"Quantification of Protein Kinase C Delta positive cells in the Bed Nucleus of the Stria Terminalis in Adolescent Mice Exposed to Intermittent Ethanol"

J.M. Pham, P.J. Logarbo, L.P. Ha, L. Albrechet-Souza, T.A. Wills

Louisiana State University Health Sciences Center, Department of Cell Biology and Anatomy, New Orleans, LA, 70112

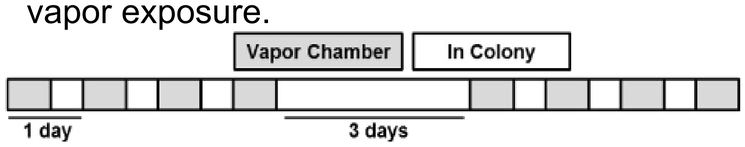
LSU Health NEW ORLEANS School of Medicine

Background

- The adolescence phase is a critical period of neural development and is vulnerable to the effect of alcohol.
- Early onset of alcohol use during adolescence increases the risk of alcohol use disorder (AUD).
- Recent research suggests a bidirectional interaction of alcohol use and pain.
- Previous published data from our lab reports a long-lasting mechanical and thermal hypersensitivity after Adolescent Intermittent Ethanol (AIE) exposure.
- The aim of this study is to investigate the possible invovlement of Protein Kinase C delta (PKCd) expressing cells in the Bed Nucleus of the Stria Terminalis (BNST) in AIE-induced hypersensitivity.

Methods

- Male and female C57BL/6J mice were either underwent AIE or water vapor exposure between post-natal day 29-38.
- AIE involves two 4-day cycles of exposure to ethanol vapor (or water vapor as control) for 16 hours followed by 8 hours out of the chamber for four days separated by three days of no vapor exposure.

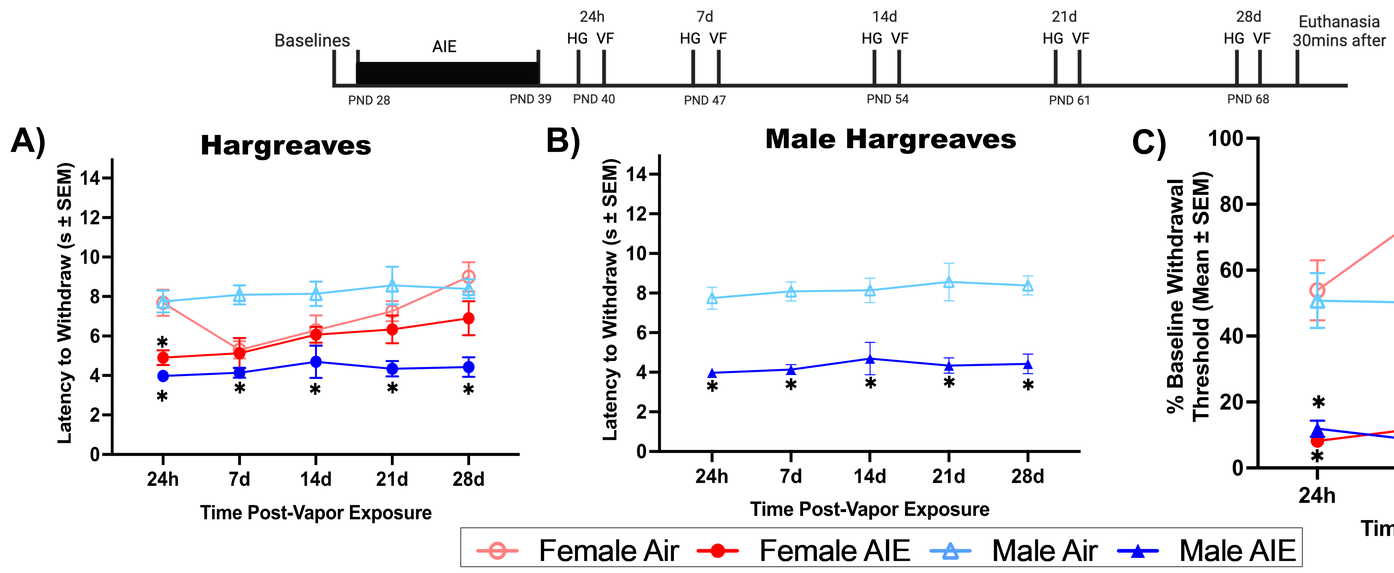


- The blood alcohol concentrations of ethanol vapor exposed animals were maintained around 200 mg/dL range.
- Mechanical (von Frey) and thermal (Hargreaves) hypersensitivity was assessed at four different time points: 7, 14, 21, and 28 days post-vapor exposure (Data not shown).

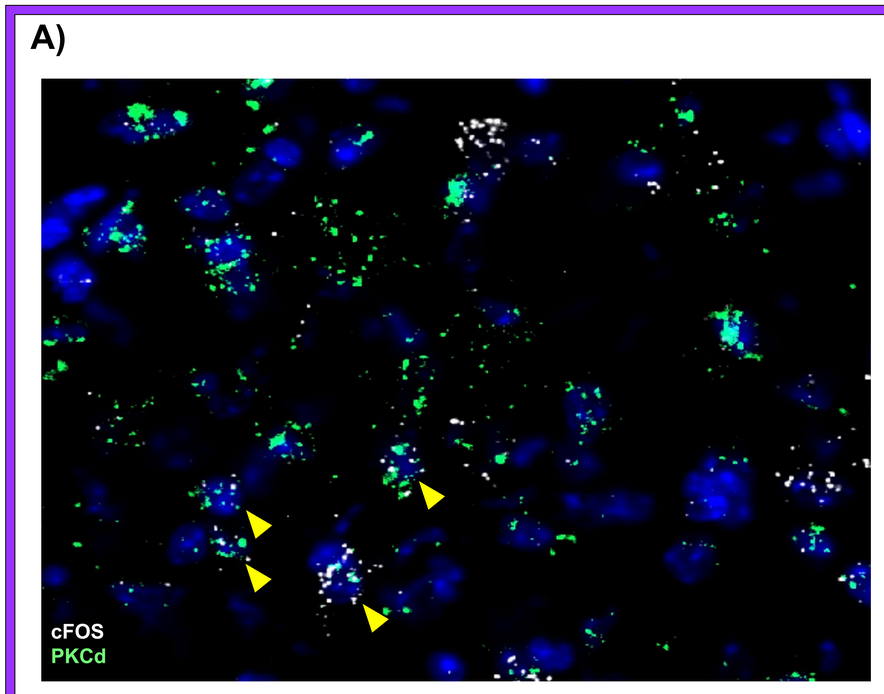
- On day 28, thirty minutes after the final von Frey assessment, mice were sacrificed and whole brains were collected.
- The obtained brains were flash-frozen and sliced at 12um for RNAscope assay.
- RNAscope was performed using probes for *prkcd* and mouse *c-fos* genes as molecular markers.

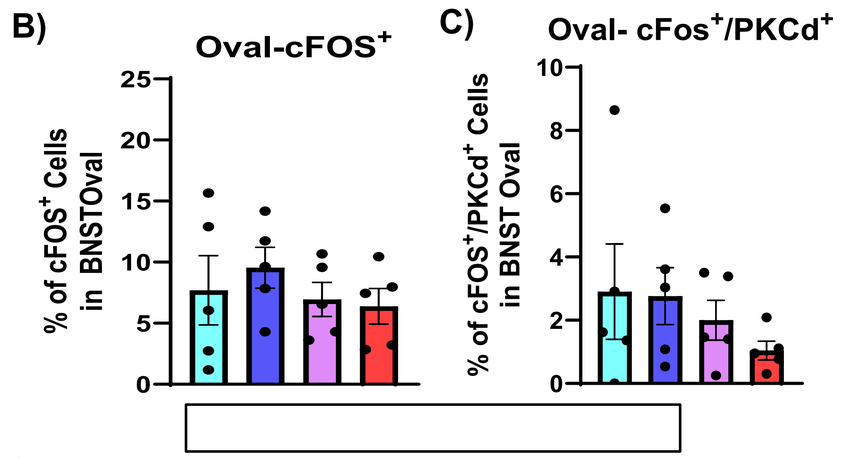
Results

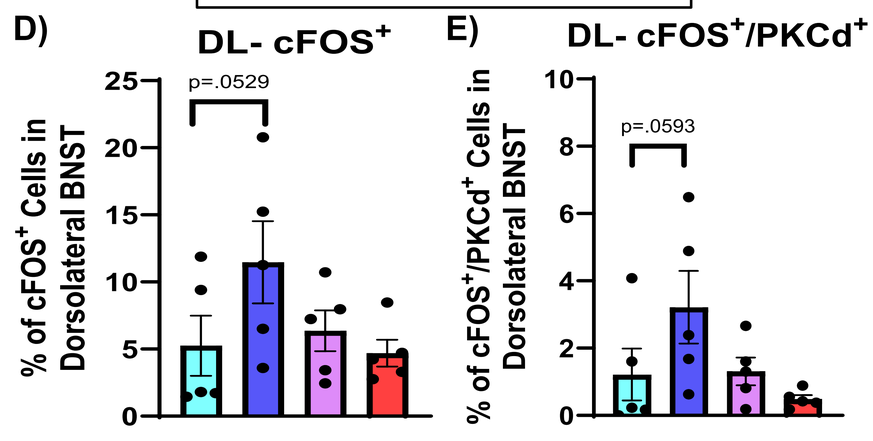
Previous Data



Adolescent Hypersensitivity. A. Adolescent ethanol exposure (AIE) produces a sex-specific difference in thermal sensitivity with male mice showing persistent decreases in paw withdrawal thresholds and no significant difference in female mice. **B.** Separation of males from (A) to illustrate the significance through all timepoints. **C.** AIE produces a persistent mechanical hypersensitivity in both sexes into adulthood.







PKCd RNAScope. A.) Representative RNAscope Image. Fluorescent signals indicate presence of Protein Kinase C delta (PKCd, green) or c-fos (white) messenger RNA within the BNST. Arrows show examples of colocalization. **B. – D.)** Quantification of c-fos+ and c-fos+/PKCd+ cells in the Oval and Dorsolateral (DL) nuclei of the BNST in Air and AIE mice following von Frey testing.

Conclusion

No differences in overall nor PKCd-mediated activity were seen in either brain region between AIE and air groups. Based on post-hoc analysis, a trend towards differences in activity in the dorsolateral BNST was observed in the M-AIE group.

Future Directions

- Quantification of activity of other neuronal populations within the BNST (such as Somatostatin) following AIE to determine altered circuitry that mediates AIE-induced hypersensitivity.
- Further increase in n-value to determine validity of trend seen in DL-BNST.

Acknowledgment

- Wills Lab and the Department of Cell Biology and Anatomy.
- Medical School Summer research.