SESSION 1B

UNMASKING BRAIN FUNCTION: FROM SINGLE MOLECULES TO COMPLEX INFORMATION PROCESSING

Session Moderator: Tom Daniel, Biology
MGH 228
12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Investigating Interactions between a Presynaptic Voltage-Gated Calcium Channel and the Extracellular Matrix Protein, Laminin, in the Synaptic Cleft
Tinhdoan Thi (Doan) Phi, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Steven Carlson, Physiology & Biophysics

At the neuromuscular synapse, the protein laminin binds the alpha subunit of the voltage-gated calcium channel (Cav2.1). Cav2.1 is a transmembrane protein of the nerve terminal; laminin is an extracellular matrix protein of the synaptic cleft. This interaction stabilizes the active zone — the cytosolic apparatus for neurotransmitter release. We hypothesize that Cav2.1 acts as an anchor for the active zone. In the B2 laminin chain, the amino acid sequence LRE plays a critical role in its interaction with the L5III extracellular loop of Cav2.1. We wish to know which amino acid residues of the L5III extracellular loop are critical for this interaction with the LRE sequence. To investigate, we are developing an assay to measure the interaction between the L5III extracellular loop, produced as a fusion protein, and a fusion protein containing the LRE sequence of the B2 laminin chain. The Cav2.1 L5III extracellular loop is laid down as a fluorescently tagged micro-patterned substrate—12 um stripes on a glass slide. Then, the slides are incubated with fluorescent beads containing the B2 laminin fusion protein with the LRE sequence. Using fluorescence microscopy, we will measure the number of beads bound to the 12 um stripes and thus measure the interaction between the two proteins. As a negative control we will use the L5III extracellular loop from Cav1.2, a calcium channel that does not bind the B2 laminin chain. By using chimeric loops containing different amounts of the Cav2.1 and Cav1.2 L5III loop sequences, we will determine which amino acid residues are critical for binding. Currently, we are using recombinant DNA methods to produce the fusion proteins for our assay. With AviTag methods we are tagging the L5III loops with biotin at a specific location which will allow us to bind the loops with a preferred orientation to a micro-patterned substrate of avidin. The active zone is critical for neuromuscular communication and our research will provide insight on the mechanism of active zone formation.

POSTER SESSION 2

Regulation of the Golgi SM Protein Sly1
Tom Duan, Senior, Biochemistry
Mary Gates Scholar, UW Honors Program, Undergraduate Research Conference Travel Awardee
Mentor: Alexey Merz, Biochemistry
Mentor: Rachael Plemel, Biochemistry

Sec1/Munc18 related proteins (SM) are essential cofactors of SNARE–mediated membrane fusion. They function in conjunction with SNARE proteins, a family of zipper-like proteins that drive the two opposing membranes toward fusion, and other SNARE cofactors. Membrane fusion is one of the most fundamental cellular processes. However, universal mechanisms of SM function and regulation have been elusive to membrane biologists for a long time. Sly1 is an SM protein that functions at the Golgi. Here, we present genetic and biochemical results, which show how a SNARE–mediated tethering mechanism of Sly1 is regulated, and which suggest that key aspects of SM function are evolutionarily conserved across the SM family. Unlike other SMs, Sly1p contains a loop that covers a portion of domain 3a on the protein. Previous studies show that mutations at the tip of the loop can suppress requirements for the Rab GTPase Ypt1 and tethering protein Uso1. It was hypothesized that the loop serves an auto–inhibitory function. Moreover, the recent crystal structure of another SM, Vps33, shows that domain 3a serves as a binding site for the cognate R–SNARE. We performed a screen for Sly1 mutants that suppress the loss of the Golgi tether, Uso1. The new mutants, as well as limited proteolysis experiments, suggest that the entire loop swings away to reveal a SNARE binding site on domain 3a, thereby activating the tethering and SNARE assembly functions of Sly1. These experiments reveal new features of Sly1 regulation, and in
addition to the results from Vps33, suggest a universal mechanism of SM–SNARE interaction during vesicle tethering.

SESSION 2D

PROTEIN CHEMISTRY AND METABOLOMICS

Session Moderator: Daniel Ratner, Bioengineering
MGH 234
3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

TRPV1 Expressed in HEK293T/17 Cells is Not Regulated by Plasma Membrane Cholesterol Content
Moshe Tsvi (Moshe) Gordon, Senior, Chemistry
Mary Gates Scholar, Undergraduate Research Conference Travel Awardee
Mentor: Sharona Gordon, Physiology and Biophysics

TRPV1 is an ion channel of great importance for pain and heat sensing. The EC50 (threshold dose) of the ion channel TRPV1 is highly variable depending on the cell type in which it is expressed. For example, the EC50 of TRPV1 in HEK293T/17 (a cultured mamalian cell line) is ~1 µM whereas the EC50 of TRPV1 in isolated DRG neurons is ~100 nM. Numerous explanations have been proposed for this phenomenon, with one possible explanation being the varying lipid contents of the plasma membrane regulating the EC50 of TRPV1. The cholesterol content of the plasma membrane is a candidate for regulating TRPV1, as it is known to vary among types of cells. We measured TRPV1 function with patch-clamp electrophysiology to determine the effect of removing cholesterol from the plasma membrane on channel function. Inside-out excise patches remain stable for tens of minutes, allowing for cholesterol removal and replacement by flowing a Methylated-β-Cyclodextrin, a ring of sugar molecules that bind cholesterol, across the patch. In intact HEK293T/17 cells, we estimated the cholesterol content to be about 30%. Thus, the Methylated-β-Cyclodextrin is expected to produce a very large change in plasma membrane properties as removes cholesterol. Recordings from these patches showed that both the EC50 for activation of TRPV1 by capsaicin and the capsaicin-induced maximum current were unaffected by patch treatment with Methylated-β-Cyclodextrin. These results are significant as they show that TRPV1 function is unaffected by a large reduction in plasma membrane cholesterol. Because removal of such an abundant component of the plasma membrane likely produces meaningful changes in the bulk properties of the bilayer, these data also indicate that lipid regulation of TRPV1 is caused by lipids which have specific, chemical interactions with the channel, rather than the bulk properties of the plasma membrane.

SESSION 2E

STEM CELLS AND REGENERATIVE MEDICINE

Session Moderator: Benjamin Freedman,
Medicine/Nephrology
MGH 238
3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Assessment of Recovery from Spinal Cord Injury using a Haptic Interface Device
Oliver Riley (Oliver) Stanley, Senior, Bioengineering, Neurobiology
Levinson Emerging Scholar, Mary Gates Scholar, UW Honors Program
Mentor: Chet Moritz, Physiology & Biophysics

Spinal cord injury (SCI) can severely impair quality of life. While there are a variety of accommodations for individuals with SCI, there are no clinically available treatments which restore pre-injury levels of function to these patients. The optimization of emerging treatments for SCI will require a large amount of detailed experimental trial data in animal models. Assessments of trained motor behavior in animal models of SCI recovery typically fail to capture information about fine gradations in the course of recovery, which interferes with the development of new restorative treatments. To better characterize these gradations during the study of an emerging method for cervical SCI rehabilitation, I monitored forelimb movements of a cohort of injured rats during a motor behavior task using a high-precision haptic interface device - a machine which simulates physical interactions with virtual objects or events by applying forces to its user. I used the motion information gathered by this device to trigger electrical stimulation of the spinal cord to enhance attempted movements and encourage Hebbian plasticity within the spinal cord. Functional recovery of strength and range of motion was assessed relative to pre-injury baseline, demonstrating the utility of 3D haptic interfaces for the assessment of promising SCI treatments. Our goal is to help individuals with disabilities due to spinal cord injury regain function and independence by quantifying, optimizing, and the accelerating the translation of potential new treatments for SCI using these precise and clinically relevant recovery measures.
TRPV1 Regulation via PI3K Pathway during Inflammatory Hyperalgesia
Ruian Yang, Senior, Biochemistry
Mary Gates Scholar
Alexander Akira (Alex) Pazevic, Senior, Neurobiology
Mentor: Anastasiia Stratiievskva, PBiO
Mentor: Sharona Gordon, Physiology and Biophysics

Transient receptor potential vanilloid receptor 1 (TRPV1) is a nonselective cation channel whose activity can be regulated by different kinds of factors including vanilloids such as capsaicin. Nerve growth factor (NGF) has been shown to cause the rapid sensitization of nociceptors neurons expressing TRPV1 under inflammatory hyperalgesia. In other words, TRPV1 is involved the sensation of pain caused by inflammation. It’s been suggested that tropomyosin receptor kinase A (trkA) and its downstream effectors including phosphoinositide 3-kinase (PI3K) play a key role in TRPV1 potentiation and we want to further examine the molecular signaling mechanism. For the purpose of our study, we utilize a system based on a light-sensitive molecule phytochrome B (PhyB) to mimic inflammatory responses in cells. Under 650 nm light, PhyB undergoes conformational change and binds to phytochrome interaction factor (PIF). By fusing proteins of interest to PIF, PhyB-PIF interaction controlled by light can be used to manipulate signaling pathways including PI3K pathway. To study the potentiating effect of PI3K on TRPV1, we examine NIH3T3 cells transfected with proteins of interest using calcium imaging. We compare cell responses to capsaicin before and after PI3K translocation activated by 650 nm light. Our experimental data suggest a significant increase of calcium signalling after translocating PI3K to the plasma membrane. In the future, we are hoping to learn more about how the downstream effectors can affect TRPV1 trafficking. We will perform similar procedures as described using dominant-negative Akt and Rac1 cells and investigate the specific mechanisms. Eventually, we hope to utilize what we know about TRPV1 in pain treatment development.

Volitional Control of Local Field Potential Oscillations in the Prefrontal Cortex for Brain-Computer Interface Applications
Camille Isabella Birch, Senior, Bioengineering
Mary Gates Scholar, UW Honors Program
Mentor: Eberhard Fetz, Physiology & Biophysics
Mentor: Tyler Libey

The prefrontal cortex has the potential to play an important role in the development of future brain-computer interface technologies, which have the potential to greatly improve mobility and quality of life for people who suffer from central nervous system injuries or diseases. Most brain-computer interface systems collect neural signals from the motor cortex to control the effector; while this has long been the standard in the field, this approach is not viable if the motor cortex is damaged (e.g. by traumatic brain injury or stroke). The prefrontal cortex plays a role in executive function; it has signals that modulate with motor planning, is susceptible to operant conditioning, and its activity could possibly control brain-computer interfaces. This project aims to explore the efficacy of volitional control of local field potential signals in the prefrontal cortex for potential brain-computer interface applications. Two macaques were bilaterally implanted over the prefrontal, premotor, and motor cortices with dual surface- and-depth electrodes to collect differential local field potential signals. Through repeated training sessions with operant conditioning, the macaques learned to control the cursor on a computer screen, performing center-out tasks by modulating the frequency of local field potentials in the prefrontal cortex. These initial results show that volitional control of local field potentials in the prefrontal cortex is possible. My role in this project is to both collect and analyze data. Current and future work includes data collection from another macaque and examination of interactions between cortical sites during task performance.

Understanding Spatial Memory in Rhesus Macaques Foraging in Virtual Reality
Albert E (Albert) Ng, Senior, Anthropology, Neurobiology
Mary Gates Scholar, Innovations in Pain Research Scholar, UW Honors Program, Washington Research Foundation Fellow
Mentor: Elizabeth Buffalo, Physiology and Biophysics

There have been major advancements in the use of Virtual Reality (VR) technologies in military, medical, and educational applications. However, it is not well understood how representations of spatial memory are encoded by the bi-modal, visual and audio sensory stimulation inherent to VR interac-
tions. Previous studies have identified the hippocampus and entorhinal cortex as structures involved in the formation of spatial memory. Because Alzheimer’s pathology affects these medial temporal structures and is characterized by deficits in memory, spatiotemporal reasoning, and route finding, better understanding of spatial representations in medial temporal areas would help create VR with potentially therapeutic effects in patients with memory deficits. Using VR, non-human primates will utilize a joystick to move a first-person avatar and perform a foraging task wherein they must collect virtual objects to obtain food rewards. We hypothesize that monkeys will use a demonstrable strategy to optimize behavior in our VR task and propose experiments and computational methods to identify unique strategies wherein memory and route planning are being used. Preliminary results indicate that the three monkeys fully trained on this task utilize unique strategies to obtain food rewards. Furthermore, the evolution of individual strategies over the training period suggests that memory and other factors may play a role in route planning. Corresponding and ongoing research is aimed at identifying neural representations of complex spaces and encoded memory and planning elicited by our task.

**Poster Session 3**
Commons East, Easel 57
2:30 PM to 4:00 PM

Zebra Finch Song Analysis as a Precursor to Understanding Modulation of Variability in Trial-and-Error Learning
Elliott Taylor Tsuyoshi (Elliott) Abe, Senior, Physics: Comprehensive Physics
Washington Research Foundation Fellow
Mentor: Adrienne Fairhall, Physiology & Biophysics

Research on the Zebra Finch song system represents a unique inter-disciplinary collaboration between physics and neurobiology to investigate trial-and-error learning. In trial-and-error, or reinforcement, learning, the “actor” explores random variations in motor outputs and is rewarded for those variations producing favorable outcomes. The potential components of a reinforcement learning scheme have been identified in the songbird system: songbirds learn their songs by trial and error, and the anatomy of this system has been well studied. The objective of this research project is to characterize the nature of the variability that remains in adult song, as this variability is presumably the basis for adult song maintenance, and the bird’s ongoing ability to learn song variations. We use the recorded vocalizations from four adult male zebra finches to analyze repetitive motifs in a song, in order to characterize song structure. By utilizing sound analysis, we aim to first identify the nature of the song syllables and then determine the variations in these syllables, particularly with respect to their pitch and timing. We will use this analysis to inform our models of the neurological activity driving such variation. The outcome sought from this research is to contribute to and constrain a mathematical model of injected variation that will allow us to predict the relationship between internal brain activity and the subsequent corresponding behaviors. By investigating such variables as timing and frequency across the songs of these four zebra finches, the identification of the degrees of freedom explored by neurally driven variation can inform our models of the manner in which trial-and-error through reinforcement learning occurs. This project demonstrates that the quantitative methods of physics can be applied within the context of neurobiology to analyze the underlying algorithms of neural computation.

**Poster Session 4**
Commons East, Easel 72
4:00 PM to 6:00 PM

Incorporation of the Non-Canonical Amino Acid L-ANAP into the Ion Channel TRPV1 in Xenopus Oocytes
Nicolas Dean Basil, Senior, Biochemistry, Chemistry (ACS Certified)
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 fluorometry. 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
In the 1960s and onward, Dr. Bruce Nicklas published a series of papers detailing the experiments he performed on the chromosomes of grasshopper sperm cells. Chromosomes are tightly condensed DNA strands that contain the information necessary for a cell to reproduce itself and carry out its designated functions. Some of Nicklas’s most notable experiments led to the findings that chromosomes correct themselves when replicated ‘sister’ chromatids are not properly oriented and will segregate incorrectly when pulled apart. This correction is based upon tension stabilization, or “pulling forces” on the chromosome. If the chromosomes of a cell are arranged improperly, this will lead to incorrect separation when the opposite ends of the cell pull to separate. If this is left uncorrected in the cell, it can lead to cell death, or birth defects like Down syndrome. Chromosome mis-segregation may also contribute to cancer. Inspired by Dr. Nicklas’s work, I seek to compare chromosome segregation in cricket spermatocytes with ingestible compounds like nicotine or aspirin, comparing them to untreated cells and cells treated with a cell phase inhibitor, MG132. Can these effects contribute to a disease like Down syndrome in offspring when the individual consumes some of those compounds? By dosing the cricket spermatocyte cell medium with one of these commonly ingested compounds we hope to observe visual differences in how these chromosomes divide and display themselves under a phase contrast microscope. Knowing whether or not these compounds can impact cell division may give the direction needed by researchers of chromosome disease to find out what can contribute to harmful diseases like Down syndrome or Fragile X syndrome.

**POSTER SESSION 4**
Commons East, Easel 71
4:00 PM to 6:00 PM

**Vesicle Reconstitution of TRPV1 for Structural Studies**
Erin Marie (Erin) Williams, Senior, Biochemistry
Mary Gates Scholar
Mentor: Sharona Gordon, Physiology and Biophysics
Mentor: Gilbert Martinez, Physiology and Biophysics

The first member of the vanilloid sensing sub-family of transient receptor potential cation channels (TRPV1) is an ion channel located primarily in the nociceptive (pain-sensing) neurons of the peripheral nervous system. It is most strongly activated by high temperatures, protons, and capsaicin, the molecule responsible for the spice of hot chili peppers. From these examples of TRPV1 activators, it has been deduced that this ion channel plays a key role in the sensory transduction of pain that results from thermal burns, acid burns, and ingestion of noxious chemicals. Thus, the TRPV1 ion channel has potential to be a drug target for innovative pain medications and therapies. Before this is feasible, physiologically relevant details of TRPV1 structure and function must be elucidated. Expanding the understanding of how TRPV1 interacts and changes in various environments is also crucial. My research aims to determine if the lipid environment surrounding TRPV1 has significant impacts on its structural conformation. Electron resonance techniques such as EPR and DEER can be used to detect structural information of proteins; however, a number of steps precede this goal. First, I expressed and purified TRPV1 in large quantities. Next, I reconstituted the purified channel into vesicles. Vesicles are spheres of phospholipid bilayer and more accurately resemble the plasma membrane environment in which TRPV1 naturally occurs. Then electron resonance techniques were used to compare the structure of detergent-stabilized TRPV1 and TRPV1 reconstituted into vesicles. The comparison of TRPV1 structure in different lipid environments has a lot of implications in the applicability of structural studies conducted outside of physiologically relevant systems and for the future study of TRPV1.

**POSTER SESSION 4**
Commons East, Easel 70
4:00 PM to 6:00 PM

**The Impact of Common Compounds on Cricket Spermatocytes during Chromosome Segregation**
Luke Michael (Luke) Johnson, Senior, Biology (General)
Mentor: Charles Asbury, Physiology and Biophysics

In the 1960s and onward, Dr. Bruce Nicklas published a series of papers detailing the experiments he performed on the chromosomes of grasshopper sperm cells. Chromosomes are tightly condensed DNA strands that contain the information necessary for a cell to reproduce itself and carry out its designated functions. Some of Nicklas’s most notable experiments led to the findings that chromosomes correct themselves when replicated ‘sister’ chromatids are not properly oriented and will segregate incorrectly when pulled apart. This correction is based upon tension stabilization, or “pulling forces” on the chromosome. If the chromosomes of a cell are arranged improperly, this will lead to incorrect separation when the opposite ends of the cell pull to separate. If this is left uncorrected in the cell, it can lead to cell death, or birth defects like Down syndrome. Chromosome mis-segregation may also contribute to cancer. Inspired by Dr. Nicklas’s work, I seek to compare chromosome segregation in cricket spermatocytes with ingestible compounds like nicotine or aspirin, comparing them to untreated cells and cells treated with a cell phase inhibitor, MG132. Can these effects contribute to a disease like Down syndrome in offspring when the individual consumes some of those compounds? By dosing the cricket spermatocyte cell medium with one of these commonly ingested compounds we hope to observe visual differences in how these chromosomes divide and display themselves under a phase contrast microscope. Knowing whether or not these compounds can impact cell division may give the direction needed by researchers of chromosome disease to find out what can contribute to harmful diseases like Down syndrome or Fragile X syndrome.
of five learning objectives: to cultivate and apply the mathematical foundations of quantum theory; to explain the properties, simplifications, and assumptions of the core quantum models; to explain the various features of relevant visual representations; to apply quantum theory to spectroscopic analysis, atoms, molecules, and chemical bonding; and to describe macroscopic phenomena using quantum theory. These objectives are intended to make quantum chemistry approachable to students in all scientific fields. We developed an initial design of the e-textbook based on the principles of minimal navigational effort, minimal clutter, maximal sense of place, and easy discoverability to keep students focused and interested. Ultimately, revising the standard quantum chemistry curriculum to emphasize core learning objectives and conceptual understanding will help students better comprehend the material and develop a stronger appreciation for the many applications of quantum mechanics.