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

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Adolescence is a time of brain maturation, lending vulnerability to the effects of alcohol.
Most alcohol use is initiated during adolescence, typically in a binge-like manner.
Adolescent alcohol use is the highest risk factor for developing an alcohol use disorder (AUD) later in life.
Current data shows that adolescent alcohol use in females is outpacing males.
Females are more motivated to consume alcohol to relieve negative affect, while males drink for the positive rewarding effects of alcohol.
Negative affect is produced during alcohol withdrawal, and this negative affect is a driver for continued alcohol use and later stress-induced relapse.
The bed nucleus of the stria terminals (BNST) is a highly sexually dimorphic brain region that is known to be critical in alcohol-mediated negative affect and subsequent relapse.

The current work will test the hypotheses that withdrawal from adolescent alcohol exposure produces sex differences in:
- Negative affect phenotypes
- BNST cellular activation, specifically in CRF and CRFR1 containing cells

**Background**

**Methods**

**Results**

**Discussion:**
This work demonstrated numerous sex differences during withdrawal from AIE.
- Anxiety-like behavior measured by the EPM was more robust in male mice.
- In the BNST, female mice have higher overall cellular activation, CRFR1, and c-fos/CRF co-expression compared to males, but these levels are not impacted by AIE. AIE appeared to increase CRFR1 and c-fos/CRF co-expression in male mice.

**Future Directions:**
- C-fos activation was expected to be higher overall in the AIE treatment group, therefore future work will explore other withdrawal timepoints and interactions with stress.
- Increase the number of mice in RNAscope experiments.
- Additional behavioral tests could be used to study if sex differences occur in other behavioral phenotypes.

**Adolescent Alcohol Exposure**

Adolescent alcohol exposure (AUD) is a critical period in the development of alcohol use disorders, with up to 1 in 5 youth and adults with AUD beginning their alcohol use prior to 12 years of age.

Adolescent alcohol exposure is associated with increased risk of AUD in adulthood, later AUD onset, more severe AUD, and higher AUD-related harm.

**Behavioral Tasks:**
- **F) Acute AIE withdrawal reduced partial open-arm entries on EPM in both males and females.**
- **G) Acute AIE withdrawal altered full open-arm entries on EPM only in males.**
- **H) Acute AIE withdrawal did not affect marble burying.**

**RNAseq:**
- Overall, RNAscope data reveals several non-significant but trending results. Female mice have greater c-fos, CRF, and c-fos/CRF co-expression compared to males but were not impacted by AIE. AIE appeared to increase CRFR1 and c-fos/CRF co-expression in male mice.

**Discussion:**
This work demonstrated numerous sex differences during withdrawal from AIE.
- Anxiety-like behavior measured by the EPM was more robust in male mice.
- In the BNST, female mice have higher overall cellular activation, CRFR1, and c-fos/CRF co-expression compared to males, but these levels are not impacted by AIE treatment.
- In male mice, AIE enhances CRFR1 expression and c-fos/CRF co-expression, demonstrating a greater sensitivity of BNST CRF-CRFR1 signaling from alcohol withdrawal in male mice.

**Future Directions:**
- C-fos activation was expected to be higher overall in the AIE treatment group, therefore future work will explore other withdrawal timepoints and interactions with stress.
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