Online Proceedings

Session 1C

Sensory Integration, Learning, and Motor Control in Animal and Human Models

Session Moderator: Horacio de la Iglesia, Biology
MGH 231
12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Exploring the Neural Mechanisms of Perceptual Rod-Cone Flicker Cancellation
Adrianne (Adree) Aguas, Senior, Neurobiology
Howard Hughes Scholar, UW Honors Program
Mentor: Fred Rieke, Physiology and Biophysics
Mentor: William Grimes, Dept. of Physiology and Biophysics

Over the course of a natural day-night cycle, mean luminance levels can span ten log units or more. Mammalian retinas effectively encode visual information over this vast range, in part, by cone photoreceptors in bright conditions. These visual signals, regardless of their origin, must pass through a common set of retinal ganglion cells- thus creating opportunities for signal interactions. The overarching goal of this research is to understand how the retina behaves under intermediate lighting conditions (e.g. dawn and dusk) when both rods and cones are active, and to relate the circuit-level retinal processing of rod and cone signals to human perception. Previous human perceptual experiments have revealed interactions between flickering rod and cone stimuli that are thought to occur in the retina. Here we explore the neural basis of rod-cone flicker interference in On and Off ganglion cells that project to the primate magnocellular visual pathways. Our recordings (from *in vitro* non-human primate retina) reveal a strong, suppressive interaction between rod and cone signals. The dependence of this interaction on the frequency and phase of the temporal modulation is similar to that observed in perceptual measurements from human subjects. This destructive interference between rod and cone signals appears to reflect a linear combination of kinetically-distinct rod and cone signals upstream of the ganglion cell synaptic inputs. Using our empirically-derived data as a foundation, we construct a mathematical model that captures known rod-cone interactions and accurately predicts retinal output in response to arbitrarily time-varying rod and cone stimuli.

Poster Session 2

MGH 206, Easel 171
1:00 PM to 2:30 PM

Using Optogenetics to Induce Grooming Behaviors in *Drosphila melanogaster*
Leyssandra (Wesley) Gaskill, Senior, Physics: Biophysics, Mathematics (Philosophy)
Mentor: John Tuthill, Physiology and Biophysics
Mentor: Anthony Azevedo, Physiology and Biophysics

In fruit flies (*Drosophila melanogaster*), grooming is an innate behavior comprised of sequential movements of particular legs over specific regions of the body. However, we know little about the neural circuits that control the locations, durations, or behavioral transitions of grooming. We sought to identify neurons in the central nervous system that control these aspects. To investigate this, we used optogenetics, which involves expressing light sensitive ion channels in defined neurons, allowing us to activate them with red light. We then recorded videos of the flies as we stimulated them with red (625nm) light for various durations (0.5, 1, 5 seconds). Finally, we manually annotated the videos by assigning binary tags to specific behaviors and aggregated the results to see trends across flies. We were able to identify neurons in the brain that influenced head grooming, neurons in the ventral nerve cord that influenced hind leg grooming as well as additional neurons that influenced other behaviors. Identifying these neurons opens up new opportunities to dissect neural circuits controlling grooming behaviors and, in the future, to understand how these circuits interact to control sequential behaviors.

Session 2B

Chemistry, Biochemistry, and Materials Science

Session Moderator: Sharona Gordon, Physiology and Biophysics
MGH 228
3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Studies of the Gating Mechanism of the Pain-Sensing Ion Channel TRPA1

Amanda Qu, Senior, Biochemistry
Levinson Emerging Scholar, Mary Gates Scholar, NASA Space Grant Scholar, UW Honors Program
Mentor: Sharona Gordon, Physiology and Biophysics
Mentor: Gilbert Martinez, Physiology and Biophysics

The protein Transient Receptor Potential Ankyrin type 1 (TRPA1) is an ion channel found in nociceptive (pain-sensing) sensory neurons. TRPA1 is activated by several noxious compounds, including those found in mustard plants, garlic, smoke, and tear gas, among others. It is responsible for the sensation of irritation and pain that these compounds cause, as well as some related chronic pain disorders. A better understanding of TRPA1 could lead to novel therapeutics against chronic pain. For this reason, the mechanism by which TRPA1 activates is an active area of research. TRPA1 contains a coiled-coil domain at its C-terminal end and several ankyrin repeat domains (ARDs) at its N-terminal end. These are both very common repeating protein motifs; ARDs in particular often modulate protein-protein interactions. Many channels in the Transient Receptor Potential (TRP) family, which includes TRPA1, contain these domains, but their role in channel activation is not fully understood. However, a recently solved atomic structure of TRPA1 provided some key insights. The structure showed that TRPA1's coiled-coil domain is tightly enveloped by its ARDs, and they appear to interact with each other. No other TRP channel with a known structure exhibits this unique structural arrangement. My project aims to better characterize the interaction between the ARDs and coiled-coil. I am studying a mutation in the ARDs of the human TRPA1 channel, which is at its interface with the coiled-coil. The mutation, K591E, changes a key lysine amino acid residue, which is positively charged, to a negatively charged glutamate. It is found in rattlesnake TRPA1, which unlike the human protein is activated by temperatures above 27 C. Current results show that the K591E mutant is active at room temperature, even without any other compounds. A stronger understanding of TRPA1's activation mechanism will be vital to the development of effective next-generation pain therapies.

POSTER SESSION 3

MGH 241, Easel 136

2:30 PM to 4:00 PM

Epidural Stimulation for Rehabilitation

Josephine Cordelia (Josephine) D'angelo, Senior, Neurobiology
Mary Gates Scholar
Mentor: Chet Moritz, Physiology & Biophysics
Mentor: Sarah Mondello, Rehabilitation Medicine

Spinal cord injury (SCI) disrupts the communication between the body and the brain. As a result, people with SCI typically have significant sensorimotor deficits leading to a lower quality of life. Electrical epidural stimulation (EES)—that is, the stimulation of the spinal cord from the surface of the cord—has shown promise for enhancing recovery of the hindlimbs when applied to the thoracic spinal cord. The goal of this study is to determine whether this same therapy applied to the cervical spinal cord in rats with cervical injuries can improve forelimb function. Prior to injury, the rats perform multiple tasks: Irvine, Beatties and Bresnahan (IBB) task, Limb-use Asymmetry Test (LUAT), Automatic-pellet-reaching task, and a Lever task. Next, rats will receive a unilateral C4 hemicontusion and below the injury the rats will receive an epidural implant for stimulating the spinal cord. All of the rats will perform the former tasks—IBB and LUAT—once a week to track their recovery. Half of the rats will be stimulated via the epidural implant while performing the automatic-pellet-reaching task and Lever task in the hopes of inducing Hebbian plasticity to enhance functional recovery. We are currently testing different epidural implants to identify a method that provides robust, long-term EES. Post-injury performance will be compared to the rat's pre-injury performance. Subsequently, the rats are perfused for tissue analysis. We are optimizing the tissue analysis protocols for identifying GAP-43 immunoreactivity to determine if there was an increase in plasticity and axonal growth. Cresyl violet and myelin staining will also be used to measure the magnitude of the injury by staining the spared neurons and myelin.

POSTER SESSION 3

MGH 241, Easel 137

2:30 PM to 4:00 PM

Optimization of Optogenetic Stimulation Methods for Spinal Cord Injury Rehabilitation

Benjamin Pedigo, Senior, Bioengineering
Mary Gates Scholar
Mentor: Chet Moritz, Physiology & Biophysics
Mentor: Sarah Mondello, Rehabilitation Medicine

Recent studies have shown that electrical stimulation of the spinal cord can manipulate spinal cord plasticity and may be effective in recovering motor function after spinal cord injury. The emerging field of optogenetics allows researchers to change a cell's membrane potential using light. Cells are made to express light-dependent ion channels (channelrhodopsins) which cause a cell to depolarize or hyperpolarize

after being triggered by specific light wavelengths. Our lab has shown that optogenetics can be used to elicit forelimb movements in rats by stimulating the cervical spinal cord. Long-term methods for providing light stimulation *in vivo* are needed to explore the treatment potential of optogenetics. Initial experiments by our group using a LED implant stimulator demonstrated that long-term optogenetic stimulation of the spinal cord results in increased GAP-43 staining. GAP-43 staining highlights areas of new neuronal growth, suggesting an increase in neuronal plasticity in optogenetically stimulated rats. Animals in this study, however, also exhibited unusual tissue morphology around the site of implant. Current research is working to understand the possible sources of this tissue disruption, including heat production from the LED and inflammation induced by LED stimulator implantation near a spinal cord injury. To investigate the possibility of heat production, I developed a new implant prototype that incorporates a thermistor to monitor temperature changes during long-term light stimulation. I tested this implant design in anesthetized and freely-moving rats to investigate how different light stimulation parameters affected implant temperature. I then performed histological tissue analysis at the site of the implants to assess the effects these temperature changes had on tissue condition. The results from this work will inform the next generation of light stimulation implants, and help to improve function following spinal cord injury via optogenetic activation of neural tissue.

POSTER SESSION 4

Balcony, Easel 115

4:00 PM to 6:00 PM

Ablation of NFkB-p65 Prevents Myocardial Injury, Pathological Remodeling, and Ventricular Dysfunction after Myocardial Infarction

Rachel Steinmetz, Senior, Biology (General)
Mentor: Qinghang Liu, Physiology and Biophysics

In addition to its known roles in regulating cell survival, inflammation, and cardiac hypertrophy, the transcription factor nuclear factor-kB (NFkB) has been implicated as a maladaptive mediator of cardiac ischemic injury, but the underlying mechanisms remain undefined. Our objective was to assess the contribution of NFkB-p65 to myocardial injury, pathological remodeling, and ventricular dysfunction after myocardial infarction using cardiac-specific knockout mice. Intriguingly, ablation of NFkB-p65 in the heart protected against adverse remodeling and heart failure following myocardial infarction for 2 weeks. NFkB-p65 knockout mice showed reduced cardiac hypertrophy, fibrosis, and pulmonary congestion compared to control mice. Better-preserved cardiac function and less ventricular dilation were also observed in NFkB-p65 knockout mice after MI. NFkB-p65 knockout and control mice were also subjected to acute ischemia/reperfusion (I/R, 60 min ischemia & 24 h reperfusion). Loss of NFkB-p65 also protected the heart against acute I/R damage as evidenced by decreased infarct size. Accumulating evidence has demonstrated that oxidative stress participates in several aspects of cardiac remodeling after myocardial infarction, including loss of cardiomyocytes by apoptosis and necrosis, inflammatory/fibrogenic responses, and hypertrophy. Here, we examined the role of NFkB-p65 in regulating cell survival/death induced by oxidative stress. We showed that NFkB-p65 silencing in mouse embryonic fibroblasts significantly reduced necrotic cell death induced by reactive oxygen species (ROS). Specifically, ablation of p65 prevented cellular uptake of propidium iodide induced by H_2O_2 or tBHP (tert-Butyl hydroperoxide), as well as the release of HMGB1 (high mobility group box 1) into culture supernatant (a biomarker of necrosis). No changes in apoptosis markers were detected under these conditions, suggesting that NFkB-p65 mediates ROS-induced necrosis, but not apoptosis. These results identified a novel role for NFkB-p65 in mediating myocardial injury and ROS-induced necrosis, suggesting that NFkB-p65 may serve as a therapeutic target for myocardial damage and remodeling following myocardial infarction.

POSTER SESSION 4

Balcony, Easel 117

4:00 PM to 6:00 PM

Investigating the Incorporation of the Non-Canonical Amino Acid *L*-ANAP into the Ion Channel TRPV1 in *Xenopus* Oocytes using Fluorescence Microscopy

Nicolas Dean Basil, Senior, Biochemistry, Chemistry (ACS Certified)
Mentor: Sharona Gordon, Physiology and Biophysics
Mentor: Mario Rosasco, Physiology and Biophysics

The family of Transient Receptor Potential (TRP) proteins contains ion channels with a wide array of functions, including invertebrate phototransduction, responding to painful stimuli, responding to temperature changes, and many others. Of particular interest is the polymodal receptor TRPV1, which responds to many stimuli, including capsaicin, heat, pH, etc. TRPV1 is known to play a role in the sensation of both pain and heat; however, the structural dynamics that underlie TRPV1's ability to transduce these signals are still incompletely understood. Therefore, an understanding of the activation, regulation, and structure of TRPV1 are of clear importance. To address these questions, I have sought to use *Xenopus laevis* oocytes as an expression platform to perform studies on the structure and function of TRPV1. Since TRPV1 is not naturally expressed in *Xenopus* oocytes, the genetic information needed for the cell to build the protein was provided for the oocyte via microinjection of RNA. After an incubation period of several days, Western blot techniques were applied to analyze the presence and strength of

expression of TRPV1. To better understand specific structural changes made when in the activated conformation, the fluorescent, non-canonical amino acid *L*-ANAP was integrated into TRPV1 using amber stop codon suppression and an engineered tRNA synthetase. Integrating the non-canonical amino acid ANAP enables structural and functional analysis of the membrane protein via fluorescence microscopy. The results of applying such methods to understand the function of TRPV1 will provide insight into the use of TRPV1 for therapeutic purposes, most prominently the reduction of the sensation of pain.

POSTER SESSION 4

MGH 241, Easel 158

4:00 PM to 6:00 PM

Electrochemical Neural Interfaces for Promoting Motor Recovery after Spinal-Cord Injury

Sarita Walvekar, Senior, Neurobiology, Biochemistry
Mentor: Steve Perlmutter, Physiology and Biophysics
Mentor: Samira Moorjani, Physiology and Biophysics

Spinal-cord injuries can lead to loss of motor function in affected limbs. Our laboratory has demonstrated enhanced recovery of forelimb motor function after spinal-cord injury using a neuroprosthetic intervention, targeted activity-dependent spinal stimulation (TADSS), to direct plasticity in spared motor pathways. TADSS uses a recurrent neural-computer interface to detect muscle activity in the affected forelimb and deliver (electromyographic) activity-triggered electrical stimulation at a functionally related spinal site below the lesion. To further enhance motor recovery outcomes and make them longer lasting, we are combining TADSS with delivery of plasticity-enhancing neuromodulators, such as brain-derived neurotrophic factor (BDNF) and quipazine, a serotonin-receptor agonist. Rats with cervical contusion injuries have been receiving TADSS in conjunction with delivery of BDNF and quipazine for 5 hours/day through chronically implanted Pt/Ir tubes. Recovery of forelimb movements over a 12-week therapy period is assessed through performance on a trained food pellet reach-grasp retrieval task, administered twice per day during the 5-hour therapy period, which further serves as physical retraining of the injured limb. We expect our combined electrochemical therapeutic strategy to lead to enhanced motor recovery outcomes compared to delivery of TADSS alone. This study may lead to development of novel therapies for targeting spinal-cord injuries.

POSTER SESSION 4

MGH 206, Easel 170

4:00 PM to 6:00 PM

The Impact of Molecule Inhibitors on Chromosome Segregation in Cricket Spermatocytes

Connor King, Sophomore, Pre-Sciences
Bill Lee, Junior, Physics: Biophysics
Mentor: Charles Asbury, Physiology and Biophysics
Mentor: Luke Johnson, Physiology and Biophysics

Aneuploidy is an irregular number of chromosomes caused by errors in cell division. Down syndrome is a common disease resulting from aneuploidy, where there are three copies of chromosome 21. I am in interested in why these errors occur and the mechanism that corrects them. Prior to anaphase, an attachment of microtubule fibers to chromosomes must occur to allow chromosomes to segregate properly. The attachments form by trial-and-error. Microtubule fibers will continue to attach and detach to chromosomes until stability is achieved. In the 1960s, Dr. Bruce Nicklas manipulated individual chromosomes using a microneedle to physically misalign chromosomes and pull on chromosome attachments to create artificial tension. The Asbury Lab is interested in recreating his experiments using tools that were not available at the time, such as small molecule inhibitors, to determine whether certain molecules such as Aurora B kinase are necessary for chromosomes to correct themselves. I use drugs such as ZM, an Aurora B kinase inhibitor, and Taxol, a microtubule polymerizer and common chemotherapy agent. I treat live cricket cells with drugs such as these, and record the treatment's effect on meiosis. One common phenotype we are expecting is a delayed transition from metaphase to anaphase due to inhibition of a protein required to allow the cell to continue into anaphase. Once my research determines whether the drugs delay cell division and the effective concentrations, the lab can test the importance of the drug's molecular targets in the error correction mechanism. Further research into the mechanisms of chromosome segregation has implications for understanding what causes aneuploidy, the mechanistic and biophysical mysteries of cell division, and the rising of cancerous cells.