Sex Differences in the Effects of Adolescent Alcohol Exposure

Rebecca K. Yoon[1], S.L. Rein[1], O.R. Brunke[1], E.B. Holmgren[2], T. Henderson[2], C.R. Kasten[2], Tiffany Wills, PhD[2]

Tulane University[1]

Dept. Cell Biology and Anatomy, LSU Health Sciences Center[2]

Introduction

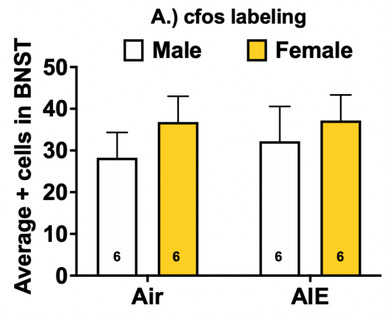
- Adolescent alcohol exposure is one of the strongest risk factors for the development of alcohol use disorder.
- Brain maturation that occurs during adolescence is vulnerable to the effects of alcohol.
- Affective disorders tend to emerge during this period of development as well.
- There is a growing body of evidence that suggests adolescent alcohol use in females is outpacing males, and that there are sex differences in various aspects of alcohol use.
  - Women are more likely to use alcohol to blunt emotions of negative affective disorders such as anxiety and depression, while men are more likely to drink alcohol for the positive reward effects.
- Negative affect can occur during alcohol withdrawal and can be a driver in alcohol induced relapse
- The bed nucleus of the stria terminalis (BNST) is a sexually dimorphic region of the brain that modulates negative affect and stress, and has proven to be critical in alcohol-induced relapse.
- BNST CRF-CRFR1 signaling is known to be involved in alcohol withdrawal, negative affect, and stress.
- **The current work will test the hypothesis that withdrawal from adolescent alcohol exposure produces sex differences in BNST cell activation, specifically CRF and CRFR1 containing cells.**

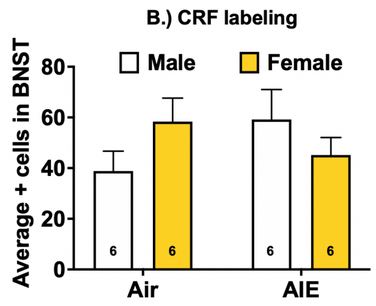
Methods

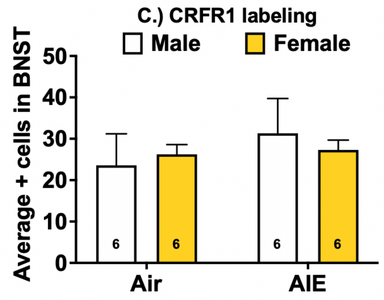
Adolescent intermittent ethanol (AIE) vapor exposure: Adolescent (PND30–41) C57Bl/6J female and male mice were given a daily injection of either pyrazole (air control, 1 mmol/kg) or pyrazole + ethanol (ethanol group, 1 mmol/kg + 0.8 g/kg, respectively) to impair the metabolism of ethanol. Thirty minutes after the injection, mice were placed in their home cages, which were then placed into a chamber filled with volatilized ethanol (20.3 ± 0.2 mg/L) or volatilized water (air group). Airflow through the chambers was maintained at 5.5 L/min, and volatilization was maintained at 1.5 L/min. After 16 hours of exposure, mice were removed from the chambers and returned to standard animal housing. Ethanol chamber exposure occurred from 1600–0800 the following day. Using these parameters, we were able to reliably obtain blood ethanol concentrations in the range of 150–185 mg/dL in adolescent mice. This protocol was run for two, 4 day cycles of 16 hours in vapor chambers and 8 hours out of vapor chambers. Five hours after the final alcohol exposure brains were collected for RNAscope.

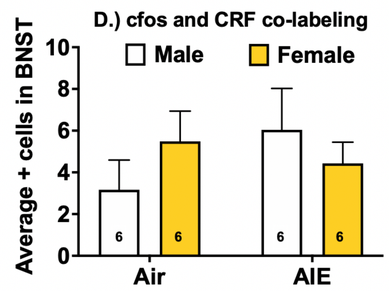
RNAscope: Brains were collected and flash frozen using isobutane. Brains were stored at -80ºC, and twenty-four hours before slicing, brains were moved to -20ºC. Brains were sliced on a Cryostat (CryoStar NX50) at 10µm and adhered to warm Fisher plus slides and immediately refrozen. RNAscope was performed on slides containing the BNST following the steps of the ACD Fluorescent Multiplex Kit. Tissues were fixed using cold 4% paraformaldehyde, and the following three probes were used for RNAscope: C1 for cfos mRNA, C2 for CRF mRNA, and C3 for CRFR1 mRNA. Twenty-four hours after completing RNAscope, slides were imaged using an Epifluorescent Slide Scanning Microscope using corresponding FITC, TX Red, and CY5 filter cubes. Images were captured using Metamorph at 20X magnification and overlayed onto each other with color using ImageJ. ImageJ was also used to count the number of cells containing specific fluorescence in order to quantify the activation of different cell populations in the BNST. Negative control probe quantification was used to detect background levels of non-specific fluorescence.

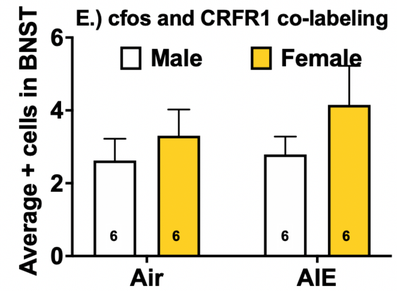
Results

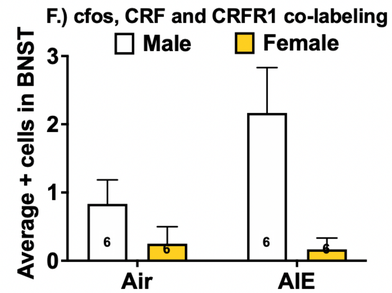




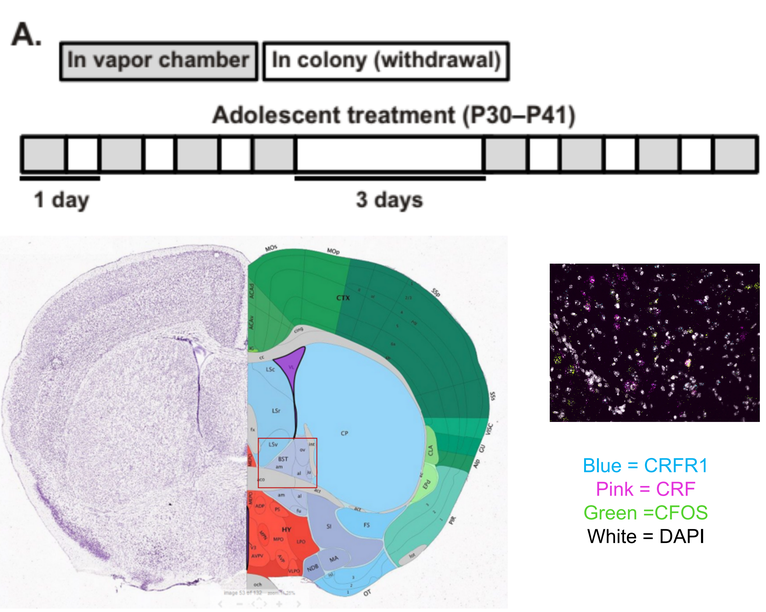








RNAscope data reveals several non-significant, but trending results. Female mice have greater cfos and cfos and crfr1 co-labeling than males, but these levels were not impacted by AIE. AIE did appear to increase CFOS/CRF co-labeling and CFOS/CFR/CRFR1 co-labeling in male mice.



Discussion

This work demonstrated sex differences during withdrawal from AIE.

- In the BNST, female mice have higher overall cellular activation (c-fos) and CRF, as well as, co-localization of c-fos CRF or CRFR1, but these levels are not impacted by AIE treatment and not statically significant.
- In male mice, AIE enhances CFOS/CRF/CRFR1 co-expression demonstrating a greater sensitivity of BNST CFOS/CRF/CRFR1 signaling from alcohol withdrawn male mice, and this expression is almost significant in terms of sex differences (p=0.0580).
- These results suggest sex differences in CRF-CRFR1 signaling and the regulation of this signaling by AIE.

C-fos activation was expected to be higher overall in the AIE treatment group, so future work will:

- Increase the animal number in each to group to see if these trends will become significant.
- Explore other withdrawal timepoints and interactions with stress.