Specific Aims

Obsessive Compulsive Disorder (OCD) affects about one percent of the nation [1]. OCD is defined by two major symptoms: obsessions, which are uncontrollable thoughts and actions, and compulsions, which are thoughts or rituals used to alleviate the anxiety that accompanies obsessions. OCD is a polygenic disorder, but one distinctive gene linked to it ultimately codes for the serotonin transporter SLC6A4. Normally this protein controls reuptake of serotonin from the synapse. However, mutations in SLC6A4 can cause its over-activation, potentially by inducing permanent phosphorylation [2]. This leads to higher rates of serotonin reuptake and dampening of serotonergic messages. The influence of phosphorylation on SLC6A4 expression gives some insight into mechanisms that may affect other proteins associated with OCD and help explain the polygenic effects that have been documented.

The heritability of OCD is roughly half, meaning that along with being polygenic, half the onset of OCD is due to environmental factors [3]. Potential contributors that lead to anxious behaviors include a lack of social bonds, threats of danger, and even behaviors that children learn from the adults around them [4]. For several decades, rats and mice that exhibit symptoms such as ritualistic chewing behaviors and anxiety-like responses have been used to study OCD [5]. They will be used as model organisms for testing our specific aims.

SLC6A4 activation and environmental stressors are known to affect OCD onset. However, changes in signal protein levels such as protein kinases in relation to over-activated SLC6A4 and how this affects other OCD-linked proteins has not been studied. **To address this lack of knowledge we will test the hypothesis that SLC6A4 over-activation causes measurable changes in protein kinase levels, in turn affecting phosphorylation of other proteins.**

The **long-term goal** of this study is to determine complex interactions between proteins as they relate to the onset of OCD. We hope to move toward this goal by using several genomic, proteomic, and bioinformatic methods.

**Aim One:** Identify novel and conserved SLC6A4 interacting proteins. **Approach:** Using tandem affinity purification (TAP) tags, novel protein-protein interactions will be tested. A cDNA library of proteins from the synaptosomes of rats will be assembled and tested for interactions with the wild-type SLC6A4 after purification. Based on STRING, any new interactions previously undocumented in rats will be entered into BLAST to find human homologs. **Hypothesis:** Many undiscovered interactions will be found because of the sensitivity of TAP tag approaches. **Rationale:** Discovering new SLC6A4 interacting proteins introduces additional proteins that may cause other behavioral changes seen in OCD.

**Aim Two:** Identify genes that are misregulated in SLC6A4 mutant rats. **Approach:** Neuronal tissue samples from healthy and SLC6A4 mutant rats will be collected and used to assess gene expression levels using RNA-seq. Genes that are differentially expressed in mutant rats will be entered into the Gene Ontology Consortium (GO) and grouped for similar biological processes. **Hypothesis:** Genes linked to signaling and modification GO terms will be under-expressed in SLC6A4 mutant rats. **Rationale:** Finding differential expression of signaling genes in SLC6A4 mutants would suggest how expression of many other genes associated with OCD could be altered, leading to characteristic OCD behaviors.

**Aim Three:** Determine how environmental factors alter the neuronal proteome in mice. **Approach:** Mice will be exposed to stressors such as isolation, threat of danger, and interaction with Slc6a4tm1Kpl mutant mice designed to already exhibit anxious tendencies. Neural proteins will be collected at time points before, during, and after each exposure. Samples will also be taken from the Slc6a4tm1Kpl mice. A proteomic assay will be performed to compare the proteomes in healthy, stressed, and mutant mice. **Hypothesis:** Mice exposed to environmental stressors will exhibit proteomes that approximate the mutant proteomes, indicating which stressors may contribute to onset of OCD. **Rationale:** Testing how environmental stressors change the proteome addresses the environmentally-based half of factors contributing to onset of OCD and indicates which have the greatest effect.

This research will determine interactions between proteins involved in OCD and lead to a greater understanding of the molecular pathways implicated in the onset of OCD symptoms. With more known about these relationships, new treatment methods could be developed. Because there are so many other anxiety disorders that are highly similar to OCD, any information discovered about its manifestation may also be applicable to others. Future revelations may lead to drug treatments or behavioral modification approaches that are effective for a wide variety of psychiatric disorders, bettering the lives of millions.

[1] Facts & Statistics | Anxiety and Depression Association of America, ADAA. (2010, January 1). Retrieved February 23, 2015, from <http://www.adaa.org/about-adaa/press-room/facts-statistics>

[2] Ramamoorthy, S., Shippenberg, T., & Jayanthi, L. (2011). Regulation of monoamine transporters: Role of transporter phosphorylation. Pharmacology & Therapeutics, 129(2), 220-238. Retrieved April 15, 2015, from <http://www.sciencedirect.com/science/article/pii/S0163725810001932>

[3] Iervolino, A., Rijsdijk, F., Cherkas, L., Fullana, M., & Mataix-Cols, D. (2011). A Multivariate Twin Study of Obsessive-Compulsive Symptom Dimensions. Archives of General Psychiatry, 68(6), 637-644. Retrieved February 5, 2015, from <http://archpsyc.jamanetwork.com.ezproxy.library.wisc.edu/article.aspx?articleid=912992>

[4] In-Depth Report: Obsessive-Compulsive Disorder. (2013, March 11). Retrieved March 16, 2015, from <http://www.nytimes.com/health/guides/disease/obsessive-compulsive-disorder/risk-factors.html>

[5] Albelda, N., & Joel, D. (2012). Review: Current animal models of obsessive compulsive disorder: An update. Neuroscience, 211, 83-106. Retrieved February 23, 2015, from <http://www.sciencedirect.com/science/article/pii/S0306452211010281>