# Adult BNST activation produces sex-specific negative affect-like behavior and changes in activation of VTA neurons in mice with adolescent alcohol history

C. K. Bailey, L. Albrechet-Souza, N. B. Bertagna, T. A. Wills
Louisiana State University Health Sciences Center, Department of Cell Biology and Anatomy

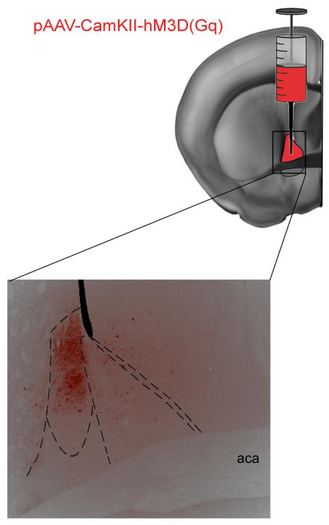
## Introduction

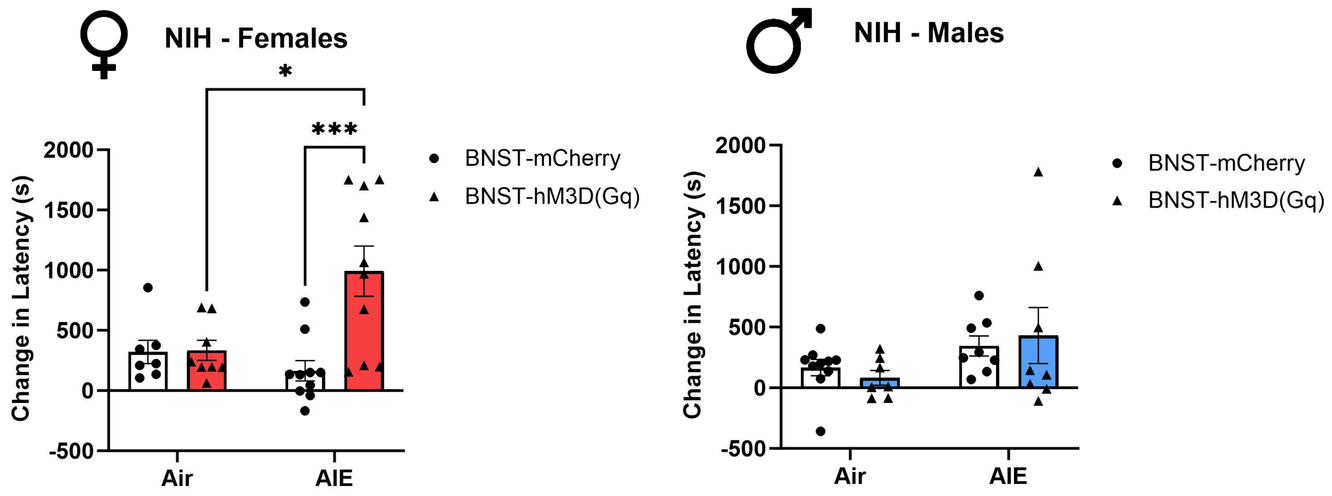
- Alcohol use commonly begins in adolescence which is a critical period for brain development.
- Adolescent alcohol use is a strong predictor for the development of alcohol use disorders in adulthood.
- Unlike male counterparts, females are more likely to drink to relieve negative affect-like behavior.
- The bed nucleus of the stria terminalis (BNST) is a sexually dimorphic brain structure implicated in emotional regulation, including negative affect-like behaviors related to alcohol withdrawal.
- The BNST projects to several other regions that modulate emotional behavior such as the ventral tegmental area (VTA). This area is characterized by a heterogenous populations of cells, including dopamine (DA) (~70%), gamma aminobutyric acid (GABA) (~30%), and glutamatergic (~2-3%) neurons.
- Previous work from our lab shows that adult females with a history of adolescent intermittent ethanol (AIE) vapor exposure exhibit negative affect-like behavior following restraint stress.

## Objectives

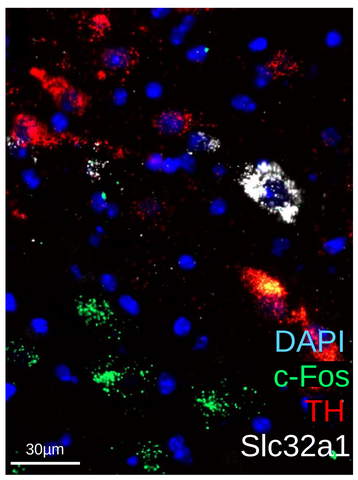
- **The current work aims to test the hypothesis that chemogenetic BNST activation produces a behavioral phenotype that mimics the stress effect in adult mice with AIE history.**
- **Furthermore, we aim to demonstrate that the increase in negative affect-like behavior triggered by BNST activation is accompanied by suppression of the mesolimbic system in mice with AIE history.**

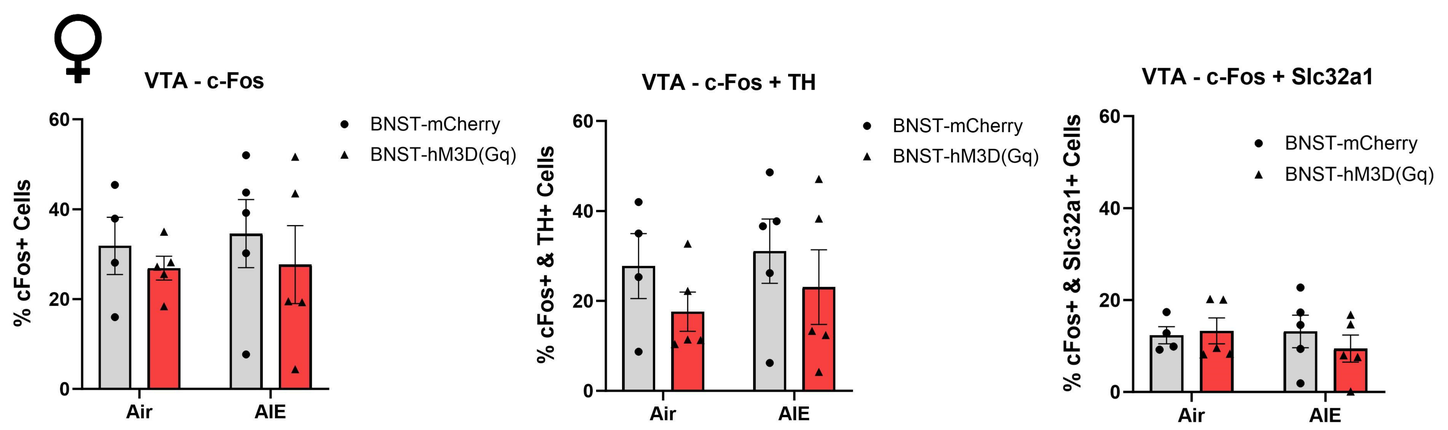
## BNST activation increases latency to consume in females with AIE history




## BNST activation produces a non-significant decrease in activation of VTA-DA neurons




## Methods

**Adolescent Intermittent Ethanol (AIE) Exposure:** C57BL/6J mice were subjected to two, 4-day cycles of 16 h in-chamber sessions of either vaporized water or 95% ethanol solution and 8 h out-of-chamber sessions separated by a 3-day break (PND 27 to 38). Prior to each 16 h session, mice were given an injection of either pyrazole (air control group) or pyrazole + ethanol (ethanol group) to impair the metabolism of ethanol. This procedure produces blood ethanol concentrations in the range of 200-250 mg/dl.


**Surgery and Viral Injections:** Mice underwent stereotaxic surgery 7-10 days following AIE protocol (PND ~45). The coordinates used in targeting the dorsolateral BNST were as follows: 0.14 mm anterior to bregma, ± 0.8 mm lateral to the midline, and 4.14 mm ventral to the skull. The mice received injections of viral vectors AAV5-CAMKIIa-mCherry or AAV5-CAMKIIa-hM3D(Gq) and were allowed to recover until adulthood (PND 70+).

**Novelty Induced Hypophagia (NIH) Task:** On PND 78, mice were given 2 h access to 50-ml bottles of Ensure nutritional shake in their home cage under low-light conditions (approx. 50 lux). The following day, latency to consume the Ensure shake (up to 30 min) was measured in their home cage. On the final day (PND 80), mice received an injection of clozapine N-oxide (CNO; 3mg/kg) 1 h prior to the beginning of testing. Mice were then individually placed into new clean cages with the same dimensions but without shavings, with bottles containing Ensure positioned. Novel cage testing took place under bright lighting conditions (approx. 420 lux), and the testing lasted for 30 min.

**In-Situ Hybridization (RNAScope):** On PND 90+, mice received an i.p. injection of CNO 1 h prior to euthanasia. Brains were flash frozen in -30°C isopentane and stored in -80°C until sectioning. Coronal sections were collected using a cryostat at 12µm. In-situ hybridization was performed using the RNAscope Multiplex Fluorescent Reagent Kit V2 with TSA Vivid Dyes acquired from ACD, Inc. The fluorescent DNA stain DAPI was used to visualize cell nuclei and the probes used were c-Fos, a marker for cell activation, tyrosine hydroxylase (TH), a marker for dopaminergic cells, and Slc32a1, a marker for GABAergic cells. Slides were imaged on ZEISS AxioScan.Z1 slide scanner and analyzed on the QuPath 0.4.3 software. mRNA expression was measured through automatic detection of puncta within automatically generated cell borders. Using a negative control, the average + SD puncta detected per cell were calculated for each probe to establish the threshold for positive cell designation. Cells that met the detection threshold requirement are expressed as a percentage of the total cell population.

## Conclusions

- Chemogenetic activation of the BNST increased latency to consume an appetitive reinforcer in females with a history of AIE.
- Neither AIE nor activation of the BNST produced a significant reduction in activation of VTA-DA or GABA neurons.

## Future Directions

- We will add more animals to the RNAscope analysis to verify if differences in cell activation reach statistical significance.
- Future work will explore regions with greater inhibitory inputs to the VTA. The rostromedial tegemental nucleus (RMTg) is a highly GABAergic region that integrates information from the extended amygdala and may be directly responsible for reducing mesolimbic DA activity.

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