Alcohol dependence is a serious public health issue and is described as a strong, often uncontrollable, desire to drink [1]. In addition to social and environmental factors, alcohol dependence is associated with a genetic contribution and one such gene, *NRXN3* was recently identified. *NRXN3* belongs to a family of Neurexin presynaptic transmembrane proteins that function as cell adhesion molecules [2]. A single nucleotide polymorphism in the 3′ region of the *NRXN3* gene distinguish individuals who are dependent on illegal substances from control individuals [3], and are associated with impulsivity and alcohol problems in men [2]. Neurexins are key factors that mediate synapse function and *NRXN3* dysfunction may play a role in diseases with a cognitive component [2]. Despite these findings, *the molecular mechanisms through which NRXN3 polymorphisms lead to alcohol dependence and synaptic dysfunction remains unclear.*

My **primary goal** is to better understand how *NRXN3* mRNA regulation contributes to synaptic dysfunction and in turn, impulsivity. My **long-term goal** of this research is to understand how *NRXN3* contributes to alcohol dependence so that effective treatments can be made for those who suffer from the disorder. My **hypothesis** is that low mRNA levels might be due to a decrease in phosphorylation at serine sites in *NRXN3*, leading to loss of protein function at the presynaptic transmembrane. *Drosophila melanogaster* will be used as a model organism because they share anatomical and molecular similarities to humans. Drosophila’s synapse is useful to investigate due to its structural accessibility and because their nerve impulses direct locomotion and behavior like in humans.

**Aim 1: Characterize conserved amino acids of *NRXN3* crucial for proper synaptic function.**

**Approach:** First, I will use BLAST to find homologs of *NRXN3*. I will then align protein sequences using Clustal Omega to identify conserved 3’ regions of amino acids among the homologs. I will then mutate the well conserved amino acids with CRISPR-Cas9 using *Drosophila* as a model to understand how these mutations affect synaptic function and alcohol dependence at different stages of development. I will then screen the mutated flies that exhibit the incoordination phenotype as well as improper synaptic function and alcohol dependence.

**Rationale:** Screening of flies with the induced gene mutations from those conserved regions should result in uncoordinated flies that will explain what mutations in *NRXN3* do to synaptic function.

**Hypothesis:** I hypothesize that specific conserved amino acids in *NRXN3* correlate with proper synaptic function and decreased alcohol dependence.

**Aim 2: Identify small molecules that rescue *NRXN3* mutant phenotypes.**

**Approach:** I will perform a high-throughput chemical genomic screen on wild type and *NRXN3* mutant flies from Aim 1 at different stages of development. This will be done by using a diversity-oriented library to identify small molecules that rescue the *NRXN3* mutant incoordination and improper synaptic function phenotypes.

**Rationale:** Treating mutant *NRXN3* flies with the identified small molecules will upregulate cell adhesion and restore wild type function of *NRXN3*.

**Hypothesis:** Small molecules that can rescue the *NRXN3* mutant phenotypes will restore proper synaptic function and decrease alcohol dependence.

**Aim 3: Identify and mutate phosphorylation sites in *NRXN3* to observe effects in the pathway.**

**Approach:** I will use STRING to find proteins that interact with *NRXN*3 and are involved in phosphorylation. NetPhos will be used to find the expected phosphorylation sites in *NRXN3*. I will specifically look for sites where serine is phosphorylated. Then I will mutate the serine sites in a wildtype *NRXN3* fly and compare protein activity levels in the mutant fly and a wildtype fly to observe how phosphorylation affects synaptic function.

**Rationale:** CASK is a serine protein kinase that helps control expression of other genes involved in brain development. Since CASK phosphorylates Neurexins, mutating *NRXN3* should result in decreased levels of CASK activity, a decrease in phosphorylation at the serine sites, and improper synaptic function.

**Hypothesis:** Mutating the most highly conserved serine phosphorylation site in *NRXN3* will result in decreased phosphorylation and improper synaptic function.

**References:**

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