Effects of Adolescent Alcohol Exposure on Glutamatergic Transmissions in Male and Female Mice

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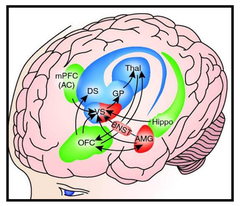
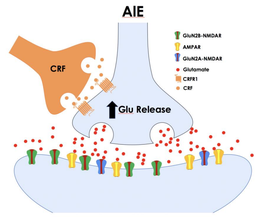
Background

Adolescence is commonly a time when alcohol use is initiated, and the manner of consumption is typically in a heavy, **binge-like manner**. Adolescent alcohol exposure has been linked to increased likelihood to develop an **alcohol use disorder (AUD)** in adulthood. Alcohol use affects the **bed nucleus of the stria terminalis (BNST)**, an important integration center for information received from cortical and hippocampal regions. Adolescent alcohol use causes long-term **changes in the BNST circuitry** that persist into adulthood. BNST is involved in negative affect and **stress induced relapse** in AUD.

During stress, both hypothalamic and extrahypothalamic (corticotropin releasing hormone) CRH are known to be activated. In adult male mice, **CRH** acts on presynaptic CRH1 receptors and **enhances glutamate release**. Following adolescent alcohol exposure, the Wills lab finds enhanced glutamate release and **sex specific changes** in excitatory plasticity (GluN2B-NMDAR mediated in males and mGluR5 mediated in females). This receptor difference may contribute to differences in how adolescent alcohol exposure affects male and female mice during stress in adulthood.





Methods

Adolescent Intermittent Ethanol Vapor Exposure (AIE)

Adolescent mice were separated into 2 groups, control air group and alcohol treated group. In the alcohol treated group, adolescent (P30-41) C57B1/6J mice were given a daily injection of pyrazole + ethanol (ethanol group, 1mmol/kg + 0.8g/kg, respectively) to impair the metabolism of ethanol. In the control air group, adolescent mice were given a daily injection of pyrazole only (air control, 1mmol/kg). 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 volatized water (air group). Airflow through the chambers was maintained at 5.5L/min, and volatilization was maintained at 1.5 L/min. The mice remained in the chambers for 16 hours, from 1600-0800, and were then removed from exposure and returned to standard animal housing for the remainder 8 hours. Using those chamber vapor parameters, the alcohol exposed mice were able to reliably reach blood ethanol concentrations in the range of 150-185 mg/dL. The mice went through the chamber exposure for 4 days in a row, and then rested for 3 days undisturbed. This process was then repeated for a second time.

Mice were then allowed to age into adulthood and then placed under one-hour restraint stress in tight conical tubes. Brain slices were taken one-hour post-stress to conduct electrophysiology.

Electrophysiology Recordings

After brain slices were taken, the slices were then transferred to a holding chamber containing heated (~29˚C), oxygenated (95%O_2 - 5% CO_2) artificial cerebrospinal fluid (ACSF). Recording electrodes were filled with Cs+gluconate internal solution. Additionally, 25 µM picrotoxin was added and recording was done at a holding potential of -70mV in normal ACSF at a fluid exchange rate of 2 ml/min to isolate spontaneous excitatory post-synaptic currents (sEPSCs). Experiments in which changes in series resistance were greater than 20% were not included in the data analysis. Five, 2-min gap free recordings were analyzed by measuring the peak amplitude and frequency of sEPSCs. Frequency indicates number of events (e.g glutamate vesicle release) occurring at the presynaptic terminal. Amplitude measures changes in post-synaptic response.

Results

sEPSCs in Adolescent Mice: AIE vs Control

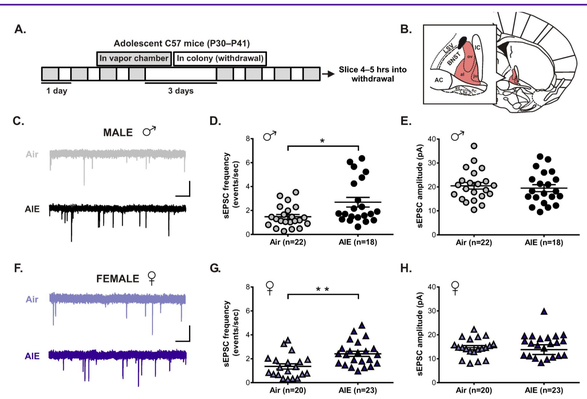


Figure 1. sEPSCs during acute withdrawal in the BNST in AIE and air-control male and female mice. A) Schedule of alcohol and air vapor chamber exposure for 16 hours for 4 days, undisturbed for 3 days, then brain slices collected 4-5 hours after their last vapor treatment during withdrawal. **B)** Coronal brain section of area of interest with BNST. **C)** Representatives of electrophysiology recordings for the male adolescent mice and **F)** female adolescent mice. **D) and G)** Graph shows an increase in frequency of sEPSCs in male and female adolescent AIE mice compared to air mice. **E) and H)** Graph shows no significant difference in amplitude of sEPSCs air and AIE mice for both male and female mice.*p<0.05,**p<0.01.

sEPSCs in Adult Mice with Restraint Stress

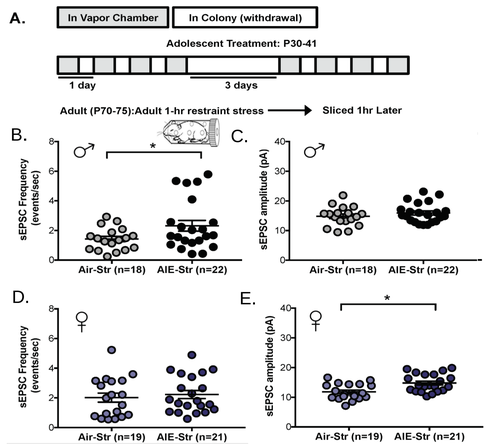


Figure 3. sEPSCs in the BNST in adult AIE and air control male and female mice after 1-hr restraint stress. A) Schedule of alcohol and air vapor chamber exposure. Mice were then allowed to mature into adulthood and placed under 1-h restraint stress with brain slicing 1 h later. **B)** Graph shows increase in sEPSC frequency in adult male AIE mice compared to air mice, but **C)** no difference in sEPSC amplitude between air and AIE mice. **D)** Graph shows no difference in sEPSC frequency in adult female AIE mice compared to air mice, but **E)** increased sEPSC amplitude in adult female AIE mice compared to air mice.*p<0.05.

sEPSCs in Adolescent Mice with CRHR1 Antagonist

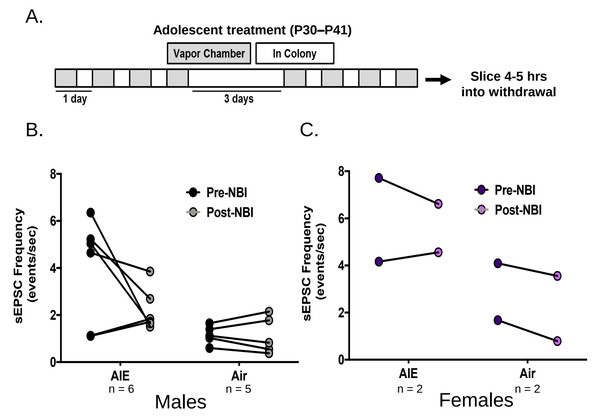


Figure 2. CRHR1 antagonist (NBI-27914) 1uM application on sEPSCs during acute withdrawal in the BNST of AIE and air-control male and female mice. A) Schedule of alcohol and air vapor chamber exposure. **B)** Graph shows frequency of sEPSCs in male AIE mice can be dampened close to baseline when NBI is used. **C)** Graph shows no effect of NBI on sEPSCs in female mice.

sEPSCs in Adult Mice with Restraint Stress and CRHR1 Antagonist

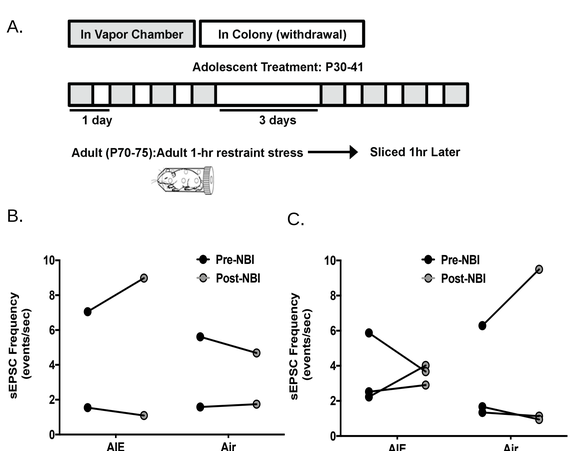


Figure 4. CRHR1 antagonist (NBI-27914) application on sEPSCs in the BNST of male and female mice with AIE history and adult stress. A) Schedule of alcohol and air vapor chamber exposure. Mice were then allowed to mature into adulthood and placed under 1 h restraint stress with brain slicing 1 h later. **B)** Graph shows frequency of sEPSCs in adult male mice after CRHR1 antagonist was applied. **C)** Graph shows frequency of sEPSCs in adult female mice after CRHR1 antagonist was applied.

Objective

1. To evaluate if CRH regulates glutamatergic transmissions in the BNST in adult mice that are stressed and were exposed to AIE during adolescence.
2. To evaluate if the effects of CRH are the same in males and females.

Conclusions

During Acute Withdrawal

- CRH-CRHR1 signaling blunts alcohol induced increase in glutamate release in male mice.
- CRH-CRHR1 signaling did not alter enhanced glutamate release in female mice.

After Adult Stress

- In adult AIE mice, CRH-CRHR1 signaling did not alter glutamate release in male or female mice.
- However, because the sample numbers are very low in these studies, additional research and experimentation is needed.

These preliminary results suggest that CRH-CRHR1 signaling is an important regulator of glutamatergic signaling during acute withdrawal in male mice but not in female mice or in adults after stress.