Towards a Better Understanding of Neuro-Related Disorders

Session Moderator: Valerie Daggett, Bioengineering
MGH 284
1:30 PM to 3:00 PM

* Note: Titles in order of presentation.

Bursting Activity Across Simulated Neural Networks
Natalie Gonzales, Senior, Biology (Bothell Campus)
Mary Gates Scholar
Mentor: Michael Stiber, Computing & Software Systems, UW Bothell

To expand my experience with the human brain beyond dissections, I worked in the Intelligent Networks Laboratory (INL) in UW Bothell’s Computing and Software System (CSS) Division to simulate activity in a biological neural network. INL has a computer software system called “Graphitti” that allows users to run simulations of large biological neural networks. The specific simulations I analyzed were of growth and development in cultures of initially dissociated cortical neurons, which produce bursts of activity that propagate as waves across the network. Networks were grown over 28 simulated days on a 100 by 100 neuron grid, producing 500,000 synaptic connections. Two previous student papers suggest that this bursting activity is stable with 10% inhibitory neurons distributed across the simulated culture. With the original raw data stored in Matlab software (www.mathworks.com), I ran other code to pull burst location, speed, and distance from four simulations with different neuron firing rates and percentage of inhibitory neurons. In one simulation, burst origin locations were scattered across the network, indicating bursting behavior throughout the network. Two other simulations had a few, stable origin locations, suggesting a discernible pattern in bursting behavior. The burst propagation speed graphs for each network demonstrated growth and stabilization of activity by the end of the simulations. Working with this neural network has provided me with a fresh and "live" perspective on the connectivity of neurons. By understanding the dynamics of the network under these conditions, the data inspires further research investigating the connectivity effects of neurodegenerative diseases such as Parkinson’s Disease.

Investigating the Amyloidogenesis of Amyloid Beta Mutations to Characterize their Role in Inherited Alzheimer’s Disease
Isiac J Orr, Senior, Bioengineering: Data Science
Mentor: Valerie Daggett, Bioengineering

As of 2021, Alzheimer’s Disease (AD) afflicts 6.2 million Americans. This number is expected to climb rapidly in the coming decades as the number of individuals at the age of increased risk rises. AD is characterized by the deposition of insoluble, β-sheet-rich amyloid plaques of the amyloid β (Aβ) peptide. However, the AD-causing toxicity of Aβ is mediated by soluble, oligomeric species of the peptide formed in the process of aggregating into plaques (amyloidogenesis). These oligomers have been shown to adopt a unique secondary structure known as “α-sheet.” The onset of AD is typically sporadic, but some cases, known as familial Alzheimer’s Disease (FAD), are inherited via mutations in Aβ. None of these mutations have been studied for their ability to adopt the pathogenic α-sheet conformation. Therefore, I am investigating the impact of the Arctic (E22G), Iowa (D23N), Flemish (A21G), and Osaka (E22Δ) mutations on the amyloidogenesis and toxicity of Aβ to explain their role in FAD. I accomplish this through a Soluble Oligomer Binding Assay (SOBA) designed in the Daggett Lab to capture and quantify α-sheet conformations. Additionally I use circular dichroism, MTT cell toxicity assays and Thioflavin-T curves to investigate the aggregation pathway of these mutants. I have found that these mutations confer unique aggregation patterns to Aβ. Many of these Aβ mutants appear to adopt a stabilized α-sheet consistent with their tie to FAD. This provides an explanation of how these mutants cause FAD and offers insight into the factors that contribute to the adoption of α-sheet structure and pathogenesis of AD.
Mutations in Glucosidase, Beta Acid 1 Increase the Spread of Protein Aggregation in Parkinson’s Disease by Dysregulation of Extracellular Vesicles
Arnab Khera, Senior, Applied & Computational Mathematical Sciences (Statistics), Neuroscience
Mentor: Marie Davis, Neurology

Mutations in the gene glucosidase, beta acid 1 (GBA) are not only the strongest genetic risk factor for Parkinson’s Disease (PD), but also accelerate the progression of PD. We hypothesize that GBA mutations accelerate disease progression by promoting propagation of Lewy pathology from cell to cell via dysregulated extracellular vesicles (EVs). To investigate this, we developed a Drosophila model of GBA deficiency (GBA	extsuperscript{el}) manifesting neurodegeneration and accelerated protein aggregation. We also developed a human neuronal model by generating human induced pluripotent stem cells (iPSCs) from an individual with PD heterozygous for a null GBA mutation (GBA	extsuperscript{el}) PD neurons differentiated from GBA	extsuperscript{el} iPSCs, isogenic GBA	extsuperscript{WT} PD iPSCs, and iPSCs from an age- and sex-matched healthy control. I performed immunocytochemistry and western blots to evaluate protein aggregation within neurons. Additionally, I isolated neuronal EVs by size exclusion chromatography and analyzed them using a ZetaView nanoparticle analyzer. We previously found the expression of wildtype GBA in muscles of GBA	extsuperscript{el} mutant flies rescued levels of protein aggregation in the brain. This non-cell autonomous rescue was accompanied by normalization of alterations observed in EVs from GBA	extsuperscript{el} flies. Similar to our fly model, I found human GBA	extsuperscript{el} PD neurons and EVs have increased ubiquitinated proteins when compared to GBA	extsuperscript{WT} PD or healthy control neurons and EVs collected from these neurons. Our results suggest that GBA deficiency mediates PD pathogenesis by accelerating propagation of pathogenic protein aggregation through the alteration of EV protein cargo. We are now further investigating how GBA influences endolysosomal trafficking and EV biogenesis and I will now test whether GBA	extsuperscript{el} PD EVs can propagate protein aggregation faster in recipient neurons than control EVs. Understanding mechanisms regulating the spread of protein aggregates could reveal novel therapeutic targets to slow the rate of progression of neurodegeneration.

Early Detection of Alpha Synuclein Toxic Oligomers
Anthony Kithya Heng, Senior, Biochemistry, Neuroscience
Mentor: Valerie Daggett, Bioengineering

The exact cellular and molecular mechanisms for the progression of amyloidogenic diseases such as Alzheimer’s and Parkinson’s disease are elusive. During amyloidogenesis, soluble protein monomers aggregate to form soluble oligomers, which further aggregate to form insoluble, ??-sheet rich fibrils. The Daggett lab investigates the involvement of a nonstandard secondary structure called ??-sheet in the aggregation pathway of amyloidogenic proteins. The ??-sheet hypothesis states that ??-sheet is formed in the soluble oligomeric species during protein aggregation. This soluble, oligomeric form of the protein with ??-sheet secondary structure is implicated to be the toxic species in amyloidogenesis and a driver of disease pathology. One amyloidogenic protein is ??-synuclein, the major constituent of Lewy bodies, which are a pathological hallmark of Parkinson’s disease. Preliminary data reveal the presence of ??-sheet in Parkinson’s disease patient samples, showing promise for detection of Parkinson’s disease earlier than previously possible utilizing ??-sheet secondary structure. I am working on connecting the ??-sheet hypothesis with the amyloidogenesis of ??-synuclein by using synthetic ??-synuclein to link together data from different types of experiments. Experiments I conducted include the purification of ??-synuclein from E. coli, thioflavin T aggregation assays to measure the progression of the protein from monomer to fibril, circular dichroism spectroscopy to measure the predominant secondary structure in a peptide solution, and a soluble oligomer binding assay to detect the amount of ??-sheet content in patient or synthetic peptide samples. The purpose of my experiments is to demonstrate that ??-sheet is formed during the amyloidogenesis of ??-synuclein prior to fibril formation by linking together the above experiments, supporting the idea that detection of ??-sheet in patient samples may lead to methods of catching the progression of amyloid diseases earlier than previously possible.

Characterizing the Ciliary Margin Zone and Potential Retinal Stem Cells
Sidnee Petter, Senior, Biology (Physiology)
Mary Gates Scholar
Mentor: Thomas Reh, Biological Structure
Mentor: Kiara Eldred, Biological Structure, University of Washington School of Medicine

In fish and amphibians there is a specialized zone of retinal stem-cells at the edge of the retina, called the ciliary margin zone (CMZ) which replenishes the retina with new cells if it is damaged in adult animals. However, the presence of these stem-cells has not been observed specifically in the CMZ of the developing human. Here, I investigate the developing human retina to understand if it contains stem-cells that could be harnessed for repair. I first utilized CMZ stem-cell markers found in fish and amphibians to assess in the developing human retina, including BLBP, C-myc, cyclin D3, Six3, SMAD1/5, and Zic1. Following the stainings, I observed expression of C-myc and BLBP. To maintain their long-term proliferation, stem-cells will replicate slowly. Therefore I analyzed cell cycle kinetics in the CMZ. Primary cultures of fetal human retina, called retinospheres, were made by dissecting the fetal retina into small pieces containing a portion of the CMZ and growing them in culture with retinal differ-
entiation media. EdU, a dye that is integrated into DNA only in replicating cells, was then added to the media for different incubation intervals with EdU being 30min, 1hr, 2hrs, 4hrs, 6hrs, 8.5hrs and 25hrs, then retinospheres were fixed in PFA. Retinospheres were IHC stained with antibodies and dyes: Pax6 (a stem-cell marker), EdU (marker of cell division), Ki67 (marker of replicating cells) and DAPI. The total Ki67 positive cells in the CMZ and of EdU and Ki67 positive cells were counted so that the S-phase of mitosis could be measured to discover how fast the cells in the CMZ were dividing. I observed that cells in the CMZ were replicating slower than those further away from the CMZ, consistent with the possibility that there is a population of stem-cells in the CMZ of the developing human retina.

In Vitro Reprogramming of Young Adult Müller Glia with Developmental Transcription Factors to Regenerate the Retina

Marlene Probst, Senior, Biology (Molecular, Cellular & Developmental), Neuroscience

UW Honors Program

Mentor: Thomas Reh, Biological Structure
Mentor: Marina Pavlou, Biological Structure

Retinal diseases such as macular degeneration and glaucoma lead to various forms of blindness as neurons in the retina die. Unlike amphibians or fish, the neurons of the mammalian retina cannot regenerate on their own and any damage is permanent. Previous research has shown that we can recover some of the lost regenerative capacity in mammalian retinas by mimicking the regeneration process in other species. This is possible by overexpressing transcription factor Ascl1 in Müller glia (MG), which are the main support cells in the retina. However, expressing Ascl1 alone can only lead to the neurogenesis of one type of neuron in the retina. This limits the therapeutic applicability of this approach because in diseases like macular degeneration or glaucoma, specific neuronal cell types are lost such as photoreceptors and ganglion cells respectively. Therefore, to regenerate these neurons we need to identify the right cocktail of transcription factors for MG reprogramming. Recent single-cell analysis from our lab has identified candidate developmental factors that could push reprogrammed cells to photoreceptors or retinal ganglion cells. This project aims to investigate the role of these factors in influencing cell fate after MG cells have been pushed to a progenitor state with Ascl1. Using lentiviral vectors, I will induce the overexpression of these candidate genes in primary cultures of young adult mouse MG that have been engineered to express Ascl1. In order to identify the nature of resulting neurons from these cultures I will perform immunocytochemistry paired with confocal microscopy, and to better understand changes in functionality of these cells I aim to perform calcium imaging. Since the electrophysiological responses of glia and neurons are distinct, cultures with re-programmed neurons would record differently. Overall, this analysis evaluates the influence of new transcription factors on mammalian retinal regeneration.