Undergraduate Research Symposium May 19, 2017 Mary Gates Hall

Online Proceedings

Poster Session 1

Balcony, Easel 91

11:00 AM to 1:00 PM

Examination of BAS/BIS and Marijuana Usage as Mediated by the Future Self

Jamie Lynn (Jamie) Crites, Senior, Interdisciplinary Arts & Sciences (Psychology), UW Tacoma

UW Post-Baccalaureate Research Education Program

Mentor: Hyoung Lee, School of Interdisciplinary Arts and Sciences

Previous research indicates that the Behavioral Activation System (BAS) in reinforcement sensitivity theory plays an important role in substance use. Research on the future self illustrates that clarity of future self can influence a person's attitudes toward marijuana usage. This study examines the relationship between reinforcement sensitivity theory (BAS and Behavioral Inhibition System [BIS]) and marijuana use, as well as determine whether this relationship is mediated by the future self (self-concept/self-esteem). The recruitment of participants is ongoing, and for now 43 undergraduate students (mean age=21, 74% females, 35% White) participated in this study. Of the 43 participants 13 had ever used marijuana. Participants completed a demographic questionnaire, marijuana use survey, Sensitivity to Reward and Sensitivity to Punishment Questionnaire; and Implicit Association Task (IAT) and semantic differential questionnaire for assessing implicit and explicit sense of future self related to marijuana use. For analysis, participants were classified into ever-used marijuana and never-used marijuana groups. ANOVA revealed significant differences between groups in BAS ($F[1, 41]=5.577$, $p<.05$), and in explicit attitudes ($F[1, 41]=5.518$, $p<.05$), but not in IAT ($F[1, 41]=0.078$, ns). Sobel test resulted in a significant direct effect between BAS and marijuana use (coefficient=0.180, $p<.05$), but not indirect effect mediated by implicit sense of future self related to marijuana use (product of coefficient=-0.006, ns). The findings of the research conducted so far indicates that there is a need to use preventative strategies that target BAS. Furthermore, attitudes of the future can precede behaviors, therefore a future self attitude that is sensation-seeking, can ultimately lead to the sensation-seeking behavior. While the Sobel test has not indicated significance for the mediation by the implicit sense of future self, preventative strategies should target the future self by addressing desirable attributes due to individuals' desire to have such attributes for their future self.

Poster Session 2

MGH 241, Easel 157

1:00 PM to 2:30 PM

A Chamber for Anesthesia-Free Long-Term Imaging in Adult Zebrafish

Micaela L Everitt, Junior, Bioengineering

Mary Gates Scholar

Mentor: Ron Kwon

It has long been known that certain species possess the capacity to regenerate bony appendages following amputation through a process called epimorphic regeneration. For instance, zebrafish possess the ability to regenerate their tail fin bones when amputated. Early regeneration involves the formation of a blastema, similar to the blastema that mediates limb regeneration in salamanders. After the blastema forms, the bone regeneration process resembles the bone developmental process in humans. Thus, a better understanding of epimorphic regeneration holds promise to enhance our understanding of regenerative biology, allow for medical advances in bone tissue engineering, and increase understanding of the skeletal developmental processes. A challenge in understanding the regeneration process is the inability to immobilize fish to generate time lapsed images of various stages of regeneration. Typical methods for anesthesia in zebrafish only enable 10-20 minutes of sedation. Several studies (including those by our lab) have developed specialized methods to do long-term imaging with the use of tricaine methanesulfonate and benzocaine. However, both anesthetics are sodium channel inhibitors, which inhibit the regeneration process itself. In order to circumvent this problem, we designed a chamber for anesthesia-free imaging with a restraint system, and a removable glass-bottom window to facilitate imaging. The chamber is coupled to peristaltic pumps so water can flow in and out. The fish is secured and unanaesthetized, thereby bypassing the issue of lack of regeneration due to the anesthesia. In pilot studies we have found that the zebrafish quickly acclimate to the chamber, suggesting that the zebrafish can remain in the chamber for several hours. Our studies indicate that this anesthesia-free, long-term imaging chamber may allow us to see details of dynamic processes that unfold over a period of 24 hours, which currently can only be seen through snapshots of short-term imaging.

POSTER SESSION 2

MGH 241, Easel 158

1:00 PM to 2:30 PM

Investigating Neuronal Regulation of De-differentiation in the Blastema Following Amputation in the Zebrafish Fin

Molly Elizabeth (Molly) Mounsey, Junior, Biology (Physiology)

Mary Gates Scholar, NASA Space Grant Scholar

Mentor: Ron Kwon

Zebrafish are widely used for the regenerative capacity of their fins and its application to human bone growth and remodeling. The purpose of this experiment is to investigate the role of neurotransmitter systems in the process of fin regeneration and their impacts on de-differentiation of bone cells proximal to the amputation plane. Neuronal signaling is vital to all processes in the human body, however a link between specific neurotransmitter systems and the formation of bone has yet to be fully determined. From our past experiments we have identified a link between botulinum toxin (BTx), a common neurotoxin that inhibits the acetylcholine neurotransmitter system, and Nicotinamide adenine dinucleotide (NADH) levels in the fin during regeneration. NADH levels are indicative of cellular metabolism and are increased in cells that are actively differentiating into mature osteocytes. From this finding we have expanded our study to two neurotransmitter systems, the cholinergic, which focuses on acetylcholine, and the adrenergic, which focuses on epinephrine. From large ligand libraries we formed a ranking of compounds based on their application to bone and prior use in vivo. The four initial compounds utilized were propranolol, galantamine, ICI 118 551, and hemicholinium. Zebrafish were subjected to fin amputations followed by seven days of dosing. Following this procedure, the levels of NADH, green fluorescent protein (GFP), and bone mineralization were analyzed using fluorescent microscopy. GFP is a protein used as a reporter of gene expression. Each condition showed a variation in at least one of these criteria. Three conditions showed an initial increase in NADH, similar to the results of BTx. These results suggest that the relationship between neurotransmitter signaling and regeneration may be composed of many complex interactions between signaling molecules and pathways, and we will continue to investigate this as the experiment progresses into the remaining neurotransmitter systems.

POSTER SESSION 2

MGH 241, Easel 156

1:00 PM to 2:30 PM

Developing a Screening Assay to Identify Osteogenic Compounds Using Post-embryonic Zebrafish

Kenza Elizabeth (Kenza) Coubrough, Senior, Materials Science & Engineering

Mary Gates Scholar, UW Honors Program

Mentor: Ron Kwon

There is a high demand in the medical research community for a high-throughput drug-screening assay to quickly and effectively identify particular osteoactive compounds of interest to make strides in osteoporosis and other bone disease research. A compound is considered osteoactive if it affects the biological processes that constitute bone formation. The goal of the following research is to develop a viable assay that can detect osteogenic compounds in less than four weeks using post-embryonic zebrafish that addresses the time-intensive nature of using alternative animal models for preliminary drug screening. Thus far, we have proved that a double fluorochrome staining technique is a viable method for tracking mineral apposition rate of zebrafish centra under the influence of a certain compound over a time period. Mineral apposition rate (MAR) is a parameter used to characterize bone formation by measuring activity of osteoblasts. Fluorescent imaging and analysis of mineralization aids in quantification of drug effects. Due to the high degree of variability in developmental stages in post-embryonic zebrafish, we are currently developing methods to stage fish in order to eliminate phenotypic variability. Once this and other challenges are addressed, an opportunity for quick breakthroughs in drug discovery pertaining to bone formation will be possible with this time-efficient screening assay.

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.

Investigating Crosstalks of Conserved Growth Regulators, AKT, TCTP, and Yki

Phillip (Phil) Zhu, Senior, Biochemistry

McNair Scholar

Mentor: Young Kwon, Biochemistry

As cancer remains a looming threat to the public, understanding the genetic and biochemical basis of how cancers operate is necessary in order to develop new medicines and treatments. A topic of critical importance when considering the biochemistry of cancers is how specific changes in gene expression control the growth of tumors. I explore this

topic by using *Drosophila* genetics in order to investigate the crosstalk amongst three key growth regulators, RAC-alpha serine/threonine-protein kinase (Akt1), Translationally Controlled Tumor Protein (TCTP), and Yorkie (Yki). The overexpression of Yki in intestinal cells is known to cause intestinal tumors, and our preliminary results indicate that Akt1, TCTP, and Yki interact to control intestinal stem cell proliferation. Nevertheless, signaling crosstalk amongst these three genes is still ambiguous. By using the advantageous genetic toolbox of *Drosophila*, I created experimental fly lines which allow me to manipulate the expression of each gene or a combination of these genes in Drosophila intestinal stem cells. My goal in this project is to anatomize this signaling crosstalk through monitoring how manipulation of these genes in combinations influence the proliferation of intestinal stem cells using confocal microscopy. Ultimately, we hope to attain a greater understanding of growth in relation to genetic networks that control it.

SESSION 2O

USING MODERN GENETIC APPROACHES TO INVESTIGATE DEVELOPMENT AND DISEASE

Session Moderator: Celeste Berg, Genome Sciences
MGH 389
3:30 PM to 5:15 PM
* Note: Titles in order of presentation.

Functional Engraftment of Murine Pre-Osteoblastic Cells in a Zebrafish Model of Epimorphic Bone Regeneration
Barrie Sue Sugarman, Senior, Biology (Physiology)
Mary Gates Scholar, Undergraduate Research
Conference Travel Awardee
Mentor: Ron Kwon

Urodeles such as newts and salamanders have the capacity to regenerate limbs following amputation through epimorphic regeneration, a process characterized by the formation of a proliferative mass of partially dedifferentiated cells called the blastema. The blastema is formed through three steps: migration of cells to the amputation site, dedifferentiation, and re-entry into the cell cycle. It is unknown whether mammalian cells possess competence to respond to blastema formation and process the inductive signals that drive migration, dedifferentiation, and proliferation. To explore this question, our goals were twofold. First, we aimed to develop a xenograft model of epimorphic regeneration by introducing mammalian pre-osteoblastic cells into the regenerating zebrafish tail fin (a tractable model of epimorphic bone regeneration that molecularly resembles amphibian limb regeneration). Next, we sought to compare the behavior of mammalian cells to embryonic zebrafish cells by developing an allograft assay. Adult zebrafish were subjected to caudal fin amputation and housed in ˜33C water. Three days post amputation, CM-DiI-labeled MC3T3-E1 murine pre-osteoblastic cells or fluorescent-dextran labeled embryonic zebrafish cells extracted from the blastula period were injected into the proximal blastema of the adult fish. Fish were subjected to daily *in vivo* imaging. Injected murine cells exhibited migration to the blastema, engraftment, and stability for up to 48 hours post injection (hpi); decreased fluorescence was observed at 2-3 days post injection potentially due to fish immune system activity. Allograft comparisons showed distal migration, engraftment, and stability for 48-72 hpi in embryonic zebrafish cells in a matter nearly identical to the behavior observed in murine cells. Because MC3T3-E1s and embryonic cells exhibit similar migration behavior, our data suggest that murine cells are able to process inductive signals driving localization to distal tissue. Provided the correct conditions, mammalian pre-osteoblastic cells may be capable of engrafting and proliferating in an epimorphic bone regeneration process.

POSTER SESSION 3

MGH 241, Easel 164
2:30 PM to 4:00 PM

Cell Extrusion: A Mechanism of Cell Elimination and Metastasis
Cecilia Minh Tran Nguyen, Senior, Biochemistry
Mary Gates Scholar
Mentor: Young Kwon, Biochemistry
Mentor: Jiae Lee, Biochemistry

Stressed cells may be able to avoid elimination but continue to respond to compensatory growth signals and accumulate, resulting in tumor formation. This ability to avoid cell elimination processes, such as apoptosis, is one of the hallmarks of cancer. The Jun N-terminal Kinase (JNK) mediates cellular stress responses by inducing apoptosis of stressed cells and promoting compensatory cell proliferation. Using immunohistochemistry and the GAL4-UAS targeted gene expression system, we are able to distinguish intestinal stem cells (ISCs) from enteroblasts, enterocytes, and other intestinal cells through a confocal microscope. We found that activation of JNK in fruit fly intestinal stem cells (ISCs) induced temporary over-proliferation. On the other hand, persistent activation of JNK resulted in elimination of the ISCs. Strikingly, we discovered that ISCs with constitutive activation of JNK 'extrude' out from the epithelial layer of fly intestines. Our findings suggest that cell extrusion may be a mechanism for JNK-mediated tumor suppression, preventing the accumulation of cancerous cells. Alternatively, cell extrusion may be involved in metastasis, enabling cancerous cells to spread to other parts of the body. We aim to further investigate the molecular mechanisms and gene expression underlying cell

extrusion in various genotypic backgrounds, which may be helpful to understand how cancerous cells evade cell elimination and undergo metastasis.