Sex Differences, Neurosteroids and the Development of Rapid Tolerance to Alcohol's Anxiolytic Effects

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Introduction

- Alcohol use disorder (AUD) is a medical condition characterized by an individual's inability to control alcohol intake regardless of its consequences to health, occupation, or social life[1].
- Approximately 15 million of individuals in the United States have AUD[3]. Although 7% of men have AUD compared to 4% of women, women are more at risk to the negative health and safety effects of alcohol.
- Biological factors interacting with sex is thus important in refining our understanding of the complex mechanisms driving alcohol related behaviors and AUD vulnerability.
- An important diagnostic criteria of AUD includes tolerance to alcohol, meaning alcohol has a diminished effect with increasing amounts, or an increased amount is needed to get the same desired effect as previously consumed[4].
- Previous work in our lab has shown that females display rapid functional tolerance to anxiolytic effects of alcohol**, therefore the aim of this project is to further investigate the role of adaptations in neurosteroid synthesis to the development of tolerance and changes to ethanol-induced anxiolysis in males and females.**

Background

- Allopregnanolone is a positive allosteric modulator of GABA and possesses a similar behavioral profile to that of ethanol. Both interact with $GABA_A$ receptors to modulate neural inhibition[5]. This endogenous neuroactive steroid may in fact mediate the anxiolytic effects of ethanol[6].
- The basolateral amygdala is an important neural structure in anxiety related and emotionally driven behaviors and has been shown to have interconnected neural circuits implicated in anxiety-related behaviors in mice with the PFC[7,8].
- Previous work from our lab demonstrated the ability of a synthetic allopregnanolone infusion to modulate network states in the BLA by producing the same anxiolytic effects as ethanol[9].
- Therefore, we hope to further investigate the role of endogenous neurosteroidogenesis mediating behavioral networks and sensitivity to ethanol-induced anxiolysis, specifically in the BLA and its projections to the PFC.

Methods

Subjects

- 13 Mice from a C57BL/6J background strain (N=3-4 mice sex/solution).

Procedure

- Female cage beddings were spiked with male urine one week before behavioral sensitivity testing. Estrous cycle phases were monitored to ensure females were cycling.
- Mice received one injection of 1g/1kg ethanol (10% v/v, I.P.) or saline daily for 2 consecutive days.
- All control mice were given 1g/1kg saline.
- Mice were placed in elevated plus maze (EPM) for 5 minutes; 30 minutes post injection.
- Mice were euthanized and brains were collected 30 minutes after completion of EPM.

Analysis

- Trunk blood samples were collected and will later be used to measure progesterone and allopregnanolone with ELISAs
- IHC completed on one mouse from each sex/treatment to compare activity of neurosteroid sensitive GABAergic interneurons in the BLA.
- BORIS software was used to analyze time spent in open arms of EPM and indicate the anxiolytic behavior of each mouse.
- ImageJ software was used to gather tracking data of each mouse in EPM. Distance traveled in cm was calculated.

Results

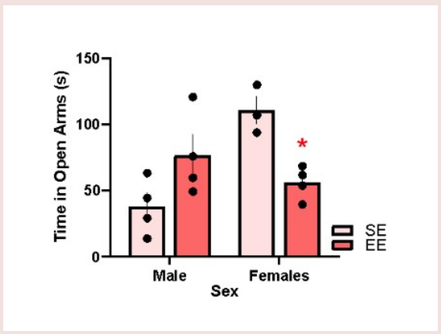

Figure 1: Rapid functional tolerance to the anxiolytic effects of ethanol. Females show rapid functional tolerance to the anxiolytic effects of ethanol. Mice received ethanol (1.5g/kg; EE) or saline (SE) one day prior to administration of ethanol (1.5gkg; all groups) and testing in EPM. Significant interaction of sex and drug history [$F(1,11)= 16.13$, $p<0.01$], only females with prior history of ethanol showed rapid functional tolerance, or a significant reduction in the anxiolytic effects of ethanol ($p<0.05$).

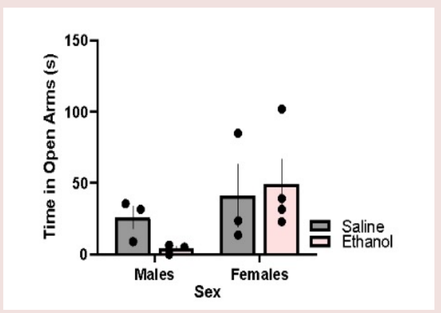

Figure 2: Time in open arms of elevated plus maze across sex and treatment on Day 1. We calculated the amount of time each mouse spent in open arm of EPM. No significant interaction of sex and treatment with time in open arms.

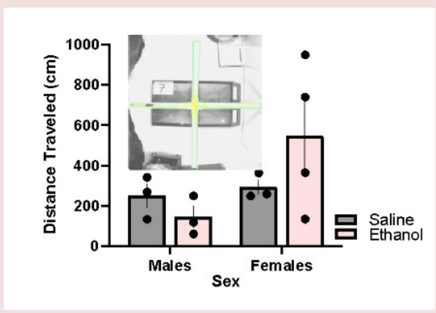

Figure 3: Distance traveled in elevated plus maze across sex and treatment on Day 1. We calculated distance traveled in cm of each mouse in EPM. No significant interaction of sex and treatment with distance traveled.

Discussion

- Females and males received saline or ethanol on Day 1 and all mice received ethanol on Day 2. Females showed rapid tolerance, or a significant decrease to alcohol's anxiolytic effect.
- Next, mice either received saline or ethanol on both days. Analysis of the first day of exposure to low dose ethanol does not support an anxiolytic effect of the drug on day 1 for either males or females.
- Numerous stressors— first time mice were handled and injected—may have affected this outcomes
- The sex difference noted in the expression of functional tolerance may in fact involve a difference in sensitivity to habituation/handling stress.

Future Directions

- Future work will continue behavioral sensitivity testing, perform IHC and ELISA assays on additional cohorts of mice. We will compare neurosteroid sensitive GABAergic interneurons in the BLA with IHC and compare circulating allopregnanolone with ELISAs to reflect differences in the sensitivity of alcohol across sex and treatment.
- In the future, we will adjust our procedure to effectively answer our question if the development of rapid tolerance and ethanol's anxiolytic effect changes across sex. We will change our procedure to have all mice receive ethanol on Day 2 to more accurately demonstrate changes in the anxiolytic effect of alcohol after a 2nd exposure in the experimental group.
- Ultimately, we would like to answer if females have a greater capacity for neurosteroid influenced neuroplasticity.

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