Characterization of Adolescent Alcohol Consumption in Preclinical Model of ADHD

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Introduction

Attention deficit hyperactivity disorder (ADHD) is a highly heritable neurodevelopment disorder characterized by deficits in attention, hyperactivity and impulsivity. In past studies, it has been shown that individuals with ADHD start drinking alcohol at an earlier age and more robustly than their non-affected peers.

ADHD and AUDs (alcohol use disorder) have shown to share multiple genetic risk factors. This includes the variants in the LPHN3 gene, which encodes a cell adhesion G-protein coupled receptor named latrophilin-3. It plays an important role in forming/maintaining glutamatergic synapses (the main excitatory synapses in the brain). The deletion of LPHN3 in rodents recapitulates symptoms of ADHD (impulsivity, attentional deficits, hyperactivity) and is considered a leading ADHD preclinical model.

Rationale

As of now, it is unknown how Lphn3 deletion affects alcohol consumption during adolescence. Gaining insight into LPHN3's role in neurological functioning using this preclinical ADHD model could lead to a greater understanding in the relationship between ADHD and AUDs.

Methods

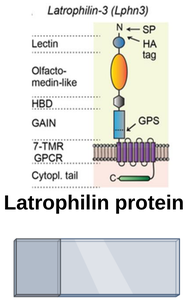
Experiment 1: Synapsin-Cre mice were crossed with floxed *Lphn3* mice in order to obtain a pan-neuronal knockout of Lphn3. Male and female mice brains (n = 12) from each genotype were collected and sectioned for fluorescent in situ hybridization assay (RNASCOPE) to determine that Lphn3 expression was reduced in neurons compared to wildtype littermates.

Experiment 2: WT, HET and MUT cKO Lphn3 mice were tested for locomotor activity and recognition memory using the 2-object novel object recognition task (NORT2)

Experiment 3: Wildtype, heterozygous, and mutant conditional KO male and female mice were given access to alcohol during adolescence (PND30 to 60) using an intermittent 2-bottle choice method between water and alcohol. We collected plasma to determine blood alcohol levels reached during drinking days.

Results

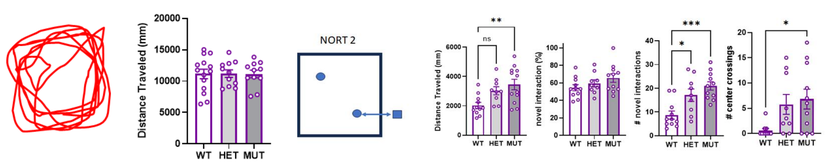
Experiment 1: Confirmation of pan-neuronal Lphn3 gene deletion



Latrophilin protein

RNAscope: *Lphn3* (ongoing)

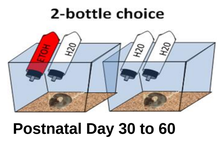
Experiment 2: Pan-neuronal deletion of Lphn3 and behavioral assessment of ADHD phenotype



Baseline locomotor activity is not different across genotypes

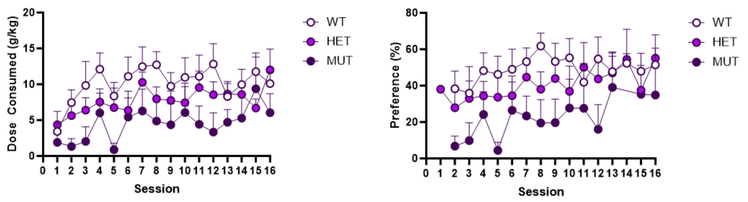
Locomotor activity, center time (%) is increased in MUTs during Novel Object Recognition Task (NORT)

Experiment 3: Pan-neuronal deletion of Lphn3 and voluntary alcohol consumption during adolescence

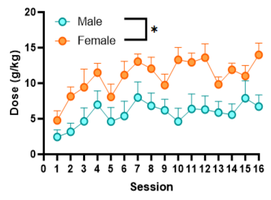


Postnatal Day 30 to 60

	WT	HET	MUT	Total
Males	3	4	3	8
Females	4	3	1	10
Total	7	7	4	**18**



We did not observe an effect of gene on alcohol dose consumed or preference



Females consumed more alcohol than males during adolescence (main effect sex, 2-way RM ANOVA, *p < 0.05). Females were underrepresented in MUT group (n = 1).

Conclusion

- **Confirmation and quantification of Lphn3 gene deletion in neurons is ongoing, however our hybridization probes appear to detect RNA.**
- **Behavioral assessment of Syn-Lphn3 cKO mice demonstrate increased locomotor activity in the presence of novel objects and increased novel object exploration.**
- **Lphn3 MUT mice cross center of arena more than WT mice, which we interpret as loss of inhibition.**
- **We did not observe any effects of gene on alcohol consumption during adolescence.**
- **Females consumed more alcohol than males during adolescence.**
- **Due to the imbalance of gender across genotypes, our results are inconclusive and require more subjects.**

Future Directions

- **Number of mice should be increased to balance gender across genotype groups**
- **We will assess blood alcohol levels**
- **We will complete RNASCOPE experiments**
- **To determine if there are non-selective effects that explain differences in alcohol consumption, we will assess sucrose consumption during adolescence and alcohol taste aversion**

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