UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 17, 2013 Mary Gates Hall

Online Proceedings

2H

Neuroscience and Genetics: From Disorders to Tools for Discovery

Session Moderator: Gwenn Garden, Neurology

248 MGH

3:45 PM to 5:15 PM

* Note: Titles in order of presentation.

Analysis of Altered miRNA Expression in a Transgenic Mouse Model of SCA7

Kt Nguyen, Senior, Neurobiology

Levinson Emerging Scholar, Undergraduate Research Conference Travel Awardee

Mentor: Gwenn Garden, Neurology

Spinocerebellar ataxia type 7 (SCA7) belongs to a family of neurodegenerative diseases. It is characterized by degeneration of the cerebellum and brainstem. The disease is caused by an expanded polyglutamine tract in ataxin 7. This type of polyglutamine mutation is a common feature in many neurodegenerative diseases. Other neurodegenerative disease including those involving polyglutamine expansions demonstrate altered microRNA (miRNA) expression. Given the supporting evidence that miRNA are differentially expressed in other polyglutamine diseases, I seek to determine the role of miRNAs in SCA7. My proposed research will analyze altered miRNA expression in Purkinje cell neurons, a selectively vulnerable neuronal population that degenerate in SCA7. I will first identify miRNAs that are upregulated or downregulated in Purkinje neurons from SCA7 mice. Next using predictive databases I will identify their potential mRNA targets. I will then test identified miRNAs in cell culture to see if they will inhibit expression of their predicted target. If expression is inhibited, I will then measure the level of mRNA and protein of the predicted target in transgenic mouse tissue. By identifying miRNAs that are differentially expressed in SCA7 mice and confirming their activity, we can build our understanding of the mechanisms of disease progression in SCA7.

Exome Sequencing of Singleton Trios and Quads Reveals *De Novo* Mutations in Schizophrenia

Amanda C (Amanda) Larson, Senior, Biology (Molecular, Cellular & Developmental)

Mary Gates Scholar, Undergraduate Research Conference Travel Awardee

Mentor: Mary-Claire King, Medicine, Genome Sciences

Schizophrenia is a debilitating neuropsychiatric disorder with a worldwide prevalence of about 1%. Although the heritability of schizophrenia is high, there are many sporadic cases; that is, affected individuals with no family history of mental illness. We are sequencing persons with schizophrenia in the absence of family history of mental illness (probands), their unaffected parents, and whenever possible, an unaffected older sibling, in order to identify *de novo* mutations that may be responsible for the disorder. Our hypothesis is that *de novo* events will be observed in many different genes, and that the functions of the impacted genes will differ between probands and their unaffected siblings. From exome sequence, we filter variants to include only those predicted to be both *de novo* and damaging to the protein product. Next, we validate all candidate variants passing these filters by Sanger sequencing, using diagnostic PCR primers to amplify genomic DNA of probands, siblings, and parents. Results to date suggest that approximately 50% of probands and siblings carry at least one damaging *de novo* mutation. The analysis of pathways in which the genes converge is in progress. Next, we will target these candidate genes and determine their complete variant profiles in 5000 cases and 5000 controls. These results can help us identify critical pathways that are affected in schizophrenia.

Examining the Localization of the 5-HT6 Receptor on Neuronal Primary Cilia

Alex Louis (Alex) Kostrinsky Thomas, Junior, Extended Pre-Major
Mentor: John Neumaier, Psychiatry
Mentor: Matthew Brodsky, Neurobiology and Behavior

The primary cilium is a sensory organelle found on most cells. In the brain, most or all neurons contain a single primary cilium, yet its function is not entirely understood. Ciliopathies are defined as a group of disorders physiologically characterized by malformation or dysfunction of primary cilia, which in turn result in a variety of symptoms including cognitive deficits, retinal blindness, situs inversus and polydactyly. The serotonin 6 receptor (5-HT6) is the only 5-HT receptor that localizes to neuronal primary cilia, and previous research has implemented the receptor in learning, memory, and drug addiction. Our experiment will investigate the link between neuronal primary cilia and 5-HT6 in an attempt to better understand the significance of this unique localization. Using an immortalized line of rat adrenal cells (PC12), we will express the 5-HT6 receptor using viral vectors, and then pharmacologically manipulate the activity of the receptors with either an agonist (WAY 399885) or an antagonist (SB 208466). We will first stain the cells using immunohistochemistry, then image using florescence microscopy, and lastly quantify the morphological change in primary cilia length. We expect to see a shortening of primary cilium in response to the introduction of an antagonist, and an increase in length with an agonist. Since the 5-HT6 receptor has been shown to be homologous between rats and humans, a practical application of this research could eventually result in a pharmaceutical solutions to some of the cognitive symptoms associated with ciliopathies like congenital cerebellar ataxia or Joubert syndrome.

In Vitro Characterization of the DREADD-1B Receptor as a Surrogate for 5-HT1B Autoreceptor Function

Jessica Ann (Jessica) Falksen, Senior, Neurobiology
Mary Gates Scholar
Mentor: John Neumaier, Psychiatry
Mentor: Yusha (Katie) Liu, Plastic Surgery

Serotonin (5-HT) is implicated in numerous emotional behaviors, including depression and anxiety. One form of serotonin receptors is the 5-HT$_1B$ autoreceptor, which is involved in regulating 5-HT transmission and uptake. 5-HT$_1B$ autoreceptors are located on serotonergic neurons and are pharmacologically indistinguishable from 5-HT$_1B$ heteroreceptors located on non-serotonergic neurons. Distinguishing between activation of the two receptor types is important as they have different functions. A novel strategy to accomplish this is to use an engineered receptor to mimic the 5-HT$_1B$ autoreceptor using a modified DREADD receptor (Designer Receptor Exclusively Activated by a Designer Drug). The DREADD-1B receptor will mimic the 5-HT$_1B$ autoreceptor function with pharmacological specificity. The DREADD-1B receptor is G$_i/o$-coupled G-protein coupled receptor that is activated by the selective ligand clozapine-N-oxide. When G$_i/o$-coupled GPCRs are activated, decreases in intracellular adenylyl cyclase and, subsequently, cAMP levels are measured. It is expected that when the DREADD-1B receptor is activated, a decrease in cAMP levels will be observed. To verify this, I created a stable DREADD-1B cell line and will use transient transfections of a luciferase plasmid to measure cAMP levels via proportional luminescence using luciferin. Optimizing the conditions for receptor activation and observing sufficient decreases in cAMP levels will confirm the general functionality of the receptor. If there are large variations in the downstream biochemistry between the DREADD-1B receptor and the 5-HT$_1B$ autoreceptor, using the DREADD-1B receptor as a surrogate for the 5-HT$_1B$ autoreceptor could have profound effects on behavioral data obtained in other projects. Creating and characterizing the DREADD-1B receptor in an *in vitro* setting will provide valuable information and tools for future projects involving the 5-HT$_1B$ autoreceptor and this novel tool.

In Vitro Analysis of Reduced Inhibition within Dravet Syndrome Interneurons

Jordan Daniel Hardman, Senior, Biology (Molecular, Cellular & Developmental)
Howard Hughes Scholar, Mary Gates Scholar
Mentor: William Catterall
Mentor: John Oakley, Neurology
Mentor: Chao Tai, Pharmocology

Dravet syndrome (DS), also known as severe myoclonic epilepsy of infancy (SMEI), is a rare genetic form of epilepsy, caused by a mutation in the neuronal voltage-gated sodium channel, NaV1.1, encoded by the SCN1A gene. This form of epilepsy is characterized by febrile seizures at infancy that often change with age into afebrile seizures, which include generalized tonic-clonic and myoclonic seizures. In most cases, DS is a debilitating disorder that renders patients with decreased cognitive function and impaired development. To study this disorder, a mouse model of DS, with heterozygous deletion of the SCN1A gene (Scn1a +/-), was developed, demonstrating a phenocopy of the DS condition, with similar seizure patterns and atypical behaviors. In vitro work in the model demonstrates selective loss of sodium current and excitability in hippocampal GABAergic interneurons. However, it remains unknown if similar changes in neocortical GABAergic interneuron excitability occur. We hypothesize that fast spiking neocortical interneurons are responsible for control of cortical excitability via GABAergic inhibition and that reduced excitability of fast spiking interneurons associated with heterozygous loss of NaV1.1 contributes to hyperexcitability and seizures. To explore this hypothesis, we will compare neocortical interneuron excitability measured from

single action potential parameters and repetitive action potential firing patterns in unaffected wild type mice and the DS mouse model, using pre-existing single-cell recordings. Excitability will be determined from the peak amplitude, width at half height, threshold for single action potentials, and from total number of action potentials in induced neuronal spike trains. If this hypothesis is correct, we expect that there will be atypical action potentials and firing patterns showing reduced excitability. This experiment will provide more insight into DS epilepsy and better therapeutic targets for the disorder.