UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 17, 2019 Mary Gates Hall

Online Proceedings

1T

BRAIN FUNCTION, DYSFUNCTION AND REPAIR

Session Moderator: Kathleen Millen, Pediatrics

JHN 175

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Using Resting-State Functional Connectivity to Detect Uncomplicated Mild Traumatic Brain Injury

Josh Wolfe, Senior, Psychology
UW Honors Program
Mentor: Tara Madhyastha, Radiology
Mentor: Christine Mac Donald, Neurological Surgery

Detection of uncomplicated mild traumatic brain injury (mTBI) is difficult because there are no visible brain lesions that are often associated with more severe forms of TBI. New biomarkers would allow doctors to more sensitively screen for mTBI using neuroimaging methods. One promising biomarker technology is resting-state functional connectivity, which is brain activity measured at rest using functional magnetic resonance imaging. One particularly salient resting-state network is the Default Mode Network (DMN). Our research focused on identifying differences in resting-state functional connectivity between individuals diagnosed with mTBI and healthy controls. We examined mTBI in 254 U.S. military personnel deployed to a combat theatre in the Middle East from 2010-2013. Each subject underwent initial magnetic resonance imaging and screening for TBI following medical evacuation to Landstuhl Regional Medical Center (LRMC), the primary triage center for all evacuated combat casualties, up to 30 days post-injury. We used four distinct groups for our analysis; Blast/Non-Blast (n=79, 44) TBI, and Blast/Non-Blast Control (n=35, 96) while covarying for age and gender. We hypothesized that resting-state networks will be disrupted in TBI and blast populations when compared to controls. We used two different methodologies; the first was a seed-based analysis examining group differences in the correlations from the Posterior Cingulate Cortex (PCC, a key hub within the DMN) to the whole brain. The second analysis used the Yeo Seven Network parcellation to compute correlations between all seven networks to the DMN. We were unable to distinguish any group from controls, suggesting that early differences in functional connectivity are not a robust biomarker of injury.

Premature Migration of Cerebellar Granule Cells due to Disrupted Fetal Mesenchymal Signaling Drives Heterotopia Formation in Dandy Walker Malformation

Danilo Dubocanin, Senior, Biochemistry
UW Honors Program
Mentor: Kathleen Millen, Pediatrics, Seattle Children's Research Institute

Heterotopia are organized structures consisting of mixed cellular and neuronal elements arranged in a clear architectural pattern inappropriate to the considered tissue. Heterotopia are observed in the cerebral cortex and the cerebellum as a feature of many neurological disorders yet the we know little about the mechanisms driving their formation. We have analyzed a substantial number of Dandy Walker malformation (DWM) human fetal cerebella and found that a significant number of cases contain heterotopia. DWM is the most common structural birth defect of the human cerebellum and is characterized by an enlarged posterior fossa, enlarged fourth ventricle, and cerebellar vermis hypoplasia. A subset of cases are caused by loss of FOXC1, a transcription factor expressed in the mesenchyme during development. Our group has previously shown that loss of FOXC1 in mice causes loss of the mesenchyme-secreted factor SDF1alpha. Further, loss of SDF1alpha is sufficient to cause cerebellar heterotopia. This emphasizes the importance of mesenchymal signaling in the maintenance and development of the clear laminar architecture of the mature cerebellum. We hypothesized that granule neuronal progenitors (GCPs) are the primary cellular target of SDF1alpha mesenchymal signaling and therefore the main cell type causing heterotopia formation. To test our hypothesis, we excised the receptor for SDF1alpha from just GCPs in mice. Our findings show that loss of SDF1alpha in GCPs causes them to prematurely migrate into the developing cerebellar anlage and also caused other cerebellar cell types to form structured heterotopia. We observed defects in cerebellar foliation in the treatment group. Our data emphasizes the importance of SDF1-alpha dependent mesenchymal signaling in cerebellar development and identifies heterotopia as a

new phenotype in DWM.

Elucidating the Kinetics of STAT1 Phosphorylation in Response to TLR4 and IFNAR Agonists in Microglia

Rachel Anne Arnold, Senior, Neurobiology
UW Honors Program
Mentor: Jonathan Weinstein, Neurology

Ischemic preconditioning (IPC) is a robust, neuroprotective phenomenon in which a brief ischemic exposure confers resistance to injury from subsequent prolonged ischemia. Characterizing IPC may provide insight into better treatment options for those at high risk of ischemic stroke. Microglia, the immune cells of the brain, play an important role in the immune response to IPC. Previously, our laboratory found that the type 1 interferon signaling pathway in microglia is important in IPC-mediated neuroprotection. This signaling pathway is dependent upon activation of Toll-like receptor 4 (TLR4) and type 1 interferon receptor (IFNAR1). We hypothesize that in this pathway, damage-induced molecular patterns (DAMPs), which are released by brain tissues under ischemic conditions, activate TLR4 resulting in a signal cascade that activates IFNAR1, leading to phosphorylation of signal transducer and activator of transcription 1 (STAT1). Phosphorylated STAT1 (pSTAT1) then forms a complex with other proteins and induces transcription of multiple interferon-stimulated genes (ISGs). ISG expression alters the microglial phenotype, leading to neuronal and axonal protection against subsequent ischemia-related brain injury. The kinetics of type 1 interferon signaling in microglia are not yet fully understood. We aimed to further characterize this pathway by culturing primary microglia from wild-type mice, exposing them to TLR4 agonists or type 1 interferons directly, and quantifying pSTAT1 levels using flow cytometry at multiple time points. A time course of STAT1 phosphorylation in response to innate immune stimuli will provide a clearer picture of the kinetics of microglial type 1 interferon signaling in the setting of ischemia. These findings will enable us to optimize experimental timing for future experiments involving more complex and physiologic stimuli. Optimization of the kinetics of the pSTAT1 assay will also allow us to investigate how genetic ablation of specific innate immune signaling pathways (like TLR4 or IFNAR1) might modulate the microglial response to ischemia.

Evaluating the Presence of Microglia Progenitor Cells in the Adult Mouse Brain

Chloe Netania Winston, Sophomore, Pre-Sciences
Mentor: Gwenn Garden, Neurology
Mentor: Katherine Prater, Neurology

Microglia, the immune cells of the central nervous system, are long lived. In mice, microglia have an average lifespan of 15.5 months. When microglia are experimentally depleted from the mouse brain, microglia populations quickly return to steady state levels. The mechanisms of this observed repopulation are unclear. More importantly, the mechanisms of microglia replenishment in the healthy brain are not well understood. The literature supports two competing hypotheses. One is that microglia proliferate simply by dividing. Another possibility is that pools of microglia progenitor cells within the central nervous system divide and differentiate into microglia. I hypothesize that microglia proliferate primarily through the differentiation of progenitor cells. Available data to date suggest CD133 as a potential marker for microglia progenitor cells. In order to study these putative progenitor populations, I used a genetic reporter mouse line in which administration of tamoxifen induces TdTomato expression specifically in CD133-expressing cells. TdTomato, a red fluorescent protein, allows these cells to be visualized under a fluorescence microscope. Importantly, all the progeny of these cells also express TdTomato, allowing us to determine whether CD133 cells generate new microglia over time. After tamoxifen treatment at the age of 10 weeks, mice were sacrificed at three and nine months of age. Brains were fixed, sectioned, and labeled with antibodies to a microglia specific protein and to TdTomato. Daughter microglia that differentiated from CD133-expressing cells express both markers. Using a fluorescence microscope, I identified several microglia daughter cells of CD133-expressing cells. This suggests that microglia populations replenish in the healthy brain at least in part through the division of CD133-expressing cells. We can apply this new knowledge about how new microglia are generated in the healthy mouse brain to further our understanding of how microglia population dynamics are affected in both health and disease.

Investigating Splice Site Variants Associated with Epilepsy

Apoorva Chowdhary, Senior, Biochemistry
Mary Gates Scholar
Mentor: Heather Mefford, Pediatrics
Mentor: Alison Muir, Pediatrics

Developmental and epileptic encephalopathies (DEEs) are an early-onset form of epilepsy characterized by intractable seizures and severe cognitive and developmental impairment. While most genetic variants that cause DEE reside in the coding regions of genes, splice-site variants can also be pathogenic. Splice-site variants are changes in the DNA close to, or on, the exon-intron boundary, which can cause aberrant splicing, resulting in exon exclusion or intron inclusion within spliced mRNA, generating a protein that is non-functional, partially functional, or aberrantly expressed. Aberrant splicing can have pathogenic consequences, but predicting which variants near splice-sites will have an effect on splicing is difficult. I am studying three potential splice-site variants in three genes associated with DEE: SYNGAP1, SCN1B, and WWOX. I used RNA extracted from fibrob-

lasts from three DEE patients, each with one of these variants, to confirm whether the splice-site variants cause aberrant splicing and what the predicted consequences of this aberrant splicing is on the protein. I have been able to discover the effects of the variants on splicing in WWOX and SYNGAP1. In SYNGAP1, a synonymous variant at the exon-intron junction caused an exon 4 deletion, resulting in a severely truncated protein (SYNGAP1:p.Glu120Alafs*20). In WWOX, a duplication which included exon 5 resulted in a transcript with the inclusion of two copies of exon 5, leading to a frameshift mutation and predicted truncated protein (WWOX:p.His173Glyfs*13). We are still investigating the effects of the intronic variant in SCN1B, which appears to decrease expression of the gene. We are using nonsense-mediated decay inhibitors in order to better understand the mechanism through which this decreased expression occurs. This research could potentially improve the care of these patients by providing genetic evidence of the causes of DEE, which can be the basis for advancing better treatments.

Analyzing Developmental Epileptic Encephalopathy Patient Genomic Data to Detect Causative Copy-Number Variations

Brian Kumar Strobel, Senior, Computer Science, Biochemistry
Mary Gates Scholar
Mentor: Heather Mefford, Pediatrics

Developmental and Epileptic Encephalopathies (DEEs) are a group of severe epilepsy disorders in children and infants characterized by prominent EEG (electroencephalography) abnormalities that disrupt brain function leading to cognitive decline. Identifying genetic causes of DEE is a key step to help researchers develop and personalize medical treatments for affected patients. Approximately 5% of DEE cases are caused by a copy number variation (CNV), where a region of DNA involving a disease gene has been duplicated or deleted. Historically, this type of mutation has been difficult to detect using sequence data. To address this, I have written a multi-step algorithm that analyzes smMIP (single molecule molecular inversion probe) targeted DNA resequencing data for known DEE genes to identify CNVs in patients' DNA that are potentially disease causing. I have run this algorithm on the large collection of smMIP data for 1158 DEE patients available in the Mefford Lab and identified several potential CNVs. Among these, three CNVs ranging in size from 250,000-2,790,000 base pairs, each involving a DEE gene - GNB1, GRIA2, and UHRF1BP1L - were validated by a second method, array CGH, the current gold standard for CNV validation. To date, the validation rate of high-confidence CNV candidates is 50% or higher. Currently I am expanding the algorithm's functionality to include the ability to selectively search for single-exon CNVs, which are as small as 500 base pairs, are more challenging to detect, have largely been missed by all CNV detection methods, but could still be pathogenic. To do this, I am leveraging the power of intersecting duplicate smMIP datasets to improve the sensitivity of single-exon CNV detection. As any disruption of a pathogenic DEE gene could be disease causing, inclusion of these smaller CNVs will increase our ability to solve DEE cases and improve patient care.

Role of Nav1.1 in the Suprachiasmatic Nucleus

Lais Lastre Conceicao, Senior, Biochemistry, Neurobiology
Mary Gates Scholar
Mentor: Horacio de la Iglesia, Biology
Mentor: Ivana Bussi, Biology

Dravet syndrome (DS) is a severe form of childhood epilepsy caused by a mutation in the SCN1A gene, which encodes the NaV1.1 voltage-gated Na+ channel. This channel is present in most GABAergic neurons, the main inhibitory neurons in the brain. Reduced activity of the channel in DS leads to loss of inhibitory activity in the brain; this, in turn, leads to seizures and developmental deficits. Through previous research using the mouse model of DS, the de la Iglesia lab has demonstrated that DS also affects circadian rhythms, which are the endogenous biological rhythms synchronized to the 24 hour day. These symptoms are likely caused by the loss of NaV1.1 in a sleep regulatory center called the suprachiasmatic nucleus (SCN), a set of cells which functions as the 'master clock' of the circadian system of mammals. However, the de la Iglesia lab found that selective deletion of the SCN1A gene from the SCN fails to replicate the abnormal circadian phenotype. We believe that these mutant mice are phenotypically normal either because there is a compensatory increase in the expression of another sodium channel, NaV1.3, or because the targeting strategy does not reach all cells within the SCN. To test the first hypothesis we employed in-situ hybridization to visualize the expression of the genes that code for NaV1.1 and NaV1.3 channels in either SCN-specific knock outs or their wild type littermates. My results will help explain the phenotype seen in the SCN-specific SCN1A mutants and determine whether developmental compensatory mechanisms are important in the SCN of DS mice.

A Fear-Entrained Oscillator in the Mouse

Luis Eduardo Salazar, Senior, Biology (Molecular, Cellular & Developmental)
Levinson Emerging Scholar, Mary Gates Scholar
Mentor: Horacio de la Iglesia, Biology
Mentor: Ivana Bussi, Biology

Most organisms show a roughly 24-h cycle in their physiological and behavioral processes, called circadian rhythms, generated endogenously through the ˜24h cyclic expression of genes known as clock genes. Clock gene expression oscillates in the master circadian clock of mammals – the suprachi-

asmatic nucleus (SCN) - and nearly every cell of the body. Typically, circadian clocks and the rhythms they sustain are 'entrained' by the 24-h light-dark (LD) cycle. Our lab has found that fear can also behave as an entraining factor. We observed that when mice or rats need to leave a safe nesting area to access a foraging area, they forage and feed during the dark phase of the LD cycle. If the foraging area is rendered dangerous with random uncued footshocks during the active dark phase, the animals' foraging and feeding activity shifts to the light phase. My goal is to understand the neural circuits and molecular processes involved in fear entrainment. I have analyzed the expression of clock genes in animals exposed to nighttime fear and control animals exposed to daytime fear; this allowed me to assess the circadian rhythm of expression of clock genes of interest (Per1 and Bmal1) in the SCN and amygdala, and I found that the amygdala entrains to fear but the SCN does not. I have also performed trials with brain-specific-knockout mice and found that nocturnal fear entrainment requires an intact molecular clock. My current experiments use a more specific knockout strategy of viral injections into the brain to determine whether a functioning circadian oscillator in the basolateral amygdala (BLA) or the SCN is needed for nocturnal fear entrainment. These experiments serve to unmask the molecular mechanism of fear entrainment and could also help understand the mechanisms linking fear and anxiety disorders to problems with circadian rhythms and sleep.

Optimizing Biocompatibility and Conductivity of Brain-Computer Interfaces

Manjari M G (Manjari) Anant, Junior, Bioengineering
Mentor: Buddy Ratner, Bioengineering

Neurological diseases like stroke, paralysis and spinal cord injuries are some of the leading causes of disability and death across the world. Current medical treatments are not effective, and there is a world-wide effort to investigate new ways to restore function in the central nervous system. A treatment option that is gaining momentum is the use of brain-computer interfaces (BCIs), which has the potential to treat neurological diseases through reading and analyzing signals from the brain and sending electrical impulses to disease-affected areas. A significant obstacle that BCI implementation faces is biocompatibility, the ability for invasive devices to coexist with living tissues. Current BCIs are metal-based interfaces; their conductive properties allow them to efficiently record and send electrical brain signals. However, the human body elicits a foreign body reaction (FBR)- an immune reaction- in response to the "foreign" metal material. As a result, a capsule of scar tissue forms around the site of implantation, which mitigates the efficiency and longevity of BCIs. Hydrogels are an exciting organic material that have the potential to reduce FBRs and create biocompatible BCIs because of their elasticity, pliable material properties, and complex network structures. My project focuses on using poly(hydroxyethyl)methacrylate (pHEMA) as the base material of BCIs due to its ability to be accepted by the brain tissue after implantation. While pHEMA is biocompatible, it can not be used as a BCI in its current form because it is not conductive (not able to send and receive electrical signals in the brain). As a result, the gel is copolymerized with the conductive monomer 3,4-ethylenedioxythiophene (EDOT). This research project balances the conductivity and biocompatibility of the pHEMA-EDOT matrix to produce a new breed of long-lasting, efficient BCIs.