Validation and Characterization of *Lphn3* Gene Deletion in a Mouse Model of ADHD

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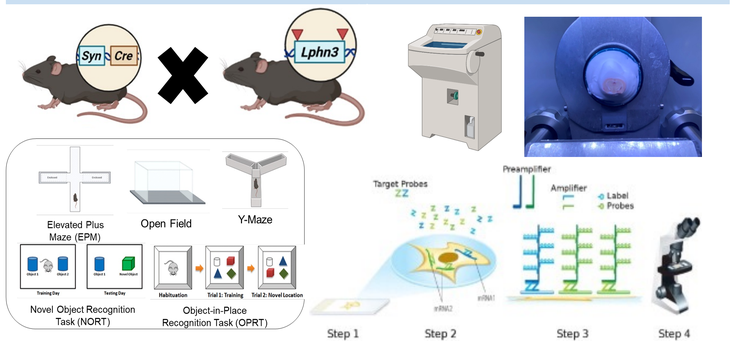
Introduction

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)[1]. Those with ADHD have been found to start drinking at a younger age and more robustly than those without ADHD[2]. Both ADHD and AUDs share common genetic risk factors, which include the *LPHN3* gene. *LPHN3* encodes latrophillin-3, a cell adhesion GPCR involved in forming and maintaining synapses that is developmentally regulated[3,4]. Constitutive genetic deletion of *LPHN3* in rodents recapitulates symptoms of ADHD (hyperactivity, impulsivity) and is considered a leading preclinical ADHD model[5]. This study will use a Cre-loxP transgenic mouse strategy to delete *Lphn3* specifically in neurons to assess ADHD-like behaviors and alcohol consumption. Proof of principle validation of this approach is required for subsequent experiments aimed at understanding the relationship between ADHD genes and alcohol with a focus on the prefrontal cortex (PFC) implicated in both ADHD and AUD.

Rationale

As of now, *Lphn3* expression and function are not well described in the cortex and it is unknown if neuronal specific deletion of *Lphn3* in mice recapitulates ADHD-like behaviors or affects alcohol consumption. We hypothesize that neuronal deletion of Lphn3 driven by the synapsin promoter will reliably delete Lphn3 from neurons in mice that will result in ADHD behaviors and increased alcohol consumption.

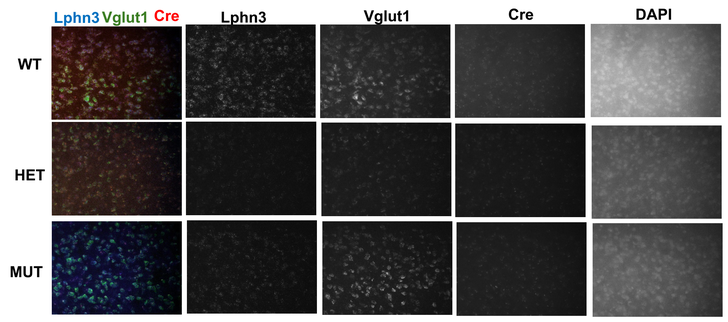
Methods



Experiment 1: Synapsin-Cre mice were crossed with floxed Lphn3 mice in order to obtain a pan-neuronal conditional knockout (cKO) of *Lphn3*. *Lphn3* WT, HET, and MUT male and female brains (n=8) were collected and sectioned for fluorescent in situ hybridization (FISH) assay (RNASCOPE) to measure Lphn3, Cre, and Vglut1 transcript expression and nuclear counterstained with DAPI.
Experiment 2: WT, HET, and MUT cKO *Lphn3* mice (n = 8) were tested in a battery of behavioral tasks to assess locomotor activity, anxiety, and memory using elevated plus maze (EPM), open field (OF), novel object recognition task (NORT), switched object recognition task (SORT), and Y-maze.
Experiment 3: Wildtype, heterozygous, and mutant conditional KO male and female mice were given access to alcohol during adolescence (PND 30 to 60) using an intermittent 2-bottle choice between water and alcohol. We collected plasma to determine blood alcohol levels reached during drinking days.

Results

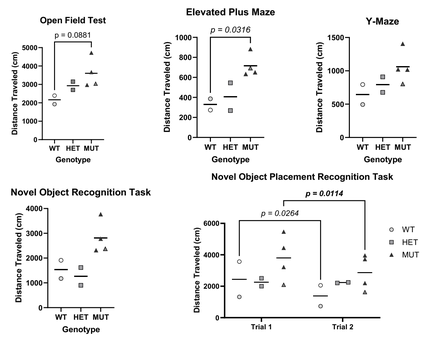
Experiment 1: Confirmation of pan-neuronal *Lphn3* gene deletion



Observations of transcript expression using FISH inconclusive due to lack of image quality

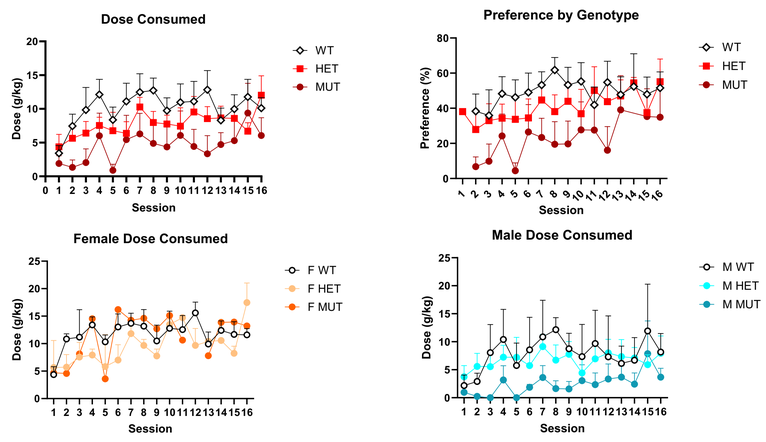
Experiment 2: Pan-neuronal deletion of *Lphn3* and behavioral assessment of ADHD phenotype in mice used for RNAscope

Locomotor Assessment across tasks **Results from behavioral tasks**



Results from behavioral experiments suggest an emerging hyperactivity phenotype in mice on each task. Task relevant outcome measures indicate an emerging trend for cognitive impairment on NORT, SORT, and Y-maze as well as decreased anxiety in HET and MUT *Lphn3* cKO mice.

Experiment 3: Pan-neuronal deletion of *Lphn3* and voluntary alcohol consumption during adolescence (separate cohort)



Adolescent alcohol consumption was not statistically significant (2-way RM ANOVA) amongst genotypes. We observed sex specific effects on dsoe consumed and therefore compared genotypes separated by sex, but these results were also not statistically significant.

Conclusion

- Signals for Lphn3, Cre, Several rounds of RNAscope experiments yielded inconclusive results related to reduction of *Lphn3* RNA in HET and MUT mice.
- Behavioral results from mice used in RNAscope validation indicate behavioral changes in *Lphn3* KO and HET mice consistent with ADHD behaviors including increased hyperactivity and poorer performance on memory tasks (although group numbers are underpowered).
- Adolescent alcohol consumption and preference were not statistically different amongst genotypes and it appeared MUT mice consumed lower amounts, against our hypothesis. This appears largely due to mutant male alcohol consumption.
- In parallel experiments, PFC deletion of *Lphn*3 increases alcohol consumption and so there may be subregional specific effects of *Lphn3* deletion on alcohol consumption

Future Directions

- We will review and troubleshoot RNAscope procedures and continue to validate gene deletion of LPHN3 in our animal model of ADHD
- We will assess LPHN3 protein expression in conditional knockout mice using immunohistochemistry procedures
- Morphological analysis of dendritic spines in LPHN3 conditional knockout mice to assess structural and physiological changes related as a result of LPHN3 deletion
- We will continue to characterize LPHN3 deletion specifically in the PFC

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