**Introduction**

- Adolescence is a time of critical brain development, a phase involving significant physical, cognitive, emotional, social, and behavioral changes.
- Most alcohol use is initiated during adolescence, typically in a binge-like manner and adolescent alcohol use is a strong predictor for the development of alcohol use disorders.
- The bed nucleus of the stria terminalis (BNST) is a sexually dimorphic brain structure implicated in emotional regulation, including negative affect-like behaviors related to alcohol withdrawal.
- Dynorphin (DYN) is an endogenous opioid peptide that can act on Kappa Opioid Receptors (KOR).
- Dyn and KOR mediate the negative affective state associated with alcohol withdrawal.

**Hypothesis:** Adolescent intermittent ethanol (AIE) induces long-term hyperalgesia, which is associated with activation of BNST Dyn and KOR expressing cells in male and female mice.

**Methods**

**Adolescent Intermittent Ethanol Vapor (AIE):** Adolescent mice were given a daily injection of either pyrazole + saline (Air-control) or pyrazole + ethanol (AIE group) to impair the metabolism of ethanol. Mice underwent two four-day cycles of AIE on postnatal day (PND) 28 to 39. This involved 16-hour periods in vapor chambers followed by 8-hour periods in regular animal housing, which allowed for the reliable obtainment of blood ethanol concentrations in the 150–185 mg/dL range.

**Behavioral testing:** Von Frey and Hargreaves occurred at 5 different time points to detect hyperalgesia in mice. Mechanical and heat sensitivity was assessed at 24 h, 7, 14, 21, 28 days post-vapor exposure.

**In-Situ Hybridization (RNASeq):** In-situ hybridization was performed using the RNAscope. The following probes were used: C1: c-fos, C2: dynorphin and C3: kappa opioid receptors. Nuclei were stained using DAPI. Slides were imaged on ZEISS AxiosScan.21 slide scanner and analyzed on the QuPath 0.4.3 software. Cells with more than 5 puncta were considered positive. To determine the percentage of cells expressing a specific mRNA with the number of positive cells divided by the total number of DAPI labeled nuclei.

**Conclusion**

- Adolescent intermittent ethanol (AIE) induces long-term hyperalgesia and produces a sex difference in activation of DYN and KOR positive cells in the dorsolateral BNST, while in the oval BNST we found sex differences in the general activation and the activation of dynorphin positive cells.
- Overall, there was no significant difference in cell activation between air and AIE exposed mice.
- For future directions, this study may be replicated with a larger cohort.

---

This research project was supported by Award Number: DBI-2349224 through the National Science Foundation (NSF), Research Experiences for Undergraduates (REU) Program, and NIH R01 AA02801.