from the DRN of P12 rats. In DRN neurons, we observed 5 times more neural activity during wake compared to sleep. These findings suggest that DRN activity follows the same pattern

across sleep and wake as seen in adults, suggesting serotonin underlies the state-dependent modulation of sensory responses in infancy.

Poster 29: The influence of spinophilin on movement by regulating postsynaptic dopamine 2 receptor

Authors: Basant Hens, and Anthony J. Baucum II

Indiana University-Indianapolis

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

Poster 30: Bilateral integration in somatosensory cortex is controlled by behavioral relevance

Authors: Hyein Park, Hayagreev Keri, Chaeyoung Yoo, Chengyu Bi, Scott Pluta

Purdue University

Abstract: Sensory perception naturally requires processing stimuli from both sides of the body. Yet, how neurons bind stimulus features across the hemispheres to create a unified perceptual experience remains unknown. To address this question, we performed large-scale recordings from neurons in both somatosensory cortices (S1) while mice shared information between their

hemispheres and discriminated between two categories of bilateral stimuli. When expert mice touched stimuli associated with reward, they moved their whiskers with greater bilateral symmetry. During this period, synchronous spiking and enhanced spike-field coupling emerged between the hemispheres. This coordinated activity was absent in stimulus-matched naïve animals, indicating that interhemispheric (IH) binding was controlled by a goal-directed, internal process. In S1 neurons, the addition of ipsilateral touch primarily facilitated the contralateral, principal whisker response. This facilitation primarily emerged for reward-associated stimuli and was lost on trials where expert mice failed to respond. Taken together, these results reveal a novel state-dependent logic underlying bilateral integration in S1, where stimulus binding and facilitation are controlled by behavioral relevance.

Poster 31: Bilateral integration in motor cortex is controlled by behavioral context and feedforward input from sensory cortex

Authors: Hayagreev V.S. Keri, Sydney J. Sneed, Scott R. Pluta

Purdue University

Abstract: Animal behavior naturally involves coordinated movements guided by sensory feedback on both sides of the body. This bilateral coordination depends on the ability to bind stimuli across the hemispheres and create a unified perceptual experience. The neural computations and brain areas supporting bilateral coordination are unclear. We recently discovered that the primary somatosensory cortex is the first stage of bilateral integration during active tactile sensation. Nonetheless, the brain areas downstream from S1 that control the flow of bilateral information and guide behavior are unknown. One possibility is the motor cortex (MC), which receives strong feedforward input from S1. We discovered that MC neurons build stimulus and task-specific representations of bilateral space, but only in mice that were deliberately coordinating tactile information between their hemispheres. MC neurons in naïve mice and mice trained on a unilateral task did not integrate stimulus features across their hemispheres. Thus, bilateral integration in MC is controlled by behavioral context. To determine the circuit mechanisms of bilateral integration in MC, we optogenetically silenced S1 axons inside MC. S1 axon silencing reduced task performance, bilateral movement symmetry, and stimulus selectivity in MC neurons. Thus, the direct feedforward flow of tactile information from S1 to MC is critical to binding stimulus features across the hemispheres and coordinating bilateral movements.

Poster 32: The emergence of value-modulated somatosensory processing in superior colliculus

Authors: Yun Wen Chu, Suma Chinta, Hayagreev V.S. Keri, Shreya Beri, Scott R. Pluta

Purdue University

Abstract: A fundamental feature of intelligent behavior is the ability to respond selectively to the stimulus with highest value. Where along the sensory hierarchy does information processing transition from a map of stimulus features to a map of stimulus value? To address this question, we recorded single-unit activity from populations of neurons in somatosensory cortex (S1) and midbrain superior colliculus (SC) in mice conditioned to respond to stimulation of a positive-valued whisker and withhold responses to stimulation of an adjacent, negative-valued whisker. The

stimulus preference of the S1 population was equally weighted towards either whisker, in line with the somatotopic map. Surprisingly, we discovered a large population of SC neurons that were disproportionately biased towards the positive-valued whisker. This disproportionate bias was largely controlled by spike suppression during the negative-valued stimulus. Removing the opportunity for mice to respond to positive-valued whisker stimulation reduced sensory responses in SC but not S1 neurons, indicating that stimulus bias in SC neurons was partially controlled by motor preparation. Similarly, the spontaneous firing rates of SC but not S1 neurons accurately predicted operant response latency, indicating that SC neurons played a persistent role in task readiness. Taken together, these data reveal that somatosensory processing transitions from a map of physical space in sensory cortex to a map of stimulus value in SC that guides target selection.

Poster 33: The joint representation of self-motion and touch in superior colliculus neurons supporting active sensation

Authors: Chinta S, and Pluta SR

Purdue University

Abstract: Localizing objects using active sensing requires the brain to integrate a map sensory space against an ongoing estimate of self-motion and body position. While such computations are often ascribed to the cerebral cortex, we examined the midbrain superior colliculus (SC), due to its close relationship with the sensory periphery as well as higher, motor-related brain regions. Using high-density electrophysiology and movement tracking, we discovered that the kinematics of active whisking and locomotion speed accurately predict the firing rate of mouse SC neurons. Neural activity was best predicted by movements occurring either in the past, present, or future, revealing that the SC population continuously estimates the trajectory of self-motion. Neural selectivity for combinations of kinematic features built an accurate representation of whisker position with high temporal resolution. Half of all self-motion encoding neurons displayed a touch response as an object entered the active whisking field. Trial-to-trial variation in the size of this response was explained by the position of the whisker upon touch. Taken together, these data indicate that SC neurons linearly combine an internal estimate of body movements with external stimulation to enable active tactile localization.

Poster 34: Traveling waves enable reliable volitional motor movement

Authors: Hammad F. Khan, Om T. Kolhe, Meisam Habibimatin, Eduard Tanase, and Krishna Jayant

Purdue University

Abstract: Traveling waves (TWs) are an emergent phenomenon observed in dynamical systems throughout nature. These TWs mediate various aspects of animal cognition, such as stimuli perception, volitional movement, and working memory. Theoretical studies have suggested that these TWs play an important role in preserving time during information transfer between two brain regions and for plasticity across long-range neural circuits. Yet their potential functional and behavioral relevance remains unknown. In this work, by implementing custom-designed flexible high-density NeuroGrids, we demonstrate that traveling waves distinctly reflect task-relevant

information in mice performing a contextual volitional motor task. Specifically in the primary motor cortex, propagating traveling wave phase-directionality reflected impending movement after an external stimulus. In contrast, the propensity of precise wave generation relied on the presence of external context. This was reflected by changes in the reliability of local spiking populations of cortical neurons across task conditions, which tightly coupled with ongoing wave dynamics within a lower dimensional state-space. A 3D temporal convolutional neural network trained on just the phase gradients of surface LFP accurately predicted behavioral outcomes, indicating that the surface TWs carry behaviorally relevant information. Using focal cooling and optogenetic inhibition, we show that the secondary motor cortex modulates the structured generation of traveling waves and correct motor execution via distinct pathways: cortical and subcortical through the motor thalamus. Thus, our results suggest that traveling waves predict task-specific computations required for reliable volitional movement and can dynamically coordinate activity between distinct brain regions via bottom-up and top-down pathways.

Poster 35: Visual cortical circuits for generating visual experience-dependent oscillations

Authors: Xi Cheng, Peiyi Zhang, Fang Huang, Alexander A. Chubykin

Purdue University

Abstract: Sensory representation, learning, and memory in the visual cortex correlate with neural oscillations across the laminar cortical layers. These oscillations are generated by circuits comprising excitatory pyramidal cells (PCs) and inhibitory interneurons (INs). Typically, INs are grouped with neighboring PCs to create specific local recurrent microcircuits, which regulate or modulate neural information flow. We found that visual familiarity induced low-frequency oscillations in the primary visual cortex (V1). Elevations of L5 PC input on L4 fast-spiking interneurons in V1, detected in ex vivo circuit mapping after visual experience, may underlie the 4-8 Hz low-frequency oscillations induced by visual familiarity. The local recurrent circuit in V1 involving excitatory PCs and parvalbumin (PV) inhibitory INs plays a crucial role in visual experience-dependent oscillations. To investigate the visual familiarity-induced synaptic plasticity in the local recurrent microcircuit between PV INs and PCs, we applied channelrhodopsin-2-assisted circuit mapping (CRACM) in acute brain slices to map the reciprocal connectivity between PV INs and PCs in mice naive or experienced with the perceptual familiarity paradigm. We found a decrease in average IPSCs after visual experience from PV+IN to PCs in L2/3 and an increase in L4, while no changes were detected in L5. The average EPSC inputs from L5 PCs to L5 and L4 PV INs increased, whereas there was no difference in L2/3 PV INs after the visual experience. Overall, perceptual experience elicited synaptic plasticity in the microcircuit that was layer-specific in V1 with distinct patterns in deep layers and superficial layers. Our studies provide direct measurements of the synaptic connectivity between PV INs and PCs in V1 before and after visual experience, revealing the mechanisms of the emergence of visual experience-dependent neural oscillations.

Poster 36: Neural correlates of perceptual motion integration and segmentation of locally paired and unpaired random-dot stimuli in middle temporal (MT) and primary visual cortex (V1)

Authors: Bikalpa Ghimire, Steven Weisner, Xin Huang

University of Wisconsin-Madison

Abstract: Primate visual system can integrate local motion vectors into a global motion pattern and segment multiple visual stimuli based on motion cues. Previous psychophysical studies showed that when random dots of overlapping stimuli moving in two directions were locally paired, human subjects perceived an integrated vector-averaged (VA) direction of the stimuli; whereas when the dots were unpaired, subjects perceived motion transparency and two component directions. The mechanism underlying this drastic perceptual change with such local spatial modulation is unknown. Neurons in higher-order areas have larger receptive fields (RFs), ideal for integrating motion information across space and stimulus features and lower visual areas with smaller RFs is ideal for processing local motion signals and can preserve different local motion signals, essential for segmentation. It remains unclear where and how motion segmentation, that is, the distinction between multiple motion surfaces, occurs. We recorded from neurons in the middle-to-higher visual area middle-temporal (MT) and lower visual area primary visual cortex (V1) of fixating monkeys. In the "paired-dot" condition, two dots moving in different directions were locally paired within a path of 0.4^{0}. In the "unpaired-dot" condition, dots moving in two directions were unpaired and had the same lifetime. Our findings reveal neural correlates of this intriguing perceptual phenomenon and have implications for the roles of local computation in early visual areas on motion integration and segmentation.

Poster 37: A zebrafish functional screen identifies leads from FDA-approved drugs for treating retinitis pigmentosa

Authors: Wang B, Ganzen L, Zhu X, James R, Tsujikawa M, and Leung YF.

Purdue University

Abstract: Retinitis pigmentosa (RP) is a genetically inherited form of retinal degeneration that affects approximately 1 in 4,000 people worldwide. RP can be caused by mutations in phototransduction genes, including RHODOPSIN (RHO). One RHO mutation, Q344X, can cause early-onset autosomal-dominant (ad) 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. To achieve this aim, we screened a SelleckChem FDA-approved drug (FDA) library (1430 compounds) on a transgenic Q344X zebrafish model, displaying an early RP onset like human patients. We used a visual-motor response (VMR) assay, where Q344X larvae showed reduced responses compared with wildtype (WT) during light offset at 7 days post-fertilization (dpf). Therefore, we expected that potential hit drugs would ameliorate Q344X responses. We exposed Q344X larvae to 10 uM compounds from 5 to 7 dpf. We first eliminated 191 drugs that showed toxicity (13.4%) and then measured the effect of 1239 non-toxic drugs in the VMR assay. The positive and negative controls were drug-carrier-treated WT and Q344X, respectively. After the screening, we identified two types of positive hits: 1) 8 compounds that increased Q344X light-Off VMR compared with the negative controls (Welch's t-test, Bonferroni-adjusted $p<0.05$), and 2) another 8 compounds induced Q344X to display a light-Off VMR profile similar to that displayed by the positive WT controls (analyzed by hierarchical clustering). We investigated one hit that induced the Q344X larvae to display a WT-like VMR and found this hit induced more rod photoreceptors in the Q344X eyeballs. These results suggest that the hit can

potentially benefit Q344X RP patients. Our future characterizations will identify more potential hits for downstream drug development.

Poster 38: Examining the origin of visually-evoked theta oscillations

Authors: Zimmerman MP, Kissinger ST, and Chubykin AA

Purdue University

Abstract: Our brains are continuously deciphering what action needs to be taken given the situation in the world around us. One line of thought proposes that prediction errors drive much of the brain's focus. At the core of this theory is a separation of stimuli into one of two categories; novel or familiar. In mice, visual experience is shown to give rise to visually evoked theta (4-8 Hz) oscillations in the primary visual cortex (V1). Recently, our lab has shown the presence of these oscillations in higher-visual areas, which are synchronized in a context-dependent manner. It remains unclear, however, where these unique oscillatory dynamics originate. To address this, we conducted paired extracellular silicon probe recordings in two visual thalamic nuclei (dorsal lateral geniculate nucleus, dLGN, and lateral posterior thalamus, LP), the retrosplenial cortex (RSC), which is a non-visual cortical area directly connected with V1, and the hippocampus (HPC), which is known to be involved in memory encoding and retention. We find that both thalamic nuclei show no oscillatory activity, however, RSC and HPC demonstrate a sparse population of neurons with theta oscillations, with the RSC activity temporally delayed. These results suggest (1) the oscillations are primarily originating in V1 and not initially in thalamic regions, and (2) the HPC activity is likely independent from V1. Overall, this work sheds light on the purpose of these visually-evoked theta oscillations and determines the role they play in cross-brain relay of information for memory encoding and retention.

Poster 39: Auditory system development in a rat model of Tuberous sclerosis complex

Authors: Inamdar M, Hart LM, Sun Z, James NP, Auerbach BD.

University of Illinois Urbana-Champaign

Abstract: Despite great progress in identifying genetic variations associated with increased prevalence of autism spectrum disorders (ASD), the challenge now is to determine if these mutations converge on common mechanisms that can account for the diverse phenotypes that define ASD. One potential convergence point may be during early life critical periods, a time in early post-natal development when neural circuits are extremely plastic and drastically shaped by sensory experience. Altered critical periods may be particularly important for atypical sensory processing in ASD, a defining feature of the disorder that greatly impacts quality of life. To test this hypothesis, we characterized the development of critical period markers in rat model of Tuberous Sclerosis Complex (TSC), one of the most common genetically defined causes of ASD. Specifically, we used neuroanatomical analysis to examine parvalbumin positive (PV+) interneuron and perineuronal nets (PNN) expression in the auditory cortex (ACx) of a Tsc2+/- Eker rat model of TSC across developmental time-points. We chose to focus on these structures because the maturation of PV+ inhibitory interneurons and their envelopment by PNNs is thought to be a major driver of critical period closure. Preliminary results suggest that there are indeed differences in PV+ expression, PNN expression, and PV+/PNN co-localization in the ACx of Tsc2+/- rat models. These

results indicate that auditory symptoms in TSC could be related to changes in the developmental time-course of PV+ and PNN expression in the ACx.

Poster 40: Tiny but mighty ears: Auditory Systems in Frog-Biting Mosquitoes, Uranotaenia lowii

Authors: Richa Singh and Ximena Bernal

Purdue University

Abstract: Hearing relies on specialized auditory sensory organs that transform mechanical vibrations caused by sound into electrical signals. In mosquitoes, hearing involves sound-induced mechanical displacement of the flagellum which stimulates the auditory efferent systems in the Johnston's organs (JO) situated at the base of their antenna. Male mosquitoes use sound for communication in close-range conspecific mating contexts, whereas frog-biting females from some mosquito species use hearing to respond to long-distance frog mating calls for host detection. This study examines the auditory system of female Uranotaenia lowii, a mosquito species that exclusively feeds on frogs by eavesdropping on their mating calls. To examine the auditory structure and function, we used a comprehensive approach involving biomechanical, neuroanatomical, and behavioral analyses of the flagellar ears. We integrate mechanical antennal vibration to sound in flagellar ears using Laser Doppler Vibrometry, auditory innervation of the JO using immunohistochemistry, and phonotaxis experiments to examine the acoustic response of this mosquito specialized on cueing on long-distance host-emitted calls. Our findings reveal the host detection-based adaptations in mosquito auditory structure and function. This study contributes to enhancing our understanding of mosquito acoustic communication in the context of host-prey interactions.

Poster 41: Love songs and blood quests: How mosquitoes hear for mating and meals

Authors: Shilpi Singh, Richa Singh and Ximena E Bernal

Purdue University

Abstract: Mosquitoes have flagellar ears that depend on the Johnston’s organ (JO) enclosed in the second segment of the antennae, to detect sound. They use sound in sex- and species-specific functions such as mating and foraging for blood meals. While the use of sound in mating is widespread among mosquitoes, the use of sound for locating hosts is less common. Wingbeats are used in close-range interactions as males locate females in mating swarms and both sexes match the upper harmonics of their wingbeats. In contrast, foraging is a far-range female-specific acoustic behavior as they approach their hosts, calling frogs. Given the emphasis on studying mosquitoes of medical importance, which use sound only in mating, little is known about the variation in acoustic neural substrate among species using sound in different functional contexts. To address this knowledge gap, we studied the Johnston’s organ of males and females of mosquito species that use sound in different contexts or do not use sound. We performed immunohistochemistry on the JOs that revealed sex- and species-specific variation in the auditory innervation. However, the innervation pattern is highly conserved in the basal plate of mosquitoes using sound either for foraging or mating. This similarity in the innervation pattern of the basal plate at the junction of the antennal flagellum and the JO might be important for sound detection. This

work highlights the neural mechanisms underlying sound detection using flagellar ears and potential hearing adaptations in mosquitoes.

Poster 42: Short-term longitudinal changes in peripheral and subcortical auditory processes in response to small arms fire-like noise exposure

Authors: Meredith Ziliak, Andres Navarro, Emily Bell, Edward L. Bartlett

Purdue University

Abstract: In 2019, Altschuler et al. identified threshold changes associated with persistent damage to outer hair cells and a reduction in cochlear synapses following exposure to noise resembling small arms fire (SAF). However, early (<10 weeks post-exposure) adaptations of peripheral and subcortical pathways, especially dynamic, region-specific changes in gain, are still not well understood. The purpose of this study is to investigate underlying mechanisms of SAF noise-induced hearing loss (SAF-NIHL) to better inform development of therapeutic strategies. We used traditional diagnostic methods to identify biomarkers and suggest mechanisms of progressive stages of SAF-NIHL. Rodent subjects were exposed to SAF noise (50 biphasic 0.3 ms pulses at 120 dB peak SPL, 1 every 3 s) and analyzed thresholds, DPOAEs, and ABRs over 8 weeks to map longitudinal changes and identify characteristics most sensitive to SAF-NIHL. Following SAF noise exposure, DPOAEs showed a persistent decrease in OHC function (greatest sensitivity at 8 kHz). Thresholds demonstrated an approximate 20 dB temporary increase, which recovered about 10 dB, but not to baseline levels. ABR amplitudes were decreased after exposure, with W1 to a lesser extent, contradicting central gain as a resulting adaptation and further supporting a distinct diagnostic profile for SAF-NIHL. Interestingly, we observed a 20-40 dB change in the sound level needed to generate “equivalent” waveform amplitudes on post-exposure days compared to baseline. Future work involves imaging cochlear and brain tissues to determine anatomical correlations to the reported functional changes.