UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 17, 2019 Mary Gates Hall

Online Proceedings

POSTER SESSION 1

MGH 241, Easel 164

11:00 AM to 1:00 PM

Microglia Involvement in the Development of the Human Fetal Retina

Kayla Marie (Kayla) Eschenbacher, Junior, Neurobiology
Mary Gates Scholar, McNair Scholar
Mentor: Thomas Reh, Biological Structure

During embryonic development of the vertebrate central nervous system, innate immune cells, called microglia, migrate into the retina and brain. Microglia are responsible for phagocytosis, monitoring tissues for pathogens, and inflammatory signaling. Several studies in model organisms show microglia also have roles in neural development, particularly in formation of synaptic circuitry. Defects of the neuronal circuitry can lead to vision loss, therefore it is probative to investigate the role and mechanisms of microglia in wiring the retina. The purpose of this project is to investigate microglia migration and activity in the human retina, where very little is understood. To understand if microglia could have similar effects in human retinal development as in model organisms, we first needed to determine when and where microglia are found at different ages. We collected donated fetal tissues from as early as developmental day 40 through 132 to determine the number and distribution of microglia in tissue sections using Iba1, a microglia-specific antibody, and confocal fluorescence microscopy. To test whether microglia are necessary for retinal development we are using our lab's fetal retina culturing technique that can be used to manipulate microglia populations. Retinal development can be visualized after deletion of microglia using markers for retinal progenitor cells (EdU) and various synaptic antibodies. So far, we have seen from whole retinal tissues that microglia are present as early as day 58, prior to the majority of synaptic development, and survive in retinal cultures. Earlier stages are being investigated, and we are currently determining methods that will deplete microglia in order to study the effects of their absence on retinal development. In conclusion, we know that microglia are present during synaptic development, and persist during retinal culturing. Therefore, microglia are potentially essential for human retinal development and could be targets for future disease research.

POSTER SESSION 1

Balcony, Easel 119

11:00 AM to 1:00 PM

Investigating the Role of Phospholipase C in Regulating the Sensitivity of TRPA1 Expressing Neuron Populations

Angela K Christman, Senior, Biology (Molecular, Cellular & Developmental)
Mary Gates Scholar
Mentor: Ajay Dhaka, Biological Structure
Mentor: Kali Esancy, Biological Structure

According to the NIH, 76.2 million Americans have suffered from pain lasting more than 24 hours, with millions more affected by chronic pain. With such a high prevalence and impact on health and quality of life, the understanding of pain is essential to effective treatment. Exploring the mechanism by which our neurons set the gain for what is perceived as painful stimuli is one way to further that knowledge. Different populations of neurons have different thresholds for activation, and oftentimes alterations to these thresholds can result in aberrant pain signaling. Phospholipase C (PLC) is a promising target to study, as it has been previously shown to potentiate the activation of pain neurons expressing Transient Receptor Potential cation channel subfamily A, member 1 (TRPA1). TRPA1 channels are responsive to noxious stimuli such as mustard oil (AITC). This study explores the effects of PLC using a PLC activator (m3m3FBS) and an inhibitor (U-71322) to examine its role in response to the noxious stimulus, AITC. We performed a locomotor assay of zebrafish larvae to explore behavioral effects, as well as neuronal imaging experiments using transgenic zebrafish with fluorescent calcium indicators in neural cells. We hypothesize that both behavioral responsiveness to pain and neuronal recruitment will likely increase with activation of PLC and decrease with deactivation of PLC because of PLC's ability to modulate TRPA1 channel activity. With data collected thus far, the results look consistent with our hypothesis. We observed increased locomotion in response to activation of PLC with painful stimulus (AITC) compared to control and inhibitor groups. Likewise, zebrafish exposed to PLC activators exhibited greater numbers of AITC responsive neurons than zebrafish exposed to control or PLC inhibitors. Together, these results indicate that PLC is an important factor in modulating the sensitivity of TRPA1 expressing neurons.

POSTER SESSION 1

Balcony, Easel 120

11:00 AM to 1:00 PM

Understanding the Role of Calcitonin Receptor-Like Receptor-Expressing Neurons in the Dorsal Horn

Dana Shauna Kamenz, Senior, Bioengineering
Innovations in Pain Research Scholar
Mentor: Ajay Dhaka, Biological Structure
Mentor: Robyn Laing, Biological Structure

Chronic pain is debilitating for millions of people worldwide and our understanding of how the central nervous system processes painful information is limited. If we are to create more effective therapeutics to treat chronic pain, it will be necessary to develop a better understanding of the circuitry and functionality of the somatosensory system. Our lab is interested in the calcitonin receptor-like (CALCRL) receptor, which is expressed throughout the central nervous system and is known to respond to pain-inducing neuropeptides. We aim to profile CALCRL receptor-expressing neurons within the dorsal horn to elucidate their functional roles in nociception and to determine how they process painful information. This was done by 1) utilizing an adeno-associated viral vector (AAV) to preferentially infect and fluorescently tag CALCRL-positive neurons in the dorsal horn, 2) utilizing a retrograde, monosynaptic rabies viral vector to infect neurons that are synaptically connected to CALCRL-positive neurons, and 3) immunohistochemical staining and analysis to detect a variety of neuronal markers of inhibitory and excitatory neurons, laminar markers, and several other known dorsal horn neuron subtypes. Thus far, CALCRL-positive neurons were successfully infected via direct injection using both AAV and rabies vectors into the dorsal horn. In addition, we have also been successful in costaining for multiple neuronal markers. We have begun to profile and classify these neurons according to known neuronal subtypes. Our next goal is to modulate the activity of this specific neuronal population followed by behavioral testing to better understand its functional role in the processing of painful stimulus. If we are able to create a clear profile of these neurons, then we may be able to identify future targets for specific therapeutics.

POSTER SESSION 1

Balcony, Easel 118

11:00 AM to 1:00 PM

Using Zebrafish to Identify Molecules with Novel Analgesic Properties

Tal Wolman, Junior, Pre-Sciences
Michael Dinh, Junior, Biochemistry
Maile Nguyen, Senior, Biochemistry
Mentor: Ajay Dhaka, Biological Structure

Both acute and chronic pain are universal, often debilitating sensations that lead to significant physiological, psychological and economic costs. Drug development and research have worked to counteract these adversities, but current therapies are often inadequate and have dangerous side effects. Targeted drug development, which relies on pre-selecting a target that is subjected to in-vitro testing, has been difficult, costly and ineffective in producing a drug that works to effectively relieve pain with minimal unfavorable consequences. An alternative approach would be to develop an untargeted screen in a system that employs complex pain behaviors. It would act as a means of modeling the nociceptive processes, which are the neural processes of encoding and processing noxious stimuli, in the organism. We utilize an unbiased, behavior-based, novel assay that uses zebrafish larvae to better understand pain sensation. We have screened thousands of small molecules on zebrafish larvae to identify ones that have analgesic properties. The potential analgesics should block sensitized temperature aversion which changes the larvae's temperature zone inclination meaning we observe no preference between the two zones. Thus far, our untargeted screen identified three novel molecules with analgesic properties. We then performed a series of pharmacology and behavioral experiments to understand the impact of the compounds and to narrow down their effects in order to confirm if it is truly impacting nociception and/or temperature aversion with the intent to validate the compounds as prospective analgesics.

POSTER SESSION 2

MGH 241, Easel 155

1:00 PM to 2:30 PM

Effects of Ebf1 on Mouse Cochlear Differentiation

Erica Airu Lee, Senior, Biochemistry
Mentor: Olivia Bermingham-McDonogh, Biological Structure
Mentor: Brent Wilkerson, Biological Structure

Hearing loss is often due to the absence or degeneration of hair cells in the cochlea, thus it is important to understand the transcriptional control mechanisms specifying the prosensory cells that generate hair cells and support cells. Early B-Cell Factor1 (Ebf1) is a transcription factor that was brought to our attention from motif enrichment in replicated ATAC-seq peaks detected in prosensory cells and non-prosensory cells. We observed that in the open chromatin regions and dynamic regions during the development of prosensory cells showed greater enrichment of Ebf motifs. This suggested that Ebf1 could be acting as a pioneer factor. To determine whether Ebf1 influences cochlear development, we generated a Sox2CreER: Ebf1 fl/fl allele by crossing Sox2-CreER mice and Ebf1-floxed mice. Administration of tamoxifen to mice orally leads to Cre-mediating deletion of Ebf1 DNA binding domain and formation of the recombinant Ebf1-GFP fusion

protein. To determine whether the Ebf1 knockout is successful we will compare Ebf1 expression between the Ebf1 knockout and wildtype through qRT-PCR. We will also compare the localization of Ebf1-GFP fusion protein in the Ebf1 knockout and wildtype. To determine whether Ebf1 affects cochlear differentiation, we will immunolabel for Sox2, Egfp, and Myo7a, this allows us to determine the presence of support cells (anti-Sox2), hair cells (anti-Myo7a) and the Ebf1 reporter (anti-Egfp) and compare support cell and hair cell counts between the Ebf1 knockout and wildtype. If the comparison shows a decrease or increase in support cell and hair cell counts in the Ebf1 knockout compared to the wildtype or the lack of support cells or hair cells in the Ebf1 knockout, then it suggests that Ebf1 is necessary for cochlear differentiation.

POSTER SESSION 2

MGH 241, Easel 164

1:00 PM to 2:30 PM

Retinal Regeneration from Müller Glia

Nick Radulovich, Senior, Biology (Physiology), French Mary Gates Scholar
Mentor: Thomas Reh, Biological Structure
Mentor: Nikolas Jorstad, Biological Structures

Retinal diseases tend to effect specific neuron subtypes, ranging from age-related macular degeneration, which is caused by the deterioration of photoreceptors near the central portion of the retina (macula), to glaucoma, which identifies abnormally high intraocular pressure resulting in the death of ganglion cells. Unfortunately, adult mammals are not able to regenerate retinal neurons. Conversely, zebrafish, frogs, and various amphibians are able to completely regenerate their retinal neurons in many different animal models of damage, and restore retinal structure and visual function. The source of regeneration stems from the resident Müller glia cells, which normally provide neuronal support and span all three retinal layers. A critical gene for the initiation of transforming Müller glia into neurons was found to be Ascl1. This led our lab to hypothesize that the introduction and upregulation of Ascl1 in mammalian Müller glia might stimulate them to become retinal neurons after damage, as occurs in these other regenerating species. Indeed, after introducing Ascl1 into the Müller glia of mice, we found newly regenerated retinal interneurons (bipolar cells) that successfully integrated into the retinal circuitry and functionally responded to light stimulus. In addition to Ascl1, we have identified another transcription factor, that when introduced in combination with Ascl1, stimulates the generation of a different retinal interneuron (amacrine cells). Ectopic expression of a proneural transcription factor to stimulate retinal regeneration provides a potential therapeutic intervention for treating blinding diseases, that even now, have few modest treatment options. In contrast to prosthetic devices and stem cell-based therapies, neuronal regeneration via viral injections is advantageous in regards to host tolerance and reducing the invasiveness of a given treatment, since the patient's own Müller glia are the source cell of the new neurons; and therefore, immunosuppression would likely not be required.

POSTER SESSION 3

MGH 206, Easel 178

2:30 PM to 4:00 PM

Optimization of Stimulus Selection for V4 Neuron Classification

Timothy Ma, Senior, Neurobiology, Applied & Computational Mathematical Sciences (Biological & Life Sciences)
Mentor: Wyeth Bair, Biological Structure

Each neuron in the visual cortex of the human brain has an affinity for specific features in our visual field. In early processing stages, these features are simple, like spots or edges of light. In deeper regions of the visual system, the features that excite neurons become complex combinations of lines, curves, color and texture that are not well understood. To better understand how combinations of visual features are encoded in the visual cortex, it is necessary to rapidly and systematically present complex visual stimuli while recording neuronal responses. To address this, we developed an online optimization approach that uses Bayes' theorem and information theory to select visual stimuli in real time to characterize neurons. Specifically, we calculated mutual information between candidate visual stimuli and a set of hypotheses about the neurons that were derived from a clustering analysis of data from previous studies of cortical area V4. We simulated our optimization system with the assumption that neuronal responses were subject to Poisson noise, and found that we were often able to classify neurons with less than one tenth the number of visual stimuli used in the original studies. We then implemented our system in the experimental lab, ensuring that the optimization calculations are performed fast enough to occur within tens of milliseconds between visual stimulus presentations. We now have a working system where signals from electrodes in the cortex are detected, digitized and transmitted to our optimizer, which then selects the next stimulus and transmits this choice to the visual display program. We are working on refinements of the optimizer algorithm in preparation for final deployment in vivo. We expect our novel system to characterize neurons more rapidly and thoroughly than ever before, and to shed new light on how cortical neurons encode complex conjunctions of visual features.

POSTER SESSION 4

Commons East, Easel 77

4:00 PM to 6:00 PM

Cell Cycle Re-Entry Delayed by UV-Induced DNA Damage in *Chlamydomonas reinhardtii*

Rebecca Schmidt, Senior, Biology (Molecular, Cellular & Developmental)
Aydan Bailey, Sophomore, AS-T, Wenatchee Valley Coll
Kai Bailey, Sophomore, AS-T, Wenatchee Valley Coll
Mentor: Sue Kane, Wenatchee Valley College
Mentor: Steve Stefanides, Biology and Chemistry, Wenatchee Valley College

Control of the cell-cycle is a topic of fundamental importance in cell biology. We are using the unicellular eukaryotic green alga *Chlamydomonas reinhardtii* to better understand how DNA damage affects transition of these cells in and out of the mitotic cell cycle. We find that UV treatment of cultures of *C. reinhardtii* gametes (in Go) delays re-entry of these cells into the mitotic cell cycle upon addition of nitrogen to cultures of gametes, a standard treatment which releases these cells to re-enter mitosis. Using a *C. reinhardtii* mutant which is unable to carry out photorepair of UV-induced DNA damage, we are characterizing the degree to which the kinetics of re-entry into mitosis depends on this very important repair system; we are thus beginning the 'parsing out' of the relative importance of the various DNA repair systems in controlling the transition from Go, a non-dividing stage, to G1, the start of DNA replication and cell division. This will potentially provide insight into other downstream effects of UV exposure, such as mutagenesis. Our work is of a very basic nature, but with application to understanding e.g. dynamics of natural populations of microalgae in a changing global environment.