Role for Hippocampal δ-GABA$_A$ Receptors in the Effects of Adolescent Alcohol Exposure on Endocrine or Behavioral Stress Reactivity in Adulthood

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Introduction

- Binge drinking is excessive alcohol use in a short amount of time
- In the U.S., it is the most common and deadly form of excessive alcohol use[1]
- Binge drinking in adolescence is linked to abnormal stress reactivity and increased vulnerability to developing anxiety disorders in adulthood
- Additionally, the ventral hippocampus is known to be important in regulating individual differences in stress and anxiety
- GABA$_A$ receptors in hippocampal interneurons contain δ subunits that are sensitive to ethanol and mediate tonic inhibitory currents[2]
- Our previous research found that in adult mice, ethanol binge drinking is associated with increased GABAergic inhibition mediated by δ-subunit-containing GABA$_A$ receptors (δ-GABA$_A$Rs) on parvalbumin (PV) positive interneurons in the dorsal hippocampus
- The same research supports this inhibition as the mechanism for the anxiety-like behavior that follows binge drinking[3]
- In adolescent mice, previous research found that binge drinking does not effect PV or δ-GABA$_A$R expression in the ventral hippocampus, but did not examine expression in the dorsal hippocampus
- Thus, the present study asks: **As in adults, is the mechanism driving abnormal stress reactivity in adulthood following binge drinking mediated by changes in δ-GABA$_A$R expression in the dorsal hippocampus in adolescents?**

Methods

Subjects

- Female (N=11) and male (N=7) adolescent (PD 30±3) C57BL/6J (B6) mice

Binge Drinking (Drinking in the Dark – Multiple Scheduled Access)

- Known to induce binge drinking
- For 14 days, mice had access to either water (N=8) or 20% v/v ethanol (N=10) for three 1 hr periods each day, with a 2 hr separation period in which all mice had ad libitum access to water

Anxiety-Like Behavioral Tests

- Followed a 2 day withdrawal period; the mice are now adults
- *Social Interaction (A):*
  - Mice were placed in the center of a three-chamber testing area, in which one chamber has a second mouse, and behavior was recorded for 5 minutes
- *Marble Burying (B):*
  - 10 marbles were placed in the mouse's home cage and the number of marbles covered in bedding material after 20 minutes was recorded

Corticosterone Measurements

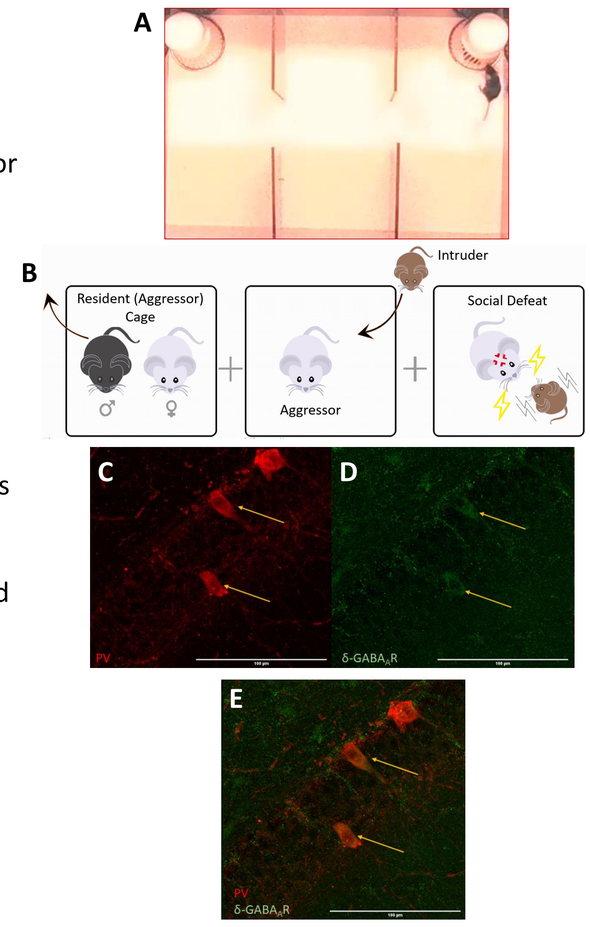
- Blood collected before and after stressors was centrifuged to isolate plasma, and corticosterone was measured using ELISA analysis

Stressors

- *Forced Swim Test:* Mice placed in bucket of water for 6 minutes
- *Social Defeat Stress:* Mice placed in a trained aggressor's cage for 5 minutes, during which the intruder was bitten

Parvalbumin and δ-GABA$_A$R Immunohistochemistry

- Mice were anesthetized, sacrificed, and brains were harvested
- Dorsal hippocampus tissue was incubated with δ and PV antibodies, as well as secondary antibodies, and imaged with a confocal for cell analysis (C-E)



Results

Binge Drinking

- 3 high ethanol (all female) and 3 low ethanol drinkers (1 female, 2 male) were identified as falling outside the upper and lower limits of the 95% confidence interval of the mean

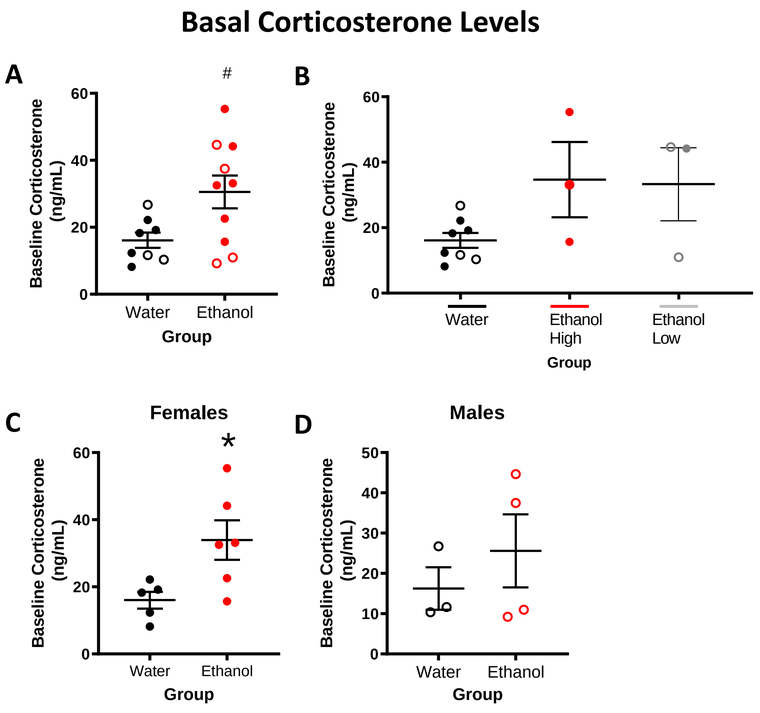


Figure 1. Basal CORT levels were marginally affected (#p = 0.054, U = 18) by adolescent drinking. Mice exposed to ethanol as adolescents had higher basal CORT in adulthood (M= 30.57ng/mL) than water exposed controls (M= 16.11 ng/mL) (A). Separating by high and low drinking did not better predict the difference in CORT (B). Separating by sex demonstrated that females (C; *p = 0.03, U = 3), but not males (D) show increased basal CORT following binge drinking.

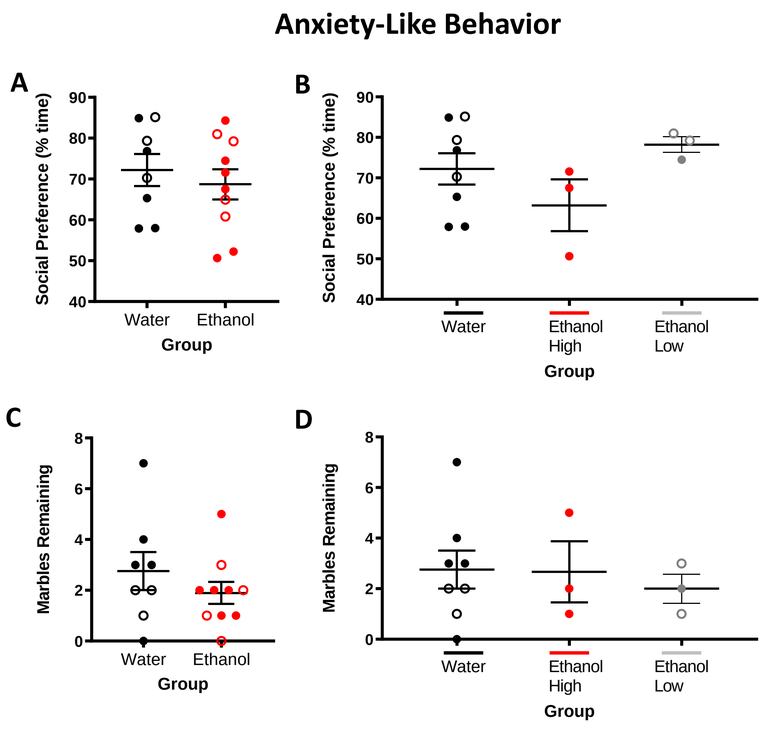


Figure 2. Anxiety-like behavior was not associated with ethanol exposure. For social preference in the three-chamber social interaction test (A-B) and the marble burying test (C-D), there was no difference between groups whether ethanol drinkers were grouped together or split into high and low drinkers. In the social interaction test, the high drinkers (M = 62.23%) spent less time on average on the side with another mouse than the low drinkers (M = 78.22%), but this difference was not significant. Separating by sex did not mirror the effect sex had on post drinking basal CORT for either measure of anxiety-like behavior.

Parvalbumin Interneuron and δ-GABA$_A$R protein Expression

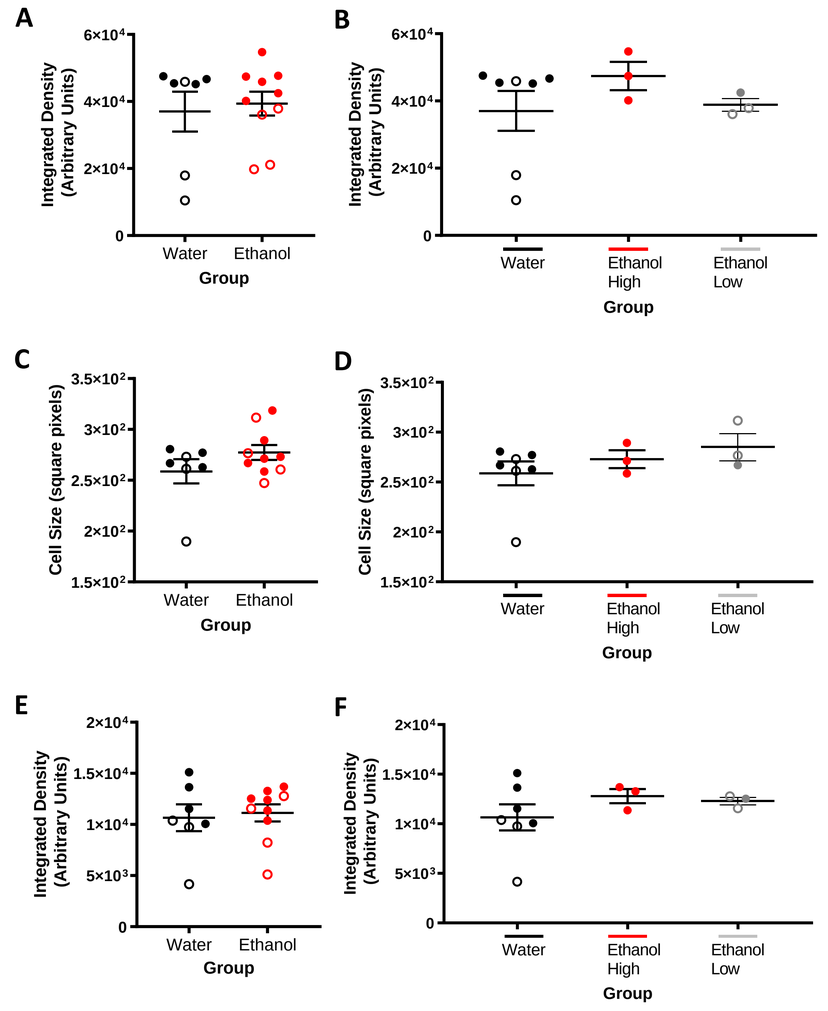


Figure 3. In the dorsal hippocampus, PV interneuron structure (including δ-GABA$_A$R expression) was not associated with ethanol exposure. For PV interneuron expression (A-B), PV cell size (C-D), and δ protein expression (E-F), there was no difference between groups whether ethanol drinkers were grouped together or split into high and low drinkers.

Discussion

- The study did not intend to assess sex differences, but sex differences were found in ethanol drinking and basal CORT levels
- Given that prior research shows ethanol intake activates the HPA axis, which stimulates release of glucocorticoids[4], the higher basal CORT levels seen only in females may be a reflection of all the high drinkers being female
- Considering that mice exposed to ethanol as adolescents had higher basal CORT in adulthood than water-exposed controls, it is surprising that adolescent binge drinking did not impact basal anxiety-like behaviors, even when separated by sex
- The lack of an effect on δ protein expression differs from prior research on adults, but corresponds to the insignificant differences in anxiety-like behavior and does not weaken the possibility that δ-GABA$_A$R expression on PV interneurons in the dorsal hippocampus mediate the heightened stress reactivity and anxiety-like behaviors associated with adolescent binge drinking
- However, the difference in mean social preference in high and low ethanol drinkers suggests that a larger sample size could show a significant relationship between anxiety-like behavior and level of binge drinking
- Though current analyses point to a difference between adolescents and adults in mechanisms for abnormal stress reactivity and anxiety following binge drinking, future research will build upon the sample size, investigate if sex differences in drinking extend to endocrine and neural mechanisms, image other brain regions, and examine anxiety-like behavior and CORT levels after acute stress

References

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