Undergraduate Research Symposium May 19, 2017 Mary Gates Hall

Online Proceedings

POSTER SESSION 2

Commons East, Easel 77

1:00 PM to 2:30 PM

Gravitational Billiards

Nvida Ange Janine (N'vida) Yotcho, Junior,
Mentor: Jayadev Athreya, Mathematics

For many, mathematics is just the language of numbers. But, what people forget is that the universe is made of numbers, thus mathematics is its language. That is why we used a mathematical approach to study the long-term behavior of a moving ball influenced by the Earth's gravitational field. For our purpose, we experimentally defined gravitational billiards to be vertical billiards in which the earth gravitational field affects the billiards ball's motion. We also assumed that the billiards boundary could be any mathematical function of our choice, and the experimental system was conservative. Once in motion, the billiards ball followed a two-dimensional projectile trajectory until collision with the boundary occurred. After that, the ball engaged in a new projectile trajectory. As a matter of fact, each trajectory between two points of ball-boundary collision could be considered as an isolated two-dimensional gravitational motion with its own set of conditions. We took advantage of that aspect to build different simulations in parabolic, circular, paraboloidal and spherical gravitational billiards. The long-term behavior of the ball under gravity in our different gravitational billiards presented some aspects that so far corroborated the physics behind our study.

SESSION 2I

MCNAIR SESSION - GOING MOLECULAR

Session Moderator: Ray Malfavon-Borja, OMAD

MGH 254

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Control of Nerve Cord Elongation by Transcription Factor Senseless-2

Abraham Philip (Abe) Shouse, Senior, Biology (General)
McNair Scholar
Mentor: Jay Parrish, Biology

As animals grow, scalar expansion of many types of neurons must be coordinated with animal growth to maintain receptive field coverage and proper connectivity, a process that is not well understood. Resulting from a genetic screen, the Parrish lab has generated and isolated a mutation that results in severe elongation of the ventral nerve cord. This mutation affects a predicted transcription factor of unknown function, senseless-2(sens-2). Although the origin of the defect is unknown, one possible explanation for the defect is that sens-2 regulates growth of the peripheral nerves. In the absence of sens-2 function, the peripheral nerves are unable to elongate properly and, as a result, larval growth leads to greater strain on the nerve cord, which leads to nerve cord elongation. An alternative explanation is that sens-2 is required in all the cells of the nerve cord (neurons and glia) for their growth. Thus, one major aim of this study is to produce antibodies that recognize the sens-2 protein. To accomplish this task, I will generate a plasmid vector for production of sens-2 protein in bacteria, perform a recombinant protein purification and submit the protein for antibody production. Upon receiving the antibody, I will perform a series of dissections accompanied by antibody staining and imaging in order to localize sens-2. A second major aim is to take an unbiased genetic approach to identify factors that interact with sens-2 to regulate nerve cord expansion. If we identify interactions with genes of known functions in growth control or patterning, it will allow us to formulate models to account for sens-2 function in nerve cord elongation. To accomplish this task, I will I will create a series of heterozygous sens-2/deficiency stocks and perform genetic screens to determine which genes in *Drosophila* interact with sens-2's molecular pathway.

SESSION 2R

ECOLOGY FROM MICROBES TO BIRDS

Session Moderator: Frieda B. Taub, Aquatic & Fishery Science

JHN 111

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Skin Lipids of the Striped Plateau Lizard (*Sceloporus virgatus*): Oleic and Stearic Acids as Potential Indicators of Mate Quality

Tiare Elaine (Tiare) Gill, Junior, Biology , University of Puget Sound
Mentor: Stacey Weiss, Biology, University of Puget Sound
Mentor: Jay Goldberg, Biology, Indiana University - Bloomington

Chemical signaling, in the form of pheromones, is an important mechanism of sexual selection in reptiles, as it provides a means of communicating mate receptivity and quality during the mating season. Previous research on the chemical profile of Striped Plateau Lizard (*Sceloporus virgatus*) skin lipids found that lower levels of oleic and stearic acid in female skin lipids correlate with larger egg clutch sizes, suggesting that low levels of the fatty acids might be an attractive mating cue for males. The purpose of this research was therefore focused around two aims: 1) Determine if male lizards respond to manipulation of oleic and stearic acid ratios, as well as between presence and absence of the fatty acids when presented as an isolated cue; 2) Investigate how altering the levels of the two fatty acids in the skin lipids of female lizards affects male courting behavior. To address these aims we recorded male chemosensory behaviors (nose taps, tongue flicks, and air tasting), as well as escape behaviors (scurrying and jumping) during 40 minute behavioral trials. Although there was no significant difference in male chemosensory behavior between low and high lipid ratio treatments when the cues were isolated, results from the manipulated female trials show that male lizards demonstrated significantly more escape behaviors when presented with cues from a fatty acid spiked female than from an un-manipulated female. This suggests that male lizards were less interested in fatty acid spiked females—possibly due to the unattractive nature of a high lipid ratio cue.

POSTER SESSION 3

MGH 206, Easel 172

2:30 PM to 4:00 PM

Epithelial Cell Gap Junctions in Dendrite-Patterning

Paige Chanin (Paige) Almond, Senior, Biology (General)
UW Honors Program
Mentor: Jay Parrish, Biology
Mentor: Nan Jiang, Biology

Cell-to-cell interactions are necessary for proper cell function. In the peripheral nervous system, interactions between sensory neurons and the epidermis influence neuronal growth, survival and function. However, mediators of these neuron-epidermis interactions are largely unknown. *Drosophilamelanogaster* provides a tractable genetic system to study these cell-cell interactions. In *Drosophila*, dendrites of larval sensory neurons are sandwiched between the epithelial cell and the extracellular matrix. Occasionally the epithelial cells form invaginations at the location of dendrites, and eventually the dendrites become embedded inside the epithelial cell. In a screen for markers that label plasma membrane-associated proteins that localize to sites of dendrite embedding, we identified components of gap junctions as candidate regulators of dendrite-epidermis interactions. Gap junctions connect two cells via their cytoplasms and allow signals to be passed from one cell to another. Innexins are a family of gap junction proteins in *Drosophilia*, one of which is encoded by the gene *innexin3* (*inx3*). Our preliminary data showed that, when overexpressed, GFP-tagged Inx3 protein can localize to sites of epithelial dendrite ensheathment, thus we hypothesize that *inx3* contributes to formation/stabilization of membrane invaginations that enclose dendrites. However, no tool exists to visualize endogenous Inx3 distribution, nor is there an Inx3 antibody for immunostaining. Using CRISPR/CAS9 genome editing we plan to generate a Drosophila line with endogenous Inx3 tagged with GFP (Inx3-GFP). Using this line, we will determine whether Inx3 indeed localizes to sites of epithelial dendrite ensheathment. If we observe Inx3 enrichment at sites of dendrite ensheathment, we will perform expansion microscopy, a technique that our lab has recently adapted for use in Drosophila, to determine whether Inx3 labels discrete domains in the epithelial membrane invaginations. Finally, using our *inx3-GFP* fly line, we will conduct genetic mosaic analysis to determine whether *inx3* is necessary for epithelial enclosure of dendrites.

POSTER SESSION 3

MGH 241, Easel 162

2:30 PM to 4:00 PM

Examining Mutations in Presenilin 2 Associated with Alzheimer's Disease

Leah Ariel Osnis, Senior, Biochemistry
UW Honors Program
Mentor: Suman Jayadev, Neurology
Mentor: Susan Fung, Neurology

Mutations in the gene Presenilin 2 (PSEN2) cause familial Alzheimer's disease (fAD). FAD shares clinical and pathological features of sporadic, late onset AD thus fAD cell models can be useful to study mechanisms relevant to all forms of AD which is critical to developing effective AD therapeutics. Presenilin 2 protein (PS2) forms the catalytic subunit of the γ-secretase complex, which cleaves amyloid precursor protein and releases Aβ1-42, considered a pathogenic contributor to Alzheimer's disease (AD). Our laboratory is interested in a fAD associated PSEN2 mutation, a frameshift two base-pair deletion (PSEN2 K115Fx) . The PSEN2 K115Fx is predicted to either lead to a truncated protein suggesting that the mutation may create a shortened peptide that interferes with normal cellular function (dominant negative or toxic gain of function) or result in degradation of the RNA transcript and subsequent loss of normal amount of PS2 protein (loss of function). To better understand how PSEN2 mutations cause disease, we have two objectives. The first aim of this project is to determine if the PSEN2 K115Fx does indeed result in a truncated protein or influence levels of wildtype PS2. I will be collecting human cultured fibroblasts isolated from AD patients with the PSEN2 mutations or controls and prepare cell lysate for analysis by Western blot. My colleague will also be analyzing mRNA levels from those same samples to determine the stability of the PSEN2 mutant and wildtype transcripts in all cases. The second aim is to determine the impact of the mutation on PS2 enzymatic activity. I will culture the cells described above, then infect with a luciferase based enzyme reporter assay to compare the impact of PSEN2 mutations on γ-secretase mediated cleavage of APP. My work will help identify the candidate mechanisms by which the PSEN2 K115Fx mutation causes AD.

POSTER SESSION 3

Balcony, Easel 117

2:30 PM to 4:00 PM

Management and Long-Term Outcomes of Esthesioneuroblastoma at a Single Institution

Andrew Wooncheng (Andrew) Lui, Senior, Biology (General)
Mary Gates Scholar
Mentor: Jay Liao, Radiation Oncology
Mentor: Upendra Parvathaneni, Radiation Oncology

Esthesioneuroblastoma (ENB) is a rare neuroendocrine sinonasal malignancy that is derived from the olfactory epithelium. Optimal management is controversial across institutions despite improvements in treatment methods of this disease. A standard of care for the management of ENB has been hampered by the relatively low incidence, which ultimately prevents the creation of large clinical studies. Most patients undergo multimodality treatment with surgery and radiotherapy with or without chemotherapy. There have been substantial advances in surgery as well as radiotherapy (RT), which may offer improved cancer control and decreased toxicity. We reviewed the management approaches and long-term outcomes at our institution. Forty-two patients with ENB were treated at a single institution from 1986-2016, with pathology reviewed. Demographic, treatment, and clinical data were retrospectively collected from medical records. Patients underwent CT and/or MRI and were staged using the Kadish staging. Event outcomes were calculated from date of surgery to time of recurrence, death, or last follow-up. Survival statistics were calculated using the Kaplan-Meier (KM) method using SPSS Statistics 19. Despite a predominance of locally advanced disease at presentation, relatively favorable long term survival outcomes were achieved for ENB with surgery followed by postoperative RT. ENB has a long natural history with risk of both early and late locoregional and distant recurrences. Local recurrence remains a major problem despite aggressive local therapy. Treatment intensification should be explored in patients with Kadish C disease, including defining the role and optimal timing of systemic therapy.

POSTER SESSION 3

MGH 241, Easel 161

2:30 PM to 4:00 PM

Mechanisms of Alzheimer's Disease Mediated by Mutations in Presenilin 2

Michelle Kaywen (Michelle) Hong, Senior, Neurobiology, Psychology
Mary Gates Scholar, UW Honors Program
Mentor: Suman Jayadev, Neurology
Mentor: Carole Smith

There is an urgent need to clarify the causes of Alzheimer's Disease (AD) so targeted treatments can be developed. Mutations in Presenilin 2 (PSEN2) cause an inherited form of AD, but the mechanism for this relationship is not fully understood. PSEN2 protein (PS2) has been found to regulate pro-inflammatory microglial response and the expression of microRNA miR146, an innate immune regulator. These findings led us to pursue the effects of PSEN2 mutations on microglia function, peripheral immunity, and AD. Our aim is to study mechanisms of microglia-induced neuron injury in AD. We hypothesize that PS2 mutation-expressing microglia contribute to neuroinflammation in AD by cytotoxicity and synaptic pruning mechanisms. To study the impact of stimulated microglia on neurons, we employ co-cultures, where microglial (BV2) and neuronal (SY5Y) cell lines are cultured together. We used a lactose dehydrogenase (LDH) cytotoxicity assay to measure levels of microglia-induced neuronal death. After initial assay optimizations, we measured LDH of a BV2/SY5Y coculture to quantify baseline LDH and optimize the ratio of neuron to microglia plating. Fluorescent microscopy was used to observe microglia and neuron

interactions morphologically and flow cytometry quantified the levels of neuron phagocytosis of microglia. Using these methods, we were able to demonstrate interactions between the cells and these same methods can be used to later study synaptic pruning. From here, we will determine whether there is a difference in synaptic pruning levels and cytotoxicity between microglia from a control mouse and a mouse expressing mutated PSEN2 transgene when co-cultured with neurons. If our hypothesis holds true, we expect to see increases in both cytotoxicity and synaptic pruning induced by mutated microglia. Determining and understanding PSEN2's role in causing AD could lead to the development of targeted treatment for patients with these mutations.

POSTER SESSION 4

MGH 206, Easel 165

4:00 PM to 6:00 PM

CRISPR Based Behavioral Screening for Genes Affecting Nociception in Zebrafish

Nicolas P Germanos, Senior, Neurobiology
Mary Gates Scholar, UW Honors Program
Mentor: Ajay Dhaka, Biological Structure
Mentor: Andrew Curtright, Biological Structure

Chronic pain affects millions of people worldwide, and current treatments are often ineffective and come with unwanted side effects. Modern research focuses on studying the mechanisms of pain sensation in the nervous system, with the goal of using our improved knowledge to develop more efficient analgesics. Our research focuses on neurons located in the Trigeminal and Dorsal Root Ganglia (TRG and DRG), as they are known to play an important role in the sensation of touch and pain, and on genes regulating the development and function of these neurons. Recent advances in gene editing methods, particularly the CRISPR/Cas9 system, allow us to mutate specific genes of interest a living animal and monitor the effect of such mutations within a matter of days. To this end, we have developed a behavioral assay which determines the effect of CRISPR induced mutations on pain sensation in zebrafish larvae. CRISPR injected larvae are exposed to noxious stimuli such as AITC (mustard oil) and harmful temperatures. The CRISPR treated larvae's response to the painful stimuli determines the impact of the specific gene we targeted on nociception. Through this assay we have identified two genes, zmat4b and zbtb7b, as potentially important to the sensation of noxious stimuli. Further experiments are now required to determine the precise mechanisms through which these genes play a role in the sensation of pain.

POSTER SESSION 4

MGH 206, Easel 166

4:00 PM to 6:00 PM

Fusion Proteins: An Approach to Distinguishing "Itch" and "Pain" Neurons

Becka Marie Warfield, Senior, Chemistry: Biochemistry (Bothell)
Innovations in Pain Research Scholar
Mentor: Ajay Dhaka, Biological Structure
Mentor: Kali Esancy, Biological Structure

Contrary to the previous belief that itch (pruritis) is simply a less intense form of pain, recent findings suggest that itch and pain are distinct sensations mediated via independent neuronal circuits. Nevertheless, itch and pain share similar molecular machinery. In mammals, itch sensations are the product of the coupling of pruritic receptors, usually GPCRs, and TRP ion channels such as TRPA1, nociceptors that normally encode noxious, painful stimuli. We identified an itch selective compound, imiquimod (IMQ), which was found to mediate pruritic responses via direct activation of TRPA1. However, other TRPA1 agonists such as allyl isothiocyanate (AITC) specifically evoke nociceptive responses. We found that IMQ is a weak agonist of TRPA1 and specifically activates itch selective neurons. These itch selective neurons are primed to respond to TRPA1 agonists while having no effect on TRPA1-expressing nociceptors. We hypothesized that the differences in sensitivity of itch-selective versus nociceptive sensory neurons to TRPA1 agonists could be caused by differences in the amount of TRPA1 channels they contain, the activation mechanism of TRPA1, or the trafficking of TRPA1 following activation. Thus, we developed a strategy to visualize TRPA1 by creating fusion proteins that tether various indicators to TRPA1. We have created a fusion construct of TRPA1 and green fluorescent protein (GFP) by using overlap polymerase chain reactions (PCR) to amplify segments of DNA and tethering the two pieces together via direct, rigid, and flexible linkers. This experiment will lead to the fusion of TRPA1 to other molecules, such as genetically-encoded calcium indicators, GCaMP and CaMPARI. These fusions will give us the ability to visualize the localization, expression, activation, and trafficking of TRPA1. These studies will help elucidate the molecular mechanisms underpinning itch versus pain sensation and show how a single ion channel can mediate distinct sensations via differential activation.