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"Negative Affect and BNST Cellular Activation during Withdrawal from Adolescent Alcohol Exposure"

Adolescence is a time of maturation when the brain is continuing to develop, leaving it vulnerable to the effects of alcohol and stress. For some adolescents, alcohol use is initiated and consumed in a binge-like manner. Alcohol exposure during adolescence is known to increase the risk of developing an alcohol use disorder (AUD) later in life. Recent evidence finds that adolescent alcohol use in females is outpacing males, highlighting the need to understand sexually dimorphic brain circuitry impacted by adolescent alcohol and the accompanying differences in negative affect. It is known that females are more motivated to consume alcohol to relieve negative affect, while males drink for the positive rewarding effects of alcohol.

Negative affect can be produced during alcohol withdrawal, and this negative affect is a driver for alcohol induced relapse. Thus, in adolescents who misuse alcohol, a cycle is created by which alcohol consumption and withdrawal produces negative affect which then drives future alcohol consumption and relapse. A brain region that is known to be critical in alcohol-induced negative affect and relapse is the bed nucleus of the stria terminalis (BNST). The BNST is also highly sexually dimorphic, so it may also be important for understanding sex differences arising from adolescent alcohol use.

Previous behavior experiments in the Wills Lab have revealed that glutamatergic signaling in the BNST is disrupted by adolescent alcohol use, however the mechanisms of this disruption are distinct between males and females. In current experiments, male and female C57Bl/6J mice underwent two 4-day cycles of alcohol vapor exposure (16 hours/day) with a 3-day period of rest between cycles from PND 30-41 (adolescent intermittent alcohol exposure: AIE). The control groups were in chambers with vaporized water. During acute withdrawal (5 hours after vapor), behavioral tests were performed to examine anxiety-like behavior using the elevated plus maze (EPM) and marble burying task. In a separate cohort of mice that underwent the same AIE protocol, brains were collected at the same acute withdrawal timepoint for RNAscope. RNAscope is used to identify BNST cell populations activated during acute withdrawal from AIE using the immediate early gene, cfos. Using RNAscope, we are quantifying cfos expression with corticotropin releasing factor (CRF) and corticotropin releasing factor receptor 1 (CRFR1). Neurons with CRFR1 receptors are activated in times of stress and anxiety. Our goal is to identify whether CRF and/or CRFR1 signaling is activated during the withdrawal from AIE and if there are sex differences in this activation between male and female mice.

Behavioral task results show that in the EPM, acute AIE withdrawal reduced partial open-arm entries in both males and females. However, acute AIE withdrawal reduced full open-arm entries in males only. Acute AIE withdrawal did not affect mice in the marble burying task. These results demonstrate sex differences in the EPM with a more robust anxiety-like phenotype in male mice. Further, the effects of AIE withdrawal were specific to the tests used, as no effects were seen with the marble burying task. Current work using RNAscope is analyzing cfos activation and its co-expression in CRF and/or CRFR1 containing cells.