Activation of CRF- and CRFR1-expressing Neurons in the Central Nucleus of the Amygdala Following Stress in Adult Mice with Adolescent Alcohol History

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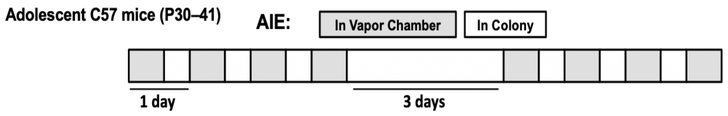
Introduction

- Adolescent alcohol exposure is a strong predictor for the onset of mental health issues and alcohol use disorder during adulthood.
- Women are more likely to drink to alleviate anxiety or other psychological distress.
- The central nucleus of the amygdala (CeA) is a key modulator of anxiety in response to drug-related stimuli.
- Corticotropin-releasing factor (CRF) is the primary pro-stress modulator in the CeA .
- Our goal is to test the hypothesis that female mice with AIE history will have higher activity of CRF- and CRFR1-expressing neurons in the CeA than male mice.

Methods

Adolescent Intermittent Ethanol (AIE) Vapor Exposure

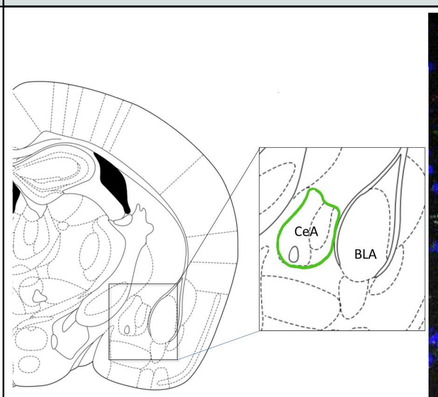
- Adolescent (PND30–41) C57Bl/6J mice were used and given a daily injection of either pyrazole (air group) or pyrazole + ethanol (ethanol group)
- Thirty minutes after the injection, mice were placed into volatilized ethanol (20.3 ± 0.2 mg/L) or volatilized water (air group) chambers.
- This protocol was run for two, four-day cycles of 16 hours in the ethanol chambers and 8 hours out of the chambers.
- Mice were left undisturbed until adulthood (PND70+) and then exposed to 1 hour of restraint stress and brains were collected 1 hour post-stress.

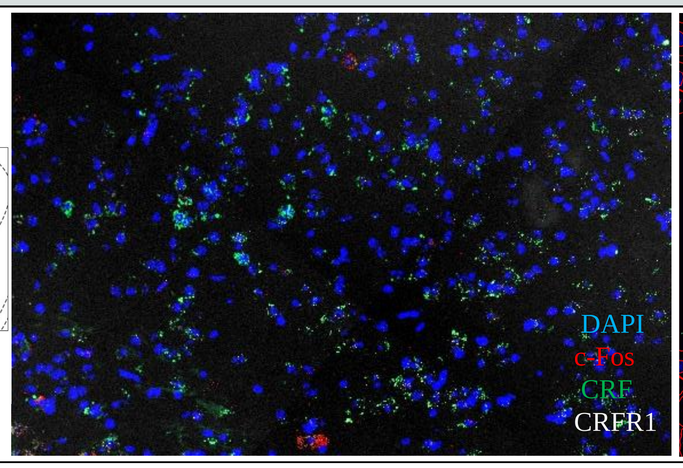


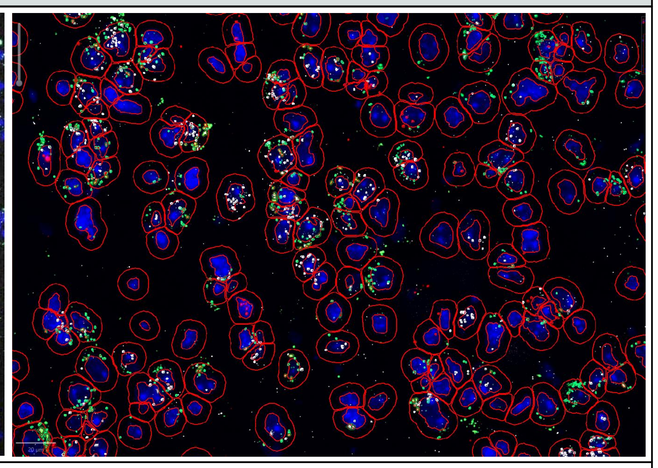
RNA In Situ Hybridization (RNAscope)

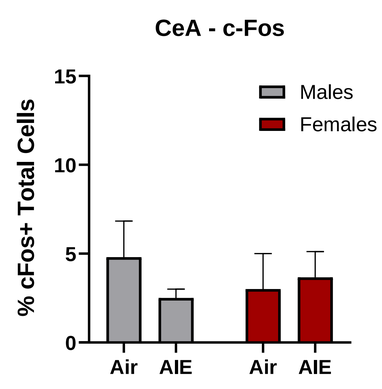
- Brains were collected, flash frozen in isopentane, and stored at -80ºC until slicing.
- Brains were sliced on a cryostat at 10 µm. Tissues were fixed using 4% paraformaldehyde. RNAscope was performed on slides containing the CeA following the steps of the ACD Fluorescent Multiplex Kit.
- The following three probes were used for RNAscope: C1: c-Fos mRNA, C2: CRF mRNA and C3: CRFR1 mRNA. Nuclei were stained using DAPI.
- Images were captured using the ZEISS AxioScan.Z1 slide scanner. QuPath software was used to count the total number of cells as well as cells containing fluorescent puncta.
- Specific mRNA positive cells were established using a threshold of 5 for all three channels. (McCullough et al. 2018)

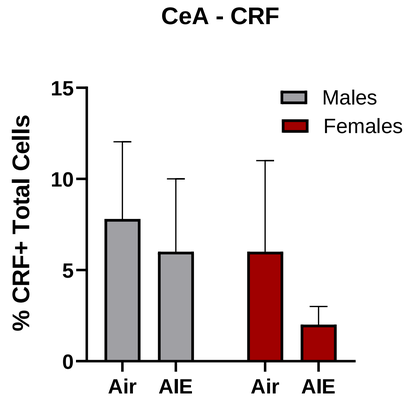
C-Fos, CRF and CRFR1 mRNA expression in the CeA

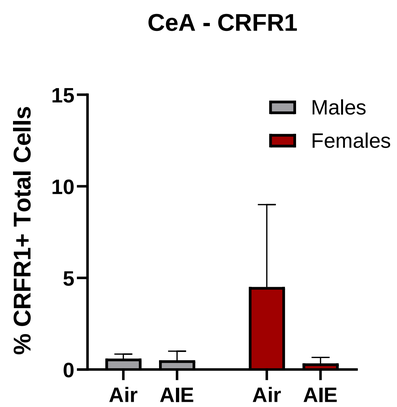


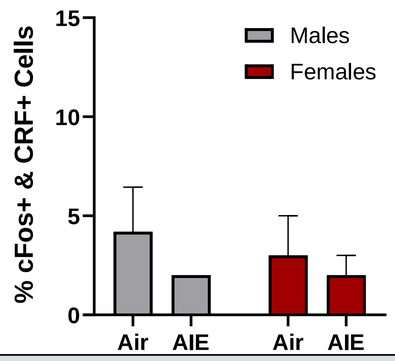


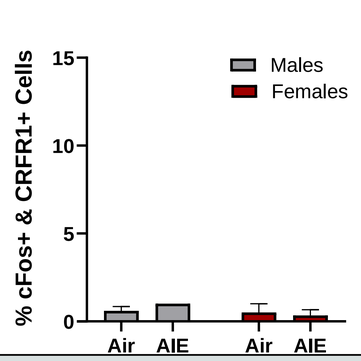












Conclusions & Future Directions

- AIE history did not produce significant sex differences in CRF or CRFR1 mRNA expression in the CeA. AIE history also did not produce significant differences in the activation of CRF- and CRFR1-positive neurons.
- Overall, c-Fos expression was expected to be higher. This is likely because slides were imaged approximately one year after the brain slicing. In the future, tissues should be imaged within 6 months of brain collection.
- Peak expression of c-Fos mRNA is approximately 30 min following the beginning of stress. In future experiments, brain collection may be done within a shorter time following restraint stress.
- This study may be replicated with a larger cohort to determine whether the results are consistent.

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