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**“Activation of DYN- and KOR-expressing Neurons in Bed Nucleus of the Stria Terminalis in Adolescent Alcohol-Induced Hyperalgesia”**

**Introduction:** Adolescent alcohol can lead to long-lasting changes in brain function by impairing healthy adolescent brain development and increasing the likelihood of developing an alcohol use disorder (AUD) over the lifetime. Most alcohol use begins during adolescence, usually in a binge-like fashion, and adolescent alcohol use is a major predictor of AUD. One behavior that contributes to alcohol use and potentially the development of AUDs is pain. Alcohol use is known to reduce acute pain, yet chronic alcohol withdrawal can produce hyperalgesia. There are also sex differences in pain sensitivity and alcohol's effect, but the interaction of these factors has not been explored. The bed nucleus of the stria terminalis (BNST) is a highly sexually dimorphic brain region involved in negative affective states, alcohol withdrawal, and, more recently, pain. BNST neurons are very heterogeneous, expressing numerous neurotransmitters and neuropeptides. One of these stress-related neuropeptides is Dynorphin. Dynorphin (Dyn) is involved in pain, AUD, and mood regulation, primarily through the action at the kappa opioid receptor (KOR). The purpose of this study is to assess the activation of Dyn- and KOR-expressing cells in the BNST of male and female mice that show adolescent intermittent ethanol (AIE) induced hyperalgesia.

**Methods:** To replicate adolescent binge drinking, both male and female C57BL/6 mice were given either AIE vapor exposure or air exposure. 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 normal animal housing, which allowed for the reliable obtainment of blood ethanol concentrations in the 150–185 mg/dL range. Von Frey and Hargreaves occurred at five different time points to detect hyperalgesia in mice. Mechanical and heat sensitivity was assessed 24 hours, 7, 14, 21, and 28 days post-vapor exposure. The brains were extracted 30 minutes after the last session of Von Frey and flashed frozen. Brains were then sliced on the cryostat collecting the BNST region and used for RNA in situ hybridization (RNAscope), which allowed for quantification of c-fos, Dynorphin, and KOR expression in the BNST. C-fos can be used as an indicator of cellular activity and quantified in the presence or absence of Dyn and KOR expression.

**Results:** AIE induced prolonged hyperalgesia (up to 28 days) in the von Frey test, decreasing paw withdrawal thresholds compared to air control in both male and female mice, with no observed sex difference. However, in the Hargreaves test, female air and AIE groups showed no differences, whereas male mice exhibited significant differences between groups across all time points. Using RNAscope, we were able to determine that female mice have higher c-fos in all cells along with higher c-fos expression in Dyn and KOR cells in the dorsal lateral BNST (dlBNST) than males with no significant effects of AIE treatment. In the oval BNST, we also see greater overall c-fos expression and c-fos/Dyn expression in female mice than in male mice. However, we did not find a significant difference in cell activation between mice exposed to air and AIE. For future directions, this study may be replicated with a larger cohort to determine whether the results are consistent.