Modeling Early Life Risk Factors for Alcohol Use Disorder Later in Life

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Introduction

- Alcohol Use Disorder (AUD) is a chronic disorder that affects 29 million people in the US and is linked to anxiety, enhanced pain sensitivity, impaired development, and long-term changes in brain function.
- Two major risk factors for AUD: Early life stress (ELS) and adolescent alcohol exposure
- The ventral tegmental area (VTA), a component of the brain's reward system, is sensitive to these influences
- VTA neuron activity is regulated in part by GIRK2 channels, which are sensitive to alcohol
- **The goal of this work is to establish preclinical models for early life risk factors for AUD**
- **We hypothesize that (1) ELS exposure decreases GIRK2 channel expression on VTA DA neurons; and (2) that adolescent alcohol exposure increases anxiety like behavior and decreases pain threshold.**

Methods

Male and female C57BL/6J mice were used for all experiments

Early Life Stress (ELS) Model

- A Limited Bedding and Nesting (LBN) paradigm was used from postnatal day 4(p4)-p11 to model ELS

Figure 1: Timeline for LBN Model. Dams and litters are transferred to a new cage with LBN (orange) or standard housing (blue); Maternal behavior is scored during the light and dark cycles (stopwatch symbols). Pups are weighed at p11 and p21 (scale symbols). Tissue samples are collected in adulthood (p120) and immunohistochemistry is performed to quantify GIRK2 expression in VTA dopaminergic neurons.

Adolescent Alcohol Exposure Models

- Voluntary alcohol intake was modeled using a Drinking in the Dark paradigm (DID); mice were given access to 20% ethanol for 2-4 hours/day from p30-p41
- Involuntary alcohol intake was modeled by adolescent intermittent exposure (AIE) to ethanol vapor for 16 hr/day for 4-day sessions to mimic binge-pattern exposure
- Anxiety-like behavior was measured using a marble burying and pain sensitivity was measured using Von Frey during protracted abstinence

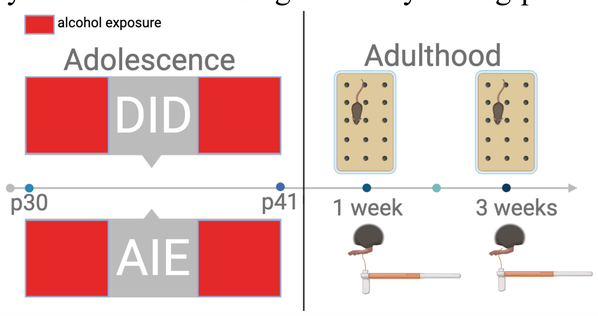

Figure 2: Timeline for Adolescent Alcohol Exposure Models.

ELS Results in Decreased Body Weight and VTA GIRK2 and TH Co-expression in Offspring

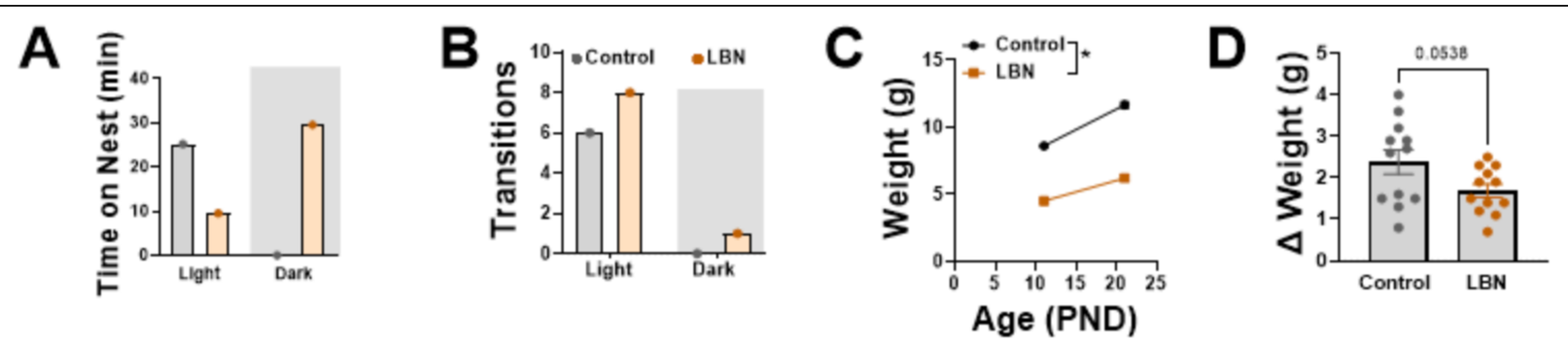

Figure 3. Maternal Behavior in LBN Paradigm. A) Time spent on nest during the light and dark cycles in control (grey) and LBN (orange) dams. B) Number of transitions on or off the nest during the light and dark cycle. C) Pup weight at p11 and p21 in LBN and control mice *p<0.05, two-way ANOVA. D) Change in pup weight from p11 and p21. LBN mice experienced decreased change in growth compared to controls (p=0.0503, two-tailed t-test).

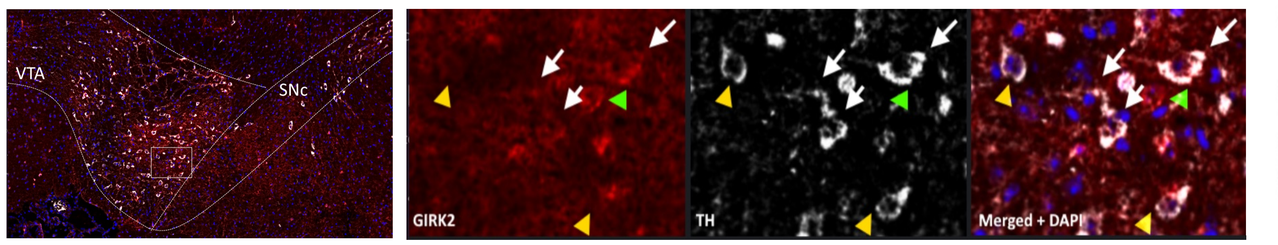

Figure 4. TH and GIRK expression in the VTA of LBN and control mice offspring. A) Representative midbrain image. GIRK2 expression (red), TH (white), DAPI (blue); VTA, ventral tegmental area; SNc, substantia nigra pars compacta. White box represents region shown in B. B) 20x image of VTA neurons. White arrows indicate GIRK2+TH+ neurons; yellow arrowheads indicate TH+GIRK2- neurons; green arrowhead indicate GIRK2-TH+ neurons C) GIRK2+TH+ cells (expressed as a percentage of all neurons) in the VTA in control (grey) vs. LBN (orange) mice (p=0.0883, two-tailed t-test).

Adolescent Alcohol Exposure Results in Lasting Impacts on Behavior During Protracted Abstinence

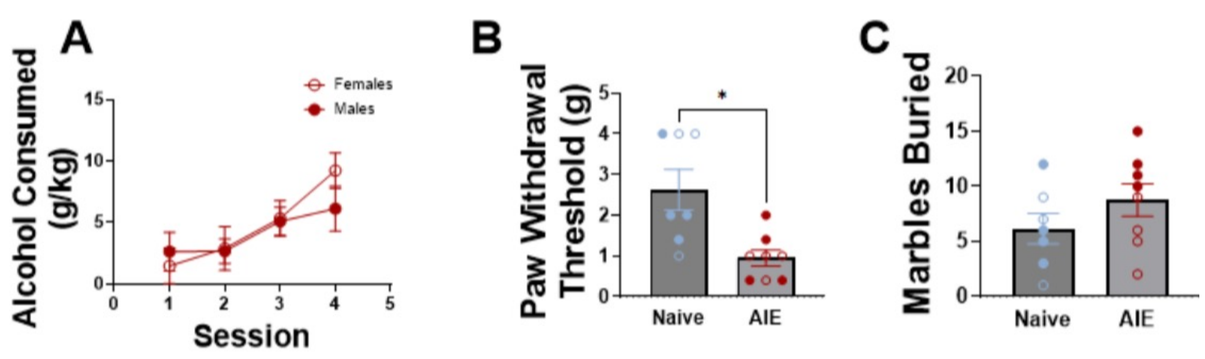

Figure 5. Adolescent Alcohol Exposure and its effects on pain threshold and anxiety-like behavior. A) Alcohol consumption during DID paradigm in female (open circles) and male (closed circles) adolescent mice. B) Paw withdrawal threshold measured using von Frey testing in mice with a history of AIE (red) tested 3 weeks post vapor session, compared to alcohol-naïve (blue) mice. *p<0.05, two-tailed t-test. C) Number of marbles buried during marble burying test in naïve (blue) and AIE (red) mice measured 3 weeks post vapor.

Conclusion

- These findings suggest that mice with a history of ELS experience decreased body weight and GIRK2 and TH co-expression in the VTA compared to controls.
- They also suggest that adolescent alcohol exposure contributes to increased anxiety-like behavior and pain sensitivity during protracted withdrawal.
- Future studies will integrate these paradigms to investigate the combined influence of ELS and adolescent alcohol on the risk for AUD later in life.

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