FROM CELLS AND MOLECULES TO SYSTEMS NEUROSCIENCE
Session Moderator: Horacio de la Iglesia, Biology
389 MGH
12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Characterization of IFNAR1 Gene Knockdown Efficiency in IFNAR<sup>f</sup>/<i>f</i> LysM<sup>Cre</sup> Line
Alexa Erdogan, Senior, Biology (Molecular, Cellular & Developmental)
UW Honors Program
Mentor: Jonathan Weinstein, Neurology

Ischemic preconditioning (IPC) in the brain is a neuroprotective phenomenon in which a brief ischemic exposure increases resistance to the injurious effects of subsequent prolonged ischemia, or inadequate blood supply to the brain. Several lines of evidence support a role for microglia in IPC. My lab recently identified expression of type 1 interferon (IFN)-stimulated genes (ISGs) as the predominant transcriptional feature of ischemia-exposed microglia. While the IFN cytokines are known primarily for their anti-viral function, they also have important function in the CNS. Type 1 IFNs (IFNo’s and IFNβ) bind to their receptor (IFNAR1) and activate a signaling cascade resulting in transcription of IFN-stimulated genes (ISGs). ISG products play an essential role in modulating immune cell function. Our lab is interested in the role of type I IFNs and IFNAR1 specifically in microglia during IPC. We hypothesize that in the absence of microglial IFNAR1, IPC-mediated neuroprotection will be attenuated. By using systemic knockout (IFNAR<sup>f</sup>/<i>f</i>) and cell type specific knockdown (IFNAR<sup>f</sup>/<i>f</i> LysM<sup>Cre</sup>) mouse models, we seek to explore the importance of microglial IFN signaling in IPC-mediated neuroprotection. To ensure the specificity of IFNAR1 knockdown, we will compare IFNAR1 expression in IFNAR<sup>f</sup>/<i>f</i> LysM<sup>Cre</sup> to that of control IFNAR<sup>f</sup>/<i>f</i> mice. I am currently performing experiments to detect the presence of IFNAR1 in both cohorts at the cell surface and mRNA levels using flow cytometry and qRT-PCR, respectively. We are also quantifying IFNβ induced release of chemokine CXCL10 in both cohorts by ELISA. Preliminary results reveal a significant reduction in cell surface IFNAR1 in the IFNAR<sup>f</sup>/<i>f</i> LysM<sup>Cre</sup> mice. We expect to see similar reductions in IFNAR1 mRNA and functional response to IFNβ. Once initial knockdown efficiency experiments have been performed, we can shift focus to the effects of cell type specific IFNAR1 knockdown on IPC-mediated neuroprotection.

Characterizing Intersections of mTOR and Kappa-Opioid Receptor Signaling Pathways
Talia Suner, Senior, Neurobiology, Biochemistry
Mary Gates Scholar, NASA Space Grant Scholar, UW Honors Program
Mentor: Selena Schattauer, Pharmacology
Mentor: Charles Chavkin, Pharmacology

Our research is centered around resolving the intersections between stress, the kappa-opioid receptor (KOR) system, and a protein kinase called mammalian target of rapamycin (mTOR). This protein kinase has regulatory functions in the cell including mediating growth and protein synthesis. Disruption of the mTOR signaling pathway has been implicated in a myriad of diseases including clinical depression. Stress has an important role in causing mTOR activation. While acute behavioral stress increases phospho-mTOR, chronically stressed animals exhibit depression-like behaviors correlated with decreased phospho-mTOR expression in the hippocampus. Additionally, post-mortem human studies show a decrease in mTOR signaling in the pre-frontal cortex of patients diagnosed with major depressive disorder. Opioid drugs also have profound effects on mood that may be mediated by mTOR activation. We are interested in determining the intersection of KOR and mTOR signaling pathways, especially under conditions of acute versus chronic stress. We use mouse and cell culture models to explore mTOR and KOR signaling using western blot analysis. We found that when treated with the KOR agonist U50488 for 30 minutes, KOR expressing HEK293 cells showed a dramatic increase of phospho-mTOR. In vivo, mTOR is activated in mouse spinal cord 30 minutes after injection with U50488. This activation of mTOR is dependent on G protein-kinase (GRK3), an enzyme activated by KOR that is required for both aversion and depression behaviors in mice. Treatment of mice with a sin-
gle dose of U50488 one day before harvesting tissue samples showed a decrease in mTOR in both the hippocampus and the spinal cord. Together, these results suggest that following a dose of kappa-opioid agonist, there may be transient activation of mTOR with a later phase of inhibition. Altogether, these findings point towards the mTOR signaling pathway as a potential target for anti-depressive pharmaceuticals.

Voltage-Gated Calcium Channel Ligands in CNS Synapses

Gregory Patrick (Greg) Sevilla, Senior, Philosophy, Biology (Molecular, Cellular & Developmental)

Mentor: Steven Carlson, Physiology & Biophysics

Vinyl chloride does not bind to the extracellular synaptic protein, the transmembrane voltage-gated calcium channel (VGCC), in the peripheral nervous system (PNS), the active zone is anchored to neurotransmitters. At the neuromuscular synapse in the peripheral nervous system (PNS) using the extracellular Leucine-Arginine-Glutamate (LRE) sequence that could physically access the VGCC to allow binding. I narrowed down these CNS proteins to three candidates: NGL1, NGL2, and reelin using these criteria. We hope to find CNS VGCC binding partners as a result of this project to help develop a deeper understanding of neuronal communication. It holds potential for novel treatments of neurological disorders.

Circadian Modulation of Neuromotor Control

Jazmine G. Perez, Senior, Gender, Women, and Sexuality Studies, Biology (Physiology)

Mentor: Jennifer Gile, Biology

Motor behavior is the result of neural programs emerging from the Primary Motor Cortex (PMC). In order for the PMC to generate behavioral outputs, it integrates exogenous and endogenous sources of variance. Electrical activity from the PMC has been effectively used to operate minimally invasive brain-machine interfaces (BMIs) that can operate prosthetic limbs to achieve basic motor outcomes such as operating a joystick. Further development of neuroprosthetic technology will rely on a deep understanding of sources of variance to the PMC and how the PMC compensates for this. The circadian system regulates physiology and behavior within the 24-hour time frame and it represents a predictable source of endogenous variance for the generation of motor behavior. The specific pathways by which the circadian clock(s) may modulate PMC motor programs is not understood, but results from our lab have shown that the circadian system modulates the PMC electrical activity associated with wheel running in mice. We implanted electrocorticographic (ECoG) electrodes onto the PMC of mice and recorded electrical activity while they ran on a wheel at different circadian times. This has shown that the PMC electrical signals associated with wheel-running are modulated in a predictable manner by the circadian system. I propose to replicate these experiments in mice with a malfunctioning circadian clock. I hypothesize that the canonical molecular circadian clock is essential for this modulation. To test this hypothesis, I will use ECoG electrodes to record PMC electrical activity of Bmal1-/- mice, which have no copies of the clock gene BMAL1, and their wildtype (Bmal1+/+) littermates. This will determine whether the circadian modulation of the PMC depends on an intact molecular circadian clock. Understanding the regulatory effects of the circadian system on PMC brain wave activity is crucial for the design of BMIs and their effective operation throughout the 24-hour day.

Toward a More Complete Model of Direction Selectivity in the Visual Cortex

Jacob Joseph (Jacob) Gile, Senior, Computer Science, Neurobiology

UW Honors Program

Mentor: Wyeth Bair, Biological Structure

Mathematical modeling is a useful tool for investigating the neural circuits that underlie perception, but discovering models that accurately describe complex processes remains a challenge. In the mammalian visual cortex, some neurons are specialized for visual features such as motion, color, or depth, but complete models to describe these processes are
unknown. For example, direction selective (DS) neurons are specialized to respond to visual stimuli that move in one direction but not in others. Previous studies of DS neurons in the macaque monkey visual cortex demonstrate that this response varies with the speed of the stimulus. In particular, when stimuli move faster, the neurons operate within a shorter window of time. This suggests that the duration of time during which DS neurons integrate their input depends on the speed of the stimulus, a phenomenon called adaptive temporal integration (ATI). A popular model for DS neurons is based on filters that change position linearly with time; however, these filters do not replicate ATI. To identify an alternative that better captures ATI, we first sought a model that is still a single filter but one that curves non-linearly with time. We also examined models with more than one filter in which the filter outputs are combined such that the most active is weighted the largest. Although we discovered single curved filters that demonstrate a change in integration window, we did not find one that also matched the direction selectivity of macaque neurons. We found that multi-filter models are more successful at simulating ATI while also preserving direction selectivity, and they qualitatively match neurophysiological data. These filters may represent multiple physical channels of input to a DS neuron and offer an alternative model that more completely characterizes the output of these neurons than do single linear filters.

The Role of Pentose Phosphate Pathway in Mitochondrial Health and Aging
Jane Jeehyun (Jane) Kwon, Senior, Biology (Molecular, Cellular & Developmental), Biochemistry
Levinson Emerging Scholar, Mary Gates Scholar, UW Honors Program
Mentor: Matt Kaeberlein, Pathology
Mentor: Christopher Bennett, Molecular and Cellular Biology

Mitochondria, the energy producing organelles in eukaryotic cells, play a critical role in cell metabolism. Mitochondrial dysfunction is implicated in various diseases ranging from severe childhood disorders to age-associated neurodegenerative diseases. Interestingly, knockdown of the components of the electron transport chain increases lifespan of Caenorhabditis elegans. One mechanism that responds to changes in mitochondrial protein homeostasis is the mitochondrial unfolded protein response (UPRmt). The UPRmt regulates the expression of several nuclear-encoded mitochondrial genes, including chaperones and other factors that assist in folding of misfolded or aggregated proteins in the mitochondria. Through a genome-wide RNAi screen in efforts to elucidate genes involved in the UPRmt pathway, we found that knockdown of genes involved in the pentose phosphate pathway (PPP) activates hsp-6 expression, a mitochondrial chaperone protein. The PPP is a metabolic shunt off of glycolysis that produces NADPH, a reducing equivalent used for glutathione and lipid metabolism, and ribose 5-phosphate for nucleotide synthesis. The knockdown of transaldolase, a gene in reductive phase of the PPP, increases the lifespan of C. elegans, and increases the level of reactive oxygen species in cells, suggesting that antioxidant systems are compromised. Through lifespan epistasis analysis, we determined that lifespan extension by transaldolase deficiency requires components of the MAP Kinase (MAPK) signaling pathway. Therefore, we hypothesize that the PPP negatively regulates MAPK signaling, and consequently, the lifespan of C. elegans by the regulation of oxidative stress. Understanding the genetics behind the UPRmt and the PPP’s role in aging has enormous benefits to human health as both the PPP and mitochondrial dysfunction are highly correlated with many age-related diseases, such as cancer and neurodegenerative diseases.

Determining the Mechanism for Regulation of HIF-1 by RHY-1 and its Effects on Aging
Alison Claire (Alison) Leonard, Senior, Biochemistry
Mary Gates Scholar, UW Honors Program
Mentor: Matt Kaeberlein, Pathology
Mentor: Scott Leiser, Pathology

Aging is a tightly regulated process involving multiple molecular mechanisms and important signaling pathways, of which many are also important responders to environmental and genetic stress. The hypoxia-inducible factor (HIF) is a transcriptional regulator of genes that respond to the stress of low-oxygen environments. The HIF protein is a heterodimer that is highly conserved between species, and in the nematode worm Caenorhabditis elegans stabilization of the regulated HIF-1α subunit (hif-1) increases health and longevity. In the canonical pathway for HIF-1 regulation, HIF-1 is hydroxylated by the prolyl hydroxylase EGL-9 and then ubiquitinated and targeted for degradation by the von Hippel Lindau protein VHL-1. Our preliminary results suggest that another protein, the regulator of hypoxia-inducible factor-1 (rhy-1), also affects both HIF-1 activity and longevity. We discovered that RHY-1 can affect longevity through a screen for age-associated autofluorescence, an accumulation of protein aggregates commonly called lipofuscin. Decreased age-associated autofluorescence frequently correlates with increased longevity, and we found that rhy-1 overexpression leads to both phenotypes. Rhy-1 is also reported to act as an inhibitor of HIF-1 through a vhl-1 independent pathway. The goal of my project is to determine how RHY-1 regulates HIF-1, and to use this information to better understand RHY-1’s function downstream of HIF-1. To explore this question we are engineering worm strains that express various tagged versions of rhy-1 and hif-1, such as tandem affinity purification and fluorescent tags. These tags will allow us to determine where HIF-1 and RHY-1 are localized in the worm, if they localize together, and to immunoprecipitate HIF-1 protein to
detect any physical modifications made by RHY-1 using mass spectroscopy. Our results will help us understand the function of downstream targets of the hypoxic response and bring us closer to identifying proteins that can be targeted therapeutically to increase health and longevity in mammals.

**Identification of Cellular Processes Associated with Aging across the Drosophila phylogeny**

Ariana Melissa (Ariana) Samuelson, Senior, Biochemistry  
NASA Space Grant Scholar  
Mentor: Daniel Promislow, Department of Pathology  
Mentor: Jessica Hoffman, University of Georgia

Age is the largest risk factor associated with mortality and morbidity. However, while many studies have determined specific molecules and pathways associated with age-related decline, few have attempted to discover the global changes that influence aging. In order to address this question, we have utilized a comparative approach involving 11 species of fruit flies in the genus *Drosophila*. These species have extremely variable natural lifespans. To discover the underlying mechanisms that result in these differences in longevity, we have employed the use of metabolomics. The metabolome consists of all the small-molecules present in an organism’s body. Metabolomics uses mass spectrometry, nuclear magnetic resonance or gas chromatography to quantify these molecules. We have measured lifespan and collected flies for metabolomics at five, 31, and 63 days of age. Samples were then run through a mass spectrometer for metabolite identification. Numerous metabolites were found to be associated with age, species, and sex, and many of these factors are also linked to specific metabolic pathways. In our future work, we will manipulate the metabolites found to be significantly associated with age and observe whether there are changes in longevity. Identifying specific cellular processes influencing longevity could then provide targets for potential therapies to alleviate the detrimental effects of aging.