UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 17, 2013 Mary Gates Hall

Online Proceedings

Poster Session 1

Commons West, Easel 26

11:00 AM to 12:30 PM

Behavioral Responses to Noxious Stimuli: The Infuence of Rearing Temperature and Analgesics on the Thermal Preference of Zebrafish (*Danio rerio*) Larvae

Song Ling (Shamii) Goh, Senior, Biology (General)
Erinn Alayne (Erinn) Wagner, Senior, Biology (Molecular, Cellular & Developmental)
Micaela (Mica) Rosser, Senior, Public Health-Global Health, Neurobiology
Mentor: Ajay Dhaka, Biological Structure

Despite much investigation, there has been relatively little progress in the development of novel analgesic compounds to treat debilitating chronic pain conditions that affect millions of people. Inherent problems in behavioral screens to identify analgesic compounds include the use of expensive and time consuming low throughput rodent based assay as well as the use of behaviors that only measure the presence or absence of acute behavioral responses to noxious stimuli, whereas assays that measure an animal's ability to choose between an innocuous and noxious environment overcome these setbacks. In this study, we aim to develop a high throughput two-choice thermal discrimination assay utilizing zebrafish (*Danio rerio*) larvae that can be used to screen for novel analgesic compounds. In order to do this, we will profile the ability of zebrafish larvae to discriminate between two temperatures. Individually arrayed 5 days post fertilization larvae will be placed in wells containing two distinct thermal zones and assayed to determine if they display a thermal preference between a testing temperature and a normal rearing temperature (28.5^{o}C). The testing temperature will range in one degree increments from noxious cold (10^{o}C) to noxious heat (38^{o}C). We will also test the limits of thermal preference by testing the ability of larvae to discriminate between two noxious temperatures, for example 36 vs 38^{o}C. To conclude if thermal preference is malleable based on the thermal environment to which larvae are acclimated, we will determine if prior incubation at a selected temperature other than 28.5^{o}C will alter the thermal preference of zebrafish larvae. Finally to determine if our assay may be appropriate for the use in a screen for novel analgesic compounds, we will investigate whether or not known noxious or analgesic compounds alter the thermal preference of zebrafish larvae.

Session 1I

Developmental Neuroplasticity

Session Moderator: Sheri Mizumori, Psychology

251 MGH

1:15 PM to 2:45 PM

* Note: Titles in order of presentation.

MicroRNA Processing by *Dicer-1* Regulates Sensory Neuronal Morphology

Marvin Eduarte Nayan, Senior, Neurobiology, Biochemistry
EIP Scholar, Howard Hughes Scholar, Levinson Emerging Scholar, Mary Gates Scholar, Undergraduate Research Conference Travel Awardee, Washington Research Foundation Fellow
Mentor: Jay Parrish, Biology

Since neuronal morphology is linked to neuronal function, the nervous system goes to great lengths to ensure that neurons achieve their proper shape, and failure to do so is associated with many cognitive disorders. However, the precise regulatory mechanisms underlying the coordinated expression of certain genes in a given cell type, at the right amount, and at the exact developmental time is one of the longstanding questions in developmental biology. MicroRNA biogenesis is a common mechanism by which cells regulate gene expression, often by "tuning" levels of target gene products, allowing for fine regulation of protein levels over a broad dynamic range. From a genetic screen for mutations in fruit flies that affect dendrite morphology in sensory neurons, we identified a novel *Dicer-1* allele that causes stereotyped dendrite defects, including deregulated growth and defects in dendrite self-avoidance. *Dicer-1* encodes a type III RNA endonuclease that is required for processing many types of small regulatory RNAs, including microRNAs. Time-lapse imaging and quantitative analysis of the dendrite patterning in *Dicer-1* mutant sensory neurons reveals that there is a significant increase in the number and overall length of dendrite branches as well as an increase in dendrite-dendrite crossing-over, especially at late stages of larval development. Interestingly, mutation in a gene (*pasha*) that functions upstream of *Dicer-1* to process a subset of miRNAs recapitulates the exuberant branching, but not the dendrite crossing phenotype of *Dicer-1* mutants. We hypothesize that different classes of miRNAs reg-

ulate dendrite branching and dendrite self-avoidance and we plan to use microarray analysis of *Dicer-1* and *pasha* mutants to identify the pathways regulated by these different classes of miRNAs.

SESSION 1R

SYNTHETIC BIOLOGY AND MOLECULAR BIOTECHNOLOGY

Session Moderator: Daniel Ratner, Bioengineering
022 JHN
1:15 PM to 2:45 PM
* Note: Titles in order of presentation.

Methods & Algorithms Refinement for Rapid, Ultra-Low-Cost, Targeted DNA Sequencing
Evan August (Evan) Boyle, Senior, Microbiology, Biochemistry
NASA Space Grant Scholar, Washington Research Foundation Fellow
Mentor: Jay Shendure, Genome Sciences

New DNA sequencing technologies have increased the throughput of sequencing by several orders of magnitude, reaching upwards of 50 GB of data per day on a single instrument. However, advancing our understanding of how genetic variation impacts human health and disease depends on studies that survey very large numbers of individuals, and as such may only be economical by analyzing specific subsets of the genome. This is complicated by the fact that traditional methods of preparing DNA samples for sequencing do not allow selection for such candidate genes. One solution is the use of "capture by circularization" by molecular inversion probes (MIPs). MIPs allow dozens of genes to be targeted and amplified simultaneously; however, individual MIPs suffer from notoriously variable performance relative to one another, which results in uneven coverage of target regions. Prior work highlighted the potential for statistical analysis to improve MIP design and selection, inviting further testing. After performing a large scale sequencing run using an unbiased set of 12,000 MIPs, logistic regression was used to build a model capable of scoring MIPs prior to synthesis. New design software incorporating this model into the selection process was developed and tested by designing MIPs to over 60 new genes. Analysis of the resulting sequence data demonstrated the successful in silico identification of challenging genomic regions and a reduction in the number of gaps in coverage, which should support the use of MIPs in future large scale studies.

POSTER SESSION 2

Commons East, Easel 82
12:45 PM to 2:15 PM

Identification of *Acsl* (Acyl-CoA Synthetase Long-Chain) as a Key Regulator of Dendrite Maintenance
Ashley Tsing Yuen (Ashley) Lau, Sophomore, Biology (Physiology)
Mentor: Jay Parrish, Biology
Mentor: Jiae Lee, Biochemistry

Neurons are specialized cells that receive and relay information throughout an organism, and the compartment that inputs the signal has multi-complex structures known as dendrites. The form of the dendrite is tightly regulated to neuron function; therefore, any abnormality that alters the structure of the dendritic arbor can lead to severe defects in neuronal function. However, the mechanisms of how dendrites develop and maintain their arborization are poorly understood. From a forward genetic screen for mutant alleles with progressive defects on dendrite patterning we identified one mutant allele that leads to a complete loss of terminal dendrites. We mapped this allele to a small genetic interval, and using complementation tests and DNA sequence analysis we identified *Acsl* (Acyl-CoA synthetase long-chain) as the gene affected by this mutation. Interestingly, Ascl is also associated with human X-linked mental retardation (XMR), but to date the role that *Ascl* plays in dendrite development has not been investigated. Here we report the phenotypic analysis of the *Acsl* mutant, effects of *Acsl* knock down in neurons, and our plans for functional analysis of *Acsl* in dendrite development.

POSTER SESSION 2

Commons East, Easel 83
12:45 PM to 2:15 PM

Characterization of a Novel Gene: A Mutation Causing Progressive Dendrite Defects in *Drosophila melanogaster*
Ranee James, Junior, Physics: Biophysics
Mentor: Jay Parrish, Biology

Dendrites are branched extensions of the cell body of a neuron that act to receive and process synaptic or sensory inputs. The elaborate branching pattern of the dendrite is a hallmark of neural type, and has a considerable impact in determining what signals a neuron receives, and how these signals are integrated. Alterations of dendrite morphology can lead to abnormalities in neuron function–dendritic defects are associated with neurological disorders such as Ret syndrome, Fragile-X syndrome, and Down syndrome, and in many cases the dendrite pathologies are progressive. Therefore we set out to identify mutations that result in progressive defects in dendrite growth and stability with the hope that the genes af-

fected by these mutations and the processes in which they regulate will provide new insight into human disease. A series of complementation tests using chromosomal deficiencies, as well as meiotic mapping have been used to map one such mutant (*cc215*) to a small chromosomal interval. In addition, we have performed a non-complementation screen to determine other alleles that affect the same gene mutated in *cc215*. Complementation tests and sequence analysis are currently being performed to classify the gene affected by this allelic series, which will allow us to investigate the underlying cause(s) of the dendrite defects in the mutants.

POSTER SESSION 2

Commons East, Easel 84

12:45 PM to 2:15 PM

Designing a Reporter Gene for *Drosophila melanogaster* Epithelial Cells

Philip Peter (Philip) Huang, Senior, Biochemistry
Mentor: Jay Parrish, Biology

The basic functional unit of the nervous system is the neuron, which contains two highly specialized compartments, the axon and dendrite. Dendrites received synaptic/sensory inputs, and the dendrite arborization pattern has a profound influence on the type and number of inputs a dendrite can receive. We are studying dendrite patterning in the *Drosophila* peripheral nervous system (PNS), with a particular focus on the role that non-neuronal signals play in shaping dendrite development. In the *Drosophila* PNS, sensory neurons grow on top of a monolayer of epithelial cells, which provide instructive cues to shape dendrite patterning. To date, there are plenty of tools available to genetically label and manipulate neurons, but few tools are available for genetic manipulation of epithelial cells. My goal is to design a reporter construct that will specifically label and facilitate targeted expression of transgenes in epithelial cells. To this end, I am assembling a modular plasmid construct which contains a minimal promoter, an enhancer sequence which we have found to be sufficient to direct epithelial expression, and the coding sequence of green fluorescent protein or other genes of interest. After assembling the construct, I will use germline transformation to generate transgenic flies and test the efficacy of the construct. If successful, this will represent the first epithelia-specific transcriptional reporter, and will provide a template for assembly of additional tools for studying dendrite-epithelia interactions.

POSTER SESSION 2

Commons East, Easel 81

12:45 PM to 2:15 PM

Development of Reporters to Monitor Spatiotemporal Patterns of *Bantam* miRNA Transcription *In Vivo*

Hui Li, Junior, Exchange - Arts & Sciences
Mentor: Jay Parrish, Biology
Mentor: Nan Jiang, Biology

MicroRNAs (miRNAs) are crucial regulators of gene expression in plants and animals and play a critical role in many biological processes, including nervous system development. From our previous study of *Drosophila melanogaster* peripheral sensory neurons, we found that miRNA *bantam* is both necessary and sufficient in epithelial cells to initiate an epithelia-derived signaling pathway that regulates dendrite growth. Using miRNA sensors, which report the location on miRNA activity, we found that *bantam* activity is developmentally regulated in epithelial cells. We hypothesize that this developmental regulation of *bantam* activity is the result of transcriptional regulation of *bantam*, and we aim to investigate this using *bantam* transcriptional reporters. To this end, we are developing *bantam* transcriptional reporter constructs in which the *bantam* transcript has been replaced by different reporter genes, such as GFP or Luciferase, while preserving the cis-regulatory landscape. We will use these reporters to monitor *bantam* transcript levels over developmental time and, pending the outcome of these studies, we will use the reporters to identify trans-acting factors that regulate *bantam* transcription.

POSTER SESSION 3

MGH 241, Easel 153

2:30 PM to 4:00 PM

Examining the Role of Dopamine Signaling in Olfactory Learning in the Yellow Fever Mosquito

Andrew Scott (Andrew) Curtright, Senior, Neurobiology, Biochemistry
Mary Gates Scholar
Mentor: Jay Parrish, Biology

Mosquitoes are the principle disease vector of malaria, yellow fever, and dengue fever, which are responsible for more than a million deaths worldwide annually. This makes controlling transmission a global health priority, and underscores the importance of understanding their behavior. Although it is known that olfaction is the main sensory modality mosquitoes use to select prey, many behavioral patterns, such as changing prey preferences, suggest that associative learning has a role in mosquito behavior. Previous research has established mosquito's affinity for human scent and characterized their olfactory receptors, but little is known about the neural substrates and molecular underpinnings that modulate olfactory learning. To gain insight into the importance of olfactory learning, I have been introducing controlled perturbations into the larval mosquito nervous system and assay-

ing their behavior using a classical conditioning model. This is achieved through genetic manipulation and injection of drugs with known targets. Initially, I have focused on the role of dopaminergic neurons, because they are known regulators of associative learning in other species. To this end, I have developed microinjection techniques that allow for delivery of silencing RNAs (siRNAs) to knockdown dopamine receptor expression. Using this approach, I have found that Dopamine receptor deficient animals, as well as those injected with Dopamine antagonists, are unable to learn like their untreated counterparts. Knockdown animals suffer a much higher mortality rate in survival experiments that test their ability to avoid predation, underscoring the importance of these receptors *in vivo*. Currently, we are using cell biological approaches to visualize the relevant neurons. Together these data show that larval mosquitoes are able to learn within days of hatching, and that dopamine plays a crucial role in facilitating aversive learning. In addition to clarifying the mechanistic basis for olfactory learning, these findings may ultimately enhance the development of vector control strategies.

POSTER SESSION 4

Balcony, Easel 114

4:15 PM to 5:45 PM

What's Washed In?: A Historical Comparison of Beached Bird Survey Data in the Gulf of Alaska

Drew Stockwell (Drew) Lyons, Junior, Biology (Ecology, Evolution & Conservation)
Xiaobin (Summer) Wang, Sophomore, Biology (Molecular, Cellular & Developmental)
Mentor: Julia Parrish, Aquatic & Fishery Sciences
Mentor: Jane Dolliver, Aquatic & Fishery Sciences

Seabird distribution and abundance can be used as indicators of the health of the coastal ecosystem. In the Gulf of Alaska over the last four decades there has been growing evidence of climate change and ocean acidification. The largest oil spill in the United States, the Exxon Valdez, occurred here. We used data from the Coastal Observation and Seabird Survey Team (COASST), a citizen science, beached bird survey program with 14 years of surveys from beaches in the Pacific Northwest, and eight years of data from Alaska. We seek to compare U.S. Fish and Wildlife Service (USFWS) data from historical surveys in the Gulf of Alaska (1970-1989), to the current beached bird data collected through the COASST program (2005-present). Historic data include monthly surveys of sites from the Kenai Peninsula to Yakutat Bay, and one offshore island, Middleton (n>250 surveys). Current COASST data cover this same range, include additional surveys from Kodiak Island, but exclude Middleton (n>1200 surveys). These data will be analyzed to examine the differences in monthly encounter rate (carcasses/km) averaged over this region, cumulative species diversity, and oiling rate (percent of recorded carcasses that are oiled). This comparison will provide insight into possible changes in the encounter rate index in the Gulf of Alaska over the past 40 years.

POSTER SESSION 4

Commons East, Easel 51

4:15 PM to 5:45 PM

Methods & Algorithms Development for Rapid, Ultra-Low-Cost, Targeted DNA Sequencing

Evan August (Evan) Boyle, Senior, Microbiology, Biochemistry
NASA Space Grant Scholar, Washington Research Foundation Fellow
Mentor: Jay Shendure, Genome Sciences

Despite recent sweeping advances in DNA sequencing technology, whole genome sequencing remains impractical as a routine medical service. Nevertheless, recent work suggests that rare genetic mutations may play large roles in modulating disease progression. More affordable targeted DNA sequencing is currently necessary to study variants of low frequency in the human population and attain sufficient statistical power to link these variants with specific disease phenotypes. Previous studies have demonstrated the effectiveness of a capture by circularization approach using molecular inversion probes (MIPs) whereby thousands of individual sites in the genome can be captured simultaneously for DNA sequencing. Yet, non-uniformity across large numbers of targeted sites persists as a major barrier to more widespread adoption. In this work, we show that statistical analysis of past MIP sequencing runs readily informs modifications to the process of probe design and selection. Additionally, logistic regression on carefully selected MIP features enhances in silico identification of ineffective probes that leave gaps in sequence coverage. Richer data sets from MIP sequencing runs representing a broader range of MIP characteristics and interactions may offer further guidance in perfecting the design of MIPs, achieving greater uniformity across targeted sites and ultimately lowering the cost of sequencing.