Modulation of Glutamatergic Signaling following Adolescent Alcohol Exposure in the Hippocampus of Male and Female Mice

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Background

- Exposure to alcohol during adolescence is proven to increase the risk of developing an alcohol use disorder.
- This increased risk is due to alcohol effects on the developing brain.
- Alcohol can produce long lasting changes within the brain by impacting the neural mechanisms of plasticity. A key mechanism involved in the induction of plasticity is through the regulation of glutamate and its receptors (specifically AMPA and NMDA receptors).

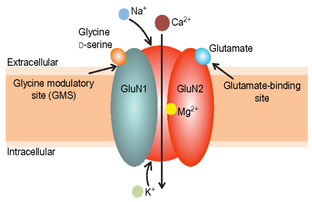


Figure 1: NMDA Receptor

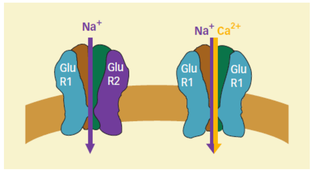


Figure 2: AMPA receptor

- NMDA and AMPA receptors are composed of multiple subunits and these subunit combinations affect receptor functions.
- Alcohol's short-term effects on NMDARs is to inhibit receptor transmission, while long-term alcohol exposure leads to a compensatory enhancement of NMDAR in adults.

The long-term effects of glutamatergic signaling from adolescent alcohol exposure and how these effects differ between the sexes are currently are not well understood.

- The Wills lab has investigated the effects of adolescent alcohol on glutamate signaling in the bed nucleus of the stria terminalis (BNST) in male and female mice.
- BNST is an important brain region, because it is a crucial region for negative affect and stress regulation, which are known causes for alcohol relapse and continued alcohol use.
- This work showed that adolescent alcohol exposure leads to an enhancement of NMDAR- plasticity in male mice but not females.

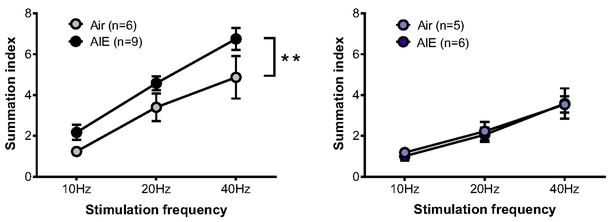


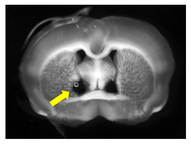
Figure 3: NMDAR Isolated Temporal summation in the BNST following AIE in Male and Female mice

Research Questions

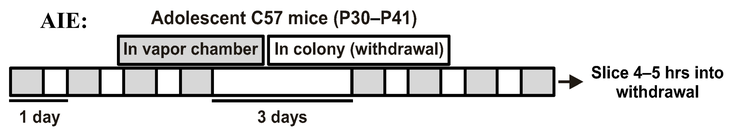
1. **Is sex-specific upregulation of NMDAR plasticity in the BNST associated with changes in NMDAR and AMPAR subunit expression?**
2. **Are these sex-specific effects of AIE isolated to the BNST or a common mechanism in other brain regions?**

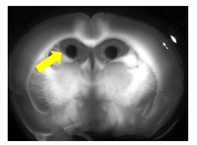
A brain region that projects to the BNST and has well characterized glutamatergic signaling is the <u>hippocampus</u>.

Results

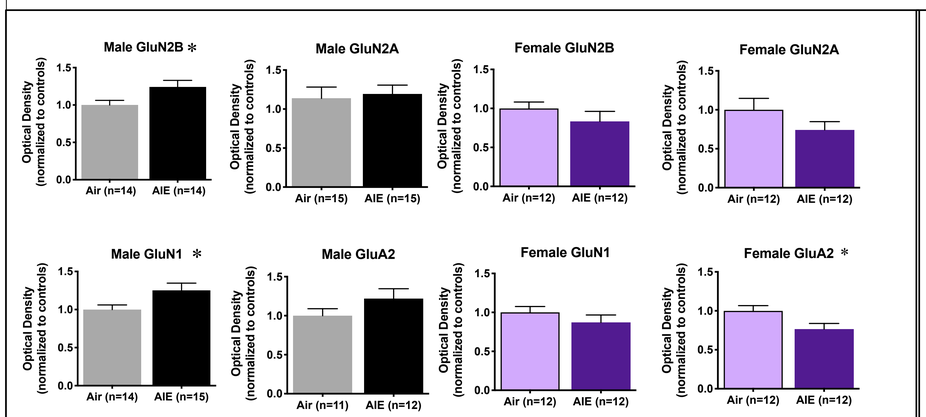


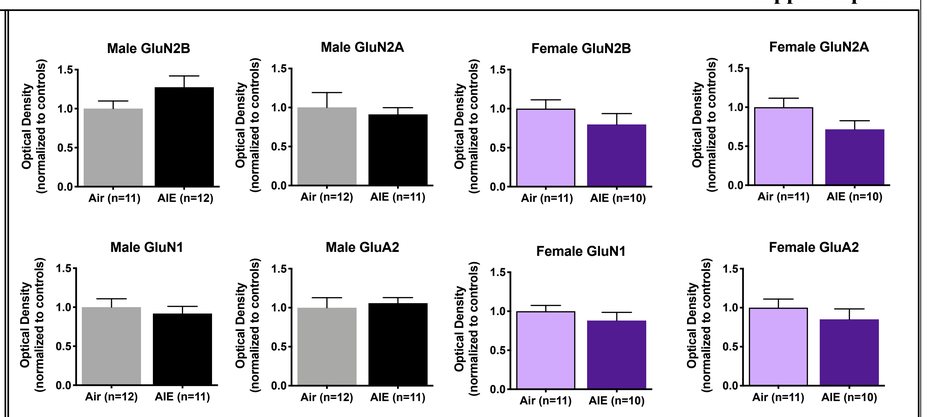
BNST





Hippocampus





Methods

<u>Alcohol vapor exposure (AIE)</u>. Adolescent (P30–34) C57Bl/6J mice were given a daily injection of either pyrazole (air control, 1 mmol/kg) or pyrazole + ethanol (ethanol group, 1 mmol/kg + 0.8 g/kg, respectively) to impair the metabolism of ethanol. Thirty minutes after the injection, mice were placed in their home cages, which were then placed into a chamber filled with volatilized ethanol (20.3 ± 0.2 mg/L) or volatilized water (air group). Airflow through the chambers was maintained at 5.5 L/min, and volatilization was maintained at 1.5 L/min. After 16 hr of exposure, mice were removed from the chambers and returned to standard animal housing. Ethanol chamber exposure occurred from 1600–0800 the following day. Using these parameters, we were able to reliably obtain blood ethanol concentrations in the range of 150–185 mg/dL in adolescent mice. This protocol was run for two, 4-day cycles of 16 hr in vapor chambers and 8 hr out of vapor chambers.

<u>Western blot analysis</u>. Tissue punches obtained from the dlBNST and dorsal hippocampus 5 hours following the final vapor chamber exposure were homogenized in homogenization buffer. Proteins were resolved by SDS-PAGE (10%) and transferred to nitrocellulose membranes, which were blocked in 5% milk in TBST and incubated with the appropriate primary and secondary antibodies. For detection using the Odyssey system (LiCor Biosciences, Lincoln, NE), infrared-conjugated secondary antibodies (LiCor) were used. Densitometry was performed using Image J (National Institutes of Health, Bethesda, MD) on images linearly adjusted for brightness and contrast. Inputs were normalized to GAPDH. The following primary antibodies were used: GluN1 (BD 1:2,000), GluN2B (BD 1:2000), GluN2A (Millipore 1:2,000), and GAPDH (ABCAM 1:10,000).

Conclusion

In the BNST,

- AIE increased expression of GluN2B and GluN1 subunit of the NMDA receptor but did not change expression levels of the GluA2 subunit of the AMPA receptor in male mice.
- AIE did not change expression of any NMDAR receptor subunits but did decrease expression levels of the GluA2 subunit of the AMPA receptor in female mice.

In the hippocampus,

- NMDA and AMPA receptor subunits were unchanged by AIE in both male and female mice

Collectively, these findings show that the BNST is especially suspectable to the AIE-induced changes in glutamatergic signaling and in the sex effects of these changes.

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