Undergraduate Research Symposium May 18, 2018 Mary Gates Hall

Online Proceedings

POSTER SESSION 1

MGH 241, Easel 160

11:00 AM to 1:00 PM

Basis for Dual-Site Modulation and Recording of Neuronal Activity *in vivo*

Mackenzie M. Andrews, Senior, Bioengineering, Neurobiology

Levinson Emerging Scholar, UW Honors Program

Mentor: Charles Chavkin, Pharmacology

Mentor: Antony Abraham, Pharmacology

Drug addiction is a highly prevalent disease with common potentiating risk factors including stress-exposure, anxiety, and depression. A number of brain regions have been linked to behaviors that drive drug seeking and abuse, however little is known about how these regions communicate to regulate those behaviors. Currently, instruments can be used to record neuronal activity *in vivo*, however these devices are typically only able to record from one brain region at a time and don't allow for specificity of neuronal stimulation. In order to improve the understanding of how brain regions communicate to drive behavior, there is a need to develop a multiple-site *in vivo* recording device that can record broad excitation signals while allowing optical stimulation of specific subtypes of neurons. In order to fill this need, I designed a dual-site optic delivering electrode or optrode. This device is small enough to mount on a mouse's skull to promote naturalistic behavior with the ability to record non-specific electrical signals from the brain regions of interest, as well as optically stimulate specific subtypes of neurons. I also designed a program to process and analyze the data recorded by the device. If this device is successful, it will improve our understanding of how brain regions communicate to drive addiction-associated behaviors which could lead to therapeutic solutions for the treatment of addiction.

POSTER SESSION 1

Balcony, Easel 90

11:00 AM to 1:00 PM

DeepSqueak: A Deep Learning Based System for Quantifying USVs and Extended Analysis Kit

Emily K Vo, Junior, Biochemistry

Mentor: John Neumaier, Psychiatry

Mentor: Kevin Coffey, Psychiatry and Behavioral Science

The ability to quantify rat and mice ultrasonic vocalizations (USVs) can provide ethological validity to animal models of affective disorders. Vocalization frequencies are key indicators of a rodent's subjective state and are closely tied to their behavior. For example, 50-kHz calls in rats are emitted during positive events such as sucrose drinking, and are attributed to a positive affective state. In contrast, 22-kHz calls are ascribed to a negative affective state, and are emitted during negative events such as shock. Current methods for manually scoring vocalizations are slow and labor intensive, while automated programs lack the accuracy to produce satisfactory scoring under non-ideal recording conditions. In order to create a more precise program for detecting these USVs, we applied deep learning, which has revolutionized the field of bioacoustics through image detection and recognition of audio files. By converting soundwaves produced by mice and rats into spectrograms, we can train convolutional neural networks to distinguish between vocalizations and noise. Using these methods, we created the software DeepSqueak©, a Deep Learning Based System for Quantifying USVs and Extended Analysis Kit, which outperforms current state-of-the-art, commercially available software. Hundreds of rat calls from the 18-75kHz were manually isolated from spectrograms and used as training data for our neural network, allowing it to differentiate calls from noise. As a result, USV detections using DeepSqueak are far more rapid and accurate in comparison to existing software. The detected calls produced from the software were then reviewed and re-entered into the neural network as another training set to consolidate its precision and efficiency. With the speed and reliability of DeepSqueak, quantification of USVs can become more widespread and lead to a wealth of new knowledge relating to affective disorders.

POSTER SESSION 1

Balcony, Easel 89

11:00 AM to 1:00 PM

Identification of Non-canonical DREADD-Receptor Signaling and Overexpression

Hafsa Abdinasir Abdinur, Sophomore, Pre-Health Sciences
Mentor: John Neumaier, Psychiatry
Mentor: Atom Lesiak, Genome Sciences

DREADD receptors (Designer Receptor Exclusively Activated by Designer Drugs) are engineered G-protein receptors which are exclusively activated by the inert-molecule CNO (clozapine-N-oxide) and to a lesser degree clozapine. DREADD receptors are often used, to identify the circuitry and cellular signals that specify behavior, perceptions, emotions, motivation, and motor functions in species ranging from flies to nonhuman primates. Heterologous overexpression of non-native genes in biological systems, is often confounded by varying degrees of overexpression and result in non-specific effects. Thus, our purpose is to determine effects on cell signaling pathways following varying levels of DREADD receptor expression. Our hypothesis is that, increased levels of DREADD receptor expression increase non-canonical cellular signaling pathway activation. We used human embryonic kidney cells (HEK cells), as our model system, then expressed various levels of DREADD receptors using Lipofectamine transfection and DNA-plasmids expressing Gs- and Gi-coupled DREADD receptors (Canonically, Gs-increases cAMP and Gi-decreases cAMP). After transfection, we treated transfected cells with 1μM of CNO or VEH (a control) for 5-30min and then lysed the cells with RIPA Buffer, and prepared samples for western blotting to detect activation of proteins of interest in the GPCR non-canonical signaling pathways (example: kinases and transcription factors). We used anti-HA antibodies to ensure dosage of receptor expression, and used a panel of antibodies to detect activated proteins in well-established non-canonical GPCR signaling pathways. Western blot results were measured using Odyssey scanner and the Image studio software suite. We expect to see an increase in non-canonical signaling with increased expression of both Gi- and Gs- coupled, specifically in the MEK/ERK and CREB signaling pathways. In summary, our findings will highlight additional considerations regarding the level of receptor expression when using DREADD receptors in vitro and in vivo experiments, and specifically measure the off-target "non-canonical" signaling pathways induced by DREADD receptor overexpression.

POSTER SESSION 1

Balcony, Easel 91

11:00 AM to 1:00 PM

Expression of the Neuropeptide PACAP: The Role of the Amygdala in Acute Stress

Zoe K. Lewis, Junior, Extended Pre-Major
UW Honors Program
Mentor: John Neumaier, Psychiatry
Mentor: Marjorie Levinstein, Psychiatry and Behavioral Sciences

Stress is highly correlated with negative physical health impacts and an estimated 75 to 90% of primary care visits are attributed to stress related conditions. Currently available treatments for anxiety are not always effective and very limited, therefore identifying new targets for the development of medication is important. Recently, neuropeptides have drawn interest as potential targets for drug therapies. Pituitary adenylate cyclase-activating polypeptide, or PACAP, is a neuropeptide that has been linked to stress responses in a variety of brain regions. For example, the amygdala is a crucial brain region for mediating chronic stress, acute stress and fear and contains numerous PACAP-expressing neurons. My research focuses on the amygdala and the expression of PACAP following an acute stress. In my experiment, I quanitfied PACAP levels both with and without acute stress. After stressing rats using a forced swim protocol, I measured PACAP and mRNA expression in the amygdala using RNAscope in situ hybridization. Brain slices were imaged using fluorescence microscopy to quantify the RNA scope signal. To analyze these images quantitatively, I have developed a CellProfiler code to accurately and rapidly analyze the proportion of neurons expressing PACAP mRNA as well as the intensity of expression within the neurons. I hypothesize that PACAP mRNA expression will be increased in the rats subjected to the acute stressor, indicating that PACAP is an important mediator for the neural stress response. By determining this relationship between neuropeptide expression and stress response, PACAP antagonists could be potentially used in drug therapies to help patients experiencing stress related health conditions.

POSTER SESSION 1

MGH 241, Easel 161

11:00 AM to 1:00 PM

Characterization of New Probes Designed to Reveal the Neural Circuitry Responsible for Stress-Induced Behaviors

Sanne Marie Casello, Sophomore, Pre-Sciences
Mentor: Charles Chavkin, Pharmacology
Mentor: Benjamin Land, Pharmacology
Mentor: Allisa Song, Pharmacology

Chronic stress induces the release of hormones and neuropeptides including dynorphin, which activates Kappa Opioid Receptors (KOR) to encode the anxiogenic components of the stress response. Previous studies have identified the spe-

cific dynorphin-KOR neurochemical signaling responses in the regions of the brain known for regulation of reward systems, and thus may be potential therapeutic targets for stress-related conditions such as depression, anxiety, and substance use disorders. Formally, cells containing dynorphin-KOR have been difficult to study due to technical limitations of the available reagents. However, the generation of new tools including mice expressing promotor-driven cre-recombinase (e.g. KOR-Cre and prodynorphin-Cre) have allowed cell-specific gene expression using Cre-dependent viral expression of fluorophores combined with in-situ staining. In this study, the reliability of a phospho-KOR antibody was tested. The specificity of the immunostaining was verified by administering a KOR agonist, U50,488, in wild-type animals or transgenic animals lacking the G protein-coupled receptor kinase 3 (GRK3), which phosphorylates the KOR. Additionally, wild-type mice with a pre-treatment of norBNI, a KOR antagonist, followed by U50 were also tested. Immunohistochemistry of animal brain tissue showed that the norBNI pre-treated, wild-type animals and GRK3 knock-out animals had lower KORp-IR fluorescence compared to their wild-type counterparts treated with U50,488, verifying that the KORp antibody was reliable. After verification, this KORp antibody was utilized to characterize the dynorphin-KOR circuitry in the mouse brain. To do so, an excitatory opsin was injected into the DR of pDyn-Cre mice with an optic fiber implant. The DR region was then optically stimulated with blue light and dynorphin-releasing projections into the ventral tegmental area were verified by measuring KORp fluorescence. This experiment details one specific dynorphin-KOR pathway in the mouse brain that was not previously studied, and future experiments will utilize this approach to map the extent of dynorphin/KOR localization within the reward circuitry.

POSTER SESSION 2

MGH 241, Easel 127

1:00 PM to 2:30 PM

Deciphering Interactors of the α1D Adrenergic Receptor

Diana Tram Anh Dinh, Senior, Biochemistry
Mentor: Chris Hague, Pharmacology, University of Washington School of Medicine
Mentor: Dorathy-Ann Harris, Pharmacology
Mentor: Eric Janezic, Pharmacology

G Protein-Coupled Receptors (GPCRs) are seven-transmembrane proteins present throughout the body that can be activated upon binding of drugs, hormones, or neurotransmitters. Acting like an inbox for messages, these multi-protein complexes play a significant role in the body by regulating cell expression and signaling, making them attractive targets for drug development. One class of GPCRs, known as the adrenergic receptors (ARs) are critical in modulating the function of numerous targets, including cardiac muscle, vascular smooth muscle, and bronchial smooth muscle. There are nine AR subtypes: three α1s, three α2s, and three βs. In my research, I focus on deciphering the interactions of proteins with the α1D-AR due to a recent publication of the Hague Lab, which discovered that α1D-AR forms a multi-PDZ protein complex. To determine the participants of this complex and identify the presence of associated proteins, I first purified the proteins that potentially bind to α1D-AR. In my purification process, I first conducted Polymerase Chain Reactions (PCRs), transformed my bacteria, grew my bacteria, and finally purified my proteins of interest. With this information, my lab and I intend to identify the complexes using mass spectrometry and Octet. Determining how α1D-ARs are organized can create a deeper understanding of how drugs work in the body and assist in new drug development.

POSTER SESSION 2

MGH 241, Easel 129

1:00 PM to 2:30 PM

Characterizing the Interaction of the hDLG1 PDZ Domains to the Alpha-1D Adrenergic Receptor

George Williams, Sophomore, Pre-Sciences
Mentor: Dorathy-Ann Harris, Pharmacology
Mentor: Chris Hague, Pharmacology, University of Washington School of Medicine

The alpha-1D adrenergic receptor (A1DAR) is a G-protein coupled receptor (GPCR) and is known for its function as a regulator for cardiovascular, urinary, and central nervous system function. This unique GPCR has a PDZ ligand at the C-terminus of this receptor. A PDZ ligand is a region on a protein that can bind to a PDZ domain on another protein and is named based on the three proteins that it was observed in. Previously, it has been discovered that this ligand interacts with the syntrophin family of PDZ domain proteins. The hDLG1 protein is a human homologue of the Drosophila disc large (DLG1) tumor suppressor protein and contains three PDZ domains that have been implicated in interacting with A1DARs. By determining how/if the PDZ domains on the hDLG1 protein bind to the A1DAR, a more accurate depiction of this macromolecular complex can eventually be determined. Several methods were used in order to determine this. First, I cloned hDLG1 PDZ domains separately as well as varying combinations of the three domains. Next, I purified the protein constructs so I could determine the binding efficiency of the hDLG1 proteins to the A1DAR via Bio-Layer Interferometry (Octet). SNAP gel assays can also be used to determine the interaction with the A1DAR. Upon reviewing the results of these techniques, the nature of the interaction between the PDZ domains of the hDLG1 protein to the A1DAR can be determined. Understanding the architecture of this structure has many implications in pharmaceuticals as this protein can be specifically targeted by medications to

treat a multitude of diseases. Side effects can be minimized by targeting specific parts of this large protein complex with medications than produce more specific responses thus limiting unwanted side effects. Medications that target the proteins involved in this complex can affect the function of the cardiovascular, urinary, and central nervous system.

POSTER SESSION 2

MGH 241, Easel 131

1:00 PM to 2:30 PM

Impact of TAF1 Missense Mutations Associated with Intellectual Disability on Histone Binding

Carolyn Brager, Junior, Pre-Sciences
UW Honors Program
Mentor: Edith Wang, Pharmacology

Gene expression constitutes the foundation of all cellular processes. The general transcription factor (TF) complex, TFIID, is essential for the proper execution of gene expression, as it binds to the promoter region of genes and initiates the recruitment of RNA Polymerase II to begin transcription. The TAF1 protein is the largest subunit of the TFIID complex and possesses histone acetylation activities. Our lab and others have shown that TAF1's ability to bind to acetylated histones is critical for regulating key steps in gene transcription and cell cycle progression. Whole genome and exome sequencing of individuals diagnosed with intellectual disability has revealed mutations in the TAF1 gene. This project is interested in introducing a disease associated missense mutation (S1600G) into TAF1 to investigate its functional consequences using biophysical approaches. We used polymerase chain reaction to amplify the domain of TAF1 containing the missense mutation. The produced product was inserted into an expression vector with an N-terminal histidine tag for purification and the resulting plasmid was transformed into competent bacterial BL21 cells for protein expression. The TAF1 protein was extracted and then purified from bacterial lysates. The purified wild type and mutant proteins will be compared using functional biophysical analysis. By using Bio-layer interferometry, an optical analytical technique for measuring binding between two molecules, we can measure the interaction between TAF1 and histones in real time as indicated by different shifts in interference patterns. Any change in the number of histone molecules bound to the TAF1 protein immobilized on the biosensor tip causes a shift in the interference pattern that can be measured as an indicator of how the missense mutations affect histone binding activity. Ultimately, significant deviance from normal histone binding activity will be an indicator of functional disruption due to these specific missense mutations possibly causal for the disease phenotype.

SESSION 2R

EXPLORING PROTEIN FUNCTION AT SCALES FROM WHOLE TISSUES TO SINGLE ATOMS

Session Moderator: Celeste Berg, Genome Sciences

JHN 111

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Functional Interaction of Cyclic Adenosine Monophosphate (cAMP) and Hippo Signaling in Tumor Suppression and Tissue Homeostasis

Annie Shoemaker, Junior, Microbiology
Mentor: Martin Golkowski, Pharmacology
Mentor: Shao-En Ong, Pharmacology

The evolutionarily conserved Hippo cell signaling pathway is important in tissue and organ development through its regulation of cell death, proliferation, and differentiation; as such, the Hippo pathway is often dysregulated in cancer. The phosphotransferases serine/threonine-protein kinase 3 and 4 (STK3 and STK4), are core components of the Hippo pathway. When active, these kinases negatively regulate the transcription factors yes-associated protein 1 (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ). The activities of STK3 and 4 are regulated by phosphorylation on multiple serine, threonine and tyrosine residues. Recently, we discovered that the central cellular signaling regulator cAMP-dependent protein kinase (PKA) can phosphorylate STK3 on serine 15 (S15). Since it has been shown before that PKA can suppress the transcriptional activity of YAP/TAZ, we hypothesized that this phosphorylation event activates STK3. To investigate the functional consequences of STK3-S15 phosphorylation, I used molecular cloning to construct active and kinase-dead (KD) FLAG-tagged STK3 mutants in which S15 was replaced by alanine (S15A, non-phosphorylatable) or aspartate (S15D, phosphomimic), respectively. I transfected plasmid DNA encoding these mutants into human embryonic kidney cells (HEK293T) to investigate if overexpression of each STK3 variant differentially affects activation of the Hippo pathway. Western blot analyses of transfected cells showed that active STK3 S15A and S15D but not the kinase-dead versions were able to phosphorylate known STK3 substrates. Further, I used anti-FLAG antibodies to enrich FLAG-tagged STK3 variants and co-precipitating protein complexes for mass spectrometric (MS) analysis to examine the effect of STK3-S15 phosphorylation on protein-protein interactions. MS analysis revealed that the STK3 S15D mutant preferentially bound to STK4, suggesting pathway activation. Insights into the mechanism of Hippo pathway regulation by PKA will have important implications

for cancer research since targeted therapeutics modulating the cAMP - PKA signaling axis may be directly applicable in tumors with aberrant YAP/TAZ activity.

POSTER SESSION 3

MGH 206, Easel 169

2:30 PM to 4:00 PM

Chemical-Genetic Manipulation of Anchored Kinase Signaling During the Cell Cycle

Akansha Bhat, Senior, Biology
Mentor: John Scott, Pharmacology
Mentor: Paula Bucko, Pharmacology

Mitosis is a process important for the regulated division of cells. In cancer cells mitosis is often dysregulated, which can cause cells to split uncontrollably. One important protein that plays a key role during mitosis is the protein kinase Polo-like Kinase 1 (plk1). The function of Plk1 is to regulate centrosome maturation, bipolar spindle assembly, and mitotic entry, making it a very important protein throughout mitosis. To understand how it functions during mitosis we can use drugs that turn off the activity of Plk1 to mimic what occurs when the protein is malfunctioning. Drugs like BI2536, a small-molecule inhibitor that turns off Plk1 activity in mitotic cells, have provided insight into how Plk1 functions; however, these drugs are not selective enough to inhibit protein activity at distinct locations in the cell. To address this problem, we use a technique called SNAP-tag to localize BI2536 to specific organelles within cells. Using genetic engineering approaches like cloning, virus generation and stable cell production, I helped make cells that express a SNAP protein linked to a PACT domain. PACT localizes to the centrosomes in dividing cells and allows us to bring SNAP protein only to that location. Our modified drug enters cells, binds to SNAP-PACT, and selectively inhibits Plk1 activity at the centrosome of cells. Through experiments I have run, we have shown that we are able to inhibit Plk1 activity in vitro and in vivo. Using immunofluorescences and super-resolution microscopy, I have shown that local targeting of CLP-BI2536 to the centrosomes causes a greater reduction in active Plk1 (p-plk1) than traditional global drug delivery. In the future, I hope to use this approach to investigate the activity of another mitotic protein, Aurora A, in order to further explore the role of mitotic kinases during the cell cycle.

POSTER SESSION 3

MGH 206, Easel 175

2:30 PM to 4:00 PM

Dopamine Subpopulations of the Ventral Tegmental Area Mediate Coordinated but Dissociable Reward Processes

Naomi Mae Mc Farland, Senior, Psychology
Mary Gates Scholar
Mentor: Larry Zweifel, Psychiatry and Pharmacology
Mentor: Gabriel Heymann

The mesolimbic dopamine pathway begins in the midbrain Ventral Tegmental Area (VTA) and projects to the nucleus accumbens (NAc) and is fundamental in regulating goal-directed behaviors. Abnormalities in this pathway are implicated in a number of neuropsychiatric disorders including drug addiction and depression. However, there is still a lot unknown about the specific circuitry and cell types that contribute to these behaviors and diseases. This project aims to provide a deeper understanding of how different dopamine populations in the VTA contribute to reward behavior. We use Cre-driver mouse lines to isolate genetically distinct VTA dopamine subpopulations. Using this strategy, we classified three different subpopulations: Corticotrophin releasing hormone receptor one (Crhr1-Cre), Cholecystokinin (Cck-Cre) and Tachykinin receptor three (Tacr3 Cre). Interestingly, these populations have inclusive and complementary projections to the NAc and it's two subdivisions, the NAcCORE and NAcSHELL. We utilize optogenetic techniques to activate each subpopulation individually. To test learning acquisition, mice without any prior training were placed into an operant box containing two levers that when pressed would result in optical excitation of the targeted VTA subpopulation. We found that optical stimulation of the Crhr1 subpopulation, which projects to the NAcCORE, can drive lever pressing instrumental learning. Activation of Cck neurons, which project to the NAcSHELL, is unable to promote learning. However, if animals first learned an instrumental behavior to obtain food reward, subsequent activation of Cck neurons would maintain instrumental responding. Interestingly, activation of Tacr3 (projects to both NAcCORE and NAcSHELL), resulted in rapid instrumental learning and maintenance of high levels of lever pressing. These results establish independent roles for Crhr1 and Cck dopamine neurons in instrumental reward learning and motivation, as well as a requirement for co-activation of NAcCORE and NAcSHELL pathways (Tacr3) for maximal reinforcement responding, thus providing more specified targets for therapeutic interventions.

POSTER SESSION 3

MGH 241, Easel 131

2:30 PM to 4:00 PM

Low Doses of Ketamine Improve Cardiac and Respiratory Rhythms in Rett Syndrome: A Clinical Pilot Study

Vy Yen Huynh, Junior, Biochemistry, Neurobiology
Mentor: Franck Kalume, Neurological Surgery and Pharmacology, UW/ Seattle Children's

Rett Syndrome (RTT) is a neurodevelopmental disorder that is first recognized in infancy and almost exclusively affects females. The syndrome is characterized by normal development in the first 6 to 18 months of life, followed by a regression of developmental milestones, particularly the loss of mobility skills and purposeful use of the hands. Additional features of RTT include severe disturbances in cardiac and respiratory functions. These are characterized by prolonged QT intervals as well as increased respiratory rate and occurrence of abnormal respiratory events such as breath holds. RTT results from mutations in the gene encoding the protein MECP2, which helps to regulate gene activity. The location and type of the MECP2 gene mutation influence the course and severity of the syndrome. Previous animal studies and clinical case reports have suggested that ketamine administered at a low dosage can reduce deficits in brain activity and improve neurological function in RTT. In this pilot study, we sought to determine the efficacy of ketamine in reducing the respiratory and cardiac phenotype observed in RTT. Electrocardiography (ECG) and respiration patterns were recorded before and after treatments of four RTT patients with low doses of ketamine. Examination of these recordings showed that ketamine decreases heart rate variability, respiratory rate, and the number of abnormal respiratory events within the 20 hours post drug administration. These results suggest that low-dose ketamine treatment may potentially serve as an effective future treatment for the cardiac and respiratory symptoms of RTT.

POSTER SESSION 4

Balcony, Easel 116

4:00 PM to 6:00 PM

Conservation of Deubiquitinating Enzyme Activity in RNA Polymerase I

Ruth Armance Groza, Senior, Biochemistry
Mentor: Richard Gardner, Pharmacology
Mentor: Sabrina Kamran, Pharmacology

Eukaryotic ribosome biogenesis is an essential and energetically demanding cellular process that must be tightly regulated. In S. *cerevisiae*, this regulation is partially achieved via ubiquitination of RPA190, the largest subunit of RNA polymerase I, and further mediated by the deubiquitinating enzyme (DUB) Ubp10. Human USP36, the ortholog of Ubp10, rescues RPA190 stability in *ubp10 delta* yeast cells, indicating DUB activity may be conserved across eukaryotes. As such, there is considerable interest in deciphering the mechanism by which RPA190 and human RPA194 are ubiquitinated. Previous work identified three lysines (K405, K408, and K410) as possible ubiquitination sites. However, whether one or a combination of these lysines are ubiquitinated has yet to be determined. We created single lysine to arginine mutant RPA190 plasmids for each site and transformed them into *rpa190 delta* cells. These constructs may be used to help determine which lysine(s) are targeted by Ubp10 and whether those site(s) are functionally analogous in RPA194. This will yield a more complete understanding of the importance of DUB activity in the context of eukaryotic ribosome biogenesis.