Binge-drinking Induced Negative Affect and Molecular Irregularities in C57BL/6J Mice

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Introduction

- Binge-drinking alcohol is the most common form of alcoholism in the United States. Among adolescents, over 90% of alcohol consumption is in the form of bingeing (3). This is of particular concern, as adolescence is a critical developmental period for brain structures that mediate emotionality and motivated behavior (10).
- Alcohol Use Disorders are significantly comorbid with Anxiety Disorders, and early onset of these disorders have been correlated with adolescent alcohol binge-drinking (2). Females are especially sensitive to these negative effects of alcohol, as compared to their male counterparts. They also have a higher prevalence of alcohol-related diseases, including liver diseases and several types of cancer (1).
- Alcohol binge-drinking behaviors and severity of withdrawal are known to vary in both primates and rodents depending on their age and sex (4,5,6). The region of the brain that mediates behavioral responses to alcohol is called the nucleus accumbens (NA). The NA also plays a significant role in regulating stress and reward-motivated behavior (9). Glutamate is an excitatory neurotransmitter, and glutamatergic dysfunctions within the NA are linked to alcohol withdrawal-induced anxious behaviors (6). The aim of this project is to better understand how changes within the NA interact with binge-drinking, withdrawal behaviors, and the subject factor interactions of sex x age.

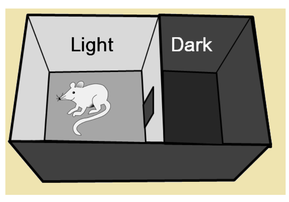
Methods

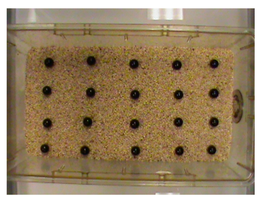
Subjects
The subjects consisted of 183 binge-drinking mice, and 160 water-drinking control mice. Both groups consisted of male, female, adolescent (28 days postnatal), and adults (56 days postnatal)

Binge-Drinking
Mice were subject to a two-hour drinking in the dark procedure for 14 days. Alcohol drinking mice were presented with sipper tubes of ethanol at concentrations of 5%, 10%, 20%, and 40% v/v. The bottles were weighed before and after the 2-hour period to determine the amount of alcohol consumed.

Blood Sampling
Blood samples were collected on Day 14 to assay for corticosterone (stress hormone) levels to use as a baseline for later negative affect assessment.

Behavioral Testing
Mice were counterbalanced between both drinking groups, and were subject to 3 behavioral tests upon withdrawal (either day 1 or day 70).





Light-Dark Box (~5 mins)
Mice are placed in a box divided into two compartments: A light side and a dark side. Non-anxious mice spend time exploring the light-side of the box while anxious mice retreat to the dark side.

Marble Burying (~15 mins)
Anxious mice spend a long time burying marbles in the sawdust, while non-anxious mice spend less time on the task and bury less marbles.

Forced Swim Test (~6 mins)
Mice are placed in a container of water. Immobility indicates low levels of anxiety while hyper-mobility is associated with anxiety.

Immunoblotting (Western Blot)
Brain tissue from the Nucleus Accumbens was collected immediately after completion of the forced swim test. The tissue was homogenized and then smeared for Homer 1a and Homer 2a, two proteins important for glutamatergic signaling (8).

Results

As expected from human models of binge-drinking and previous experiments, alcohol intake differed as a function of sex and age. Females drank more than males, and adolescents drank more than adults.

In the light-dark box test, a long latency to enter the light side of the box is an anxiety-like behavior. Conversely, a short latency to bury in the Marble Burying test is an anxiety-like behavior

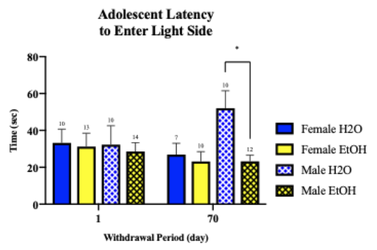


Figure 1: Adolescent male water-drinking controls on withdrawal day 70 had a longer latency to enter the light-side than their binge-drinking counterparts [t (38) = 2.60, p = .013]

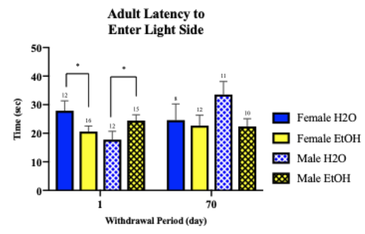


Figure 2: Female adult water-drinking controls in early withdrawal have a longer latency to enter light side than binge-drinking counterparts. [t(26) = 1.97, p = .06] Adult male water-drinking controls in early WD had shorter latency to enter LS than binge-drinking counterparts [t(25) = 1.91, p = .07]

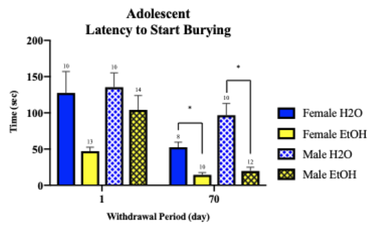


Figure 3: Irrespective of sex, adolescent water-drinking controls in late withdrawal (70-days) had a longer latency to start burying than their binge-drinking counterparts.

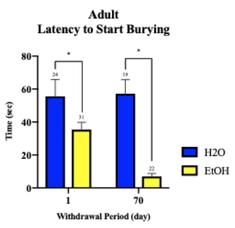


Figure 4: Adult binge-drinking mice in early withdrawal had a significantly shorter latency to bury than water-drinking counterparts [t (53) = 1.96, p = .06]. This effect was more significant in protracted withdrawal [t (39) = 6.14, p <.001]

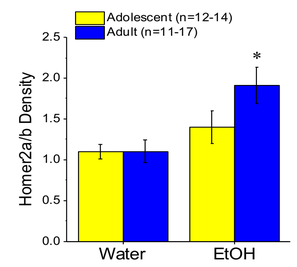


Figure 5: Density of Homer 2a/b in the NaSh on withdrawal day 1 was significantly higher in binge-drinking adults compared to water-drinking controls

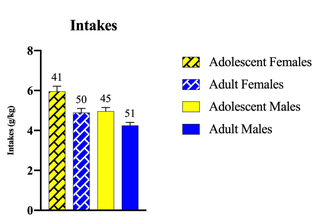


Figure 6: Adolescents consumed significantly more alcohol than adults[t (185) = 4.12, p <.0001]. Across both age groups, females consumed significantly more alcohol than males [t (185) = -3.73, p <.0001].

Discussion

- Alcohol withdrawal-induced negative affect in adults persist past 1-day withdrawal and is more robust in late withdrawal.
- Irrespective of sex, adolescent binge-drinkers show an incubation of negative affect in late withdrawal as supported by Figure 1.
- In previous studies including only males, adolescents did not exhibit negative affect in early withdrawal. In this study, however, negative affect in early withdrawal was observed among male adolescents. The novel inclusion of female mice in this study may have been a factor in the observed anxiety-like behaviors

Conclusions and Future Work

- The sex difference in alcohol intake is not a significant factor in regulating the manifestation of alcohol withdrawal-induced negative affect
- Irrespective of sex, age of drinking onset is not a consistent predicter of when negative affect is observable in withdrawal
- Our model of binge-drinking is a valid model because it adheres to the NIAAA standards of binge-drinking
- Future work will investigate the role of female pheromones in early alcohol-withdrawal anxiety among adolescent males

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