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"Validation and characterization of *Lphn3* gene deletion in a mouse model of ADHD"

Background: Attention deficit hyperactivity disorder (ADHD) is a highly heritable neurodevelopmental disorder characterized by deficits in attention, hyperactivity and impulsivity. Previous studies have found that ADHD is a major risk factor for addictive behaviors, such as alcohol use disorder (AUD). Individuals with ADHD have been shown to initiate alcohol drinking earlier and more robustly in adolescence than their non-affected peers which also accelerates the development of an AUD. AUDs are also highly heritable and in fact ADHD and AUDs share several genetic risk factors including the variants in the *LPHN3* gene. *LPHN3* encodes a cell adhesion G-protein coupled receptor (GPCR) known as latrophillin-3 which has a prominent role in forming and maintaining glutamatergic synapses. Constitutive deletion of *Lphn3* in rats and mice leads to impulsivity, attentional deficits, and hyperactivity compared to their wildtype littermates and is considered a leading ADHD preclinical model. We are interested in the relationship between ADHD and AUD and are assessing the effectiveness of neuronal and brain-region specific deletion of *Lphn3* as a model of ADHD and for studying the interaction of ADHD and alcohol. We hypothesize that neuron specific deletion of *Lphn3* using synapsin-Cre strategy results in reduced *Lphn3* transcript in neurons and behavioral changes that include increased hyperactivity, cognitive deficits, and increased alcohol consumption. Gaining insight into LPHN3's role in neurological functioning could lead to a greater understanding in the relationship between ADHD and AUD.

Methods: To obtain a pan-neuronal knockout of *Lphn3*, we crossed Synapsin-Cre mice with floxed *Lphn3* mice. We used fluorescent in situ hybridization (FISH) to determine if transcripts to *Vglut1* and *Lphn3* were present in our area of interest, the prefrontal cortex (PFC), and if *Lphn3* transcription was reduced in *Vglut1* neurons containing Cre recombinase compared to wildtype littermates. *Lphn3* wildtype (WT), heterozygous (HET), and mutant (MUT) conditional KO male and female mice were tested on behavioral task: elevated plus maze, open field, novel object recognition task, object in place recognition task, and Y-maze). *Lphn3* WT, HET, KO were also given access to alcohol during adolescence (PND30 to 60) using an intermittent 2-bottle choice method and alcohol dose consumed and alcohol preference were evaluated.

Results: Our FISH results indicate uninterpretable results related to genetic deletion of *Lphn3* in neurons and that troubleshooting of RNAscope procedures is necessary to evaluate our collected tissue. Behavioral results indicate behavioral changes in *Lphn3* KO and HET mice consistent with ADHD behaviors (increased hyperactivity, reduced recognition memory). Alcohol consumption and preference were not statistically different amongst genotypes.

Conclusion: Our pan-neuronal deletion of *Lphn3* leads to observable differences in behavior consistent with an ADHD phenotype, for instance increased locomotor activity and increased novelty seeking behaviors, however alcohol consumption during adolescence was not affected. Our proof-of-principle, FISH data are inconclusive and require further work improving our methodologies.