# EVALUATING SEX DIFFERENCES IN VULNERABILITY TO BINGE DRINKING

Jonah Yas, Quantitative Analysis Center, Wesleyan University
Faculty Sponsor: Dr. Laverne Melón, Graduate Mentor: Bilge Büyükdemirtaş

## Introduction

While excessive alcohol consumption is associated with increased mortality and disease burden, economic costs, and Alcohol Use Disorder (AUD), knowledge of binge drinking history may give insight into behavioral responses to chronic stress. Following a binge drinking period of 14 or 7 days, mice were placed in an elevated plus maze (EPM) or underwent a social interaction test or forced test (FST) after a period of withdrawal.

Their responses were analyzed by investigating different factors, such as history of *binge alcohol drinking*, preference for alcohol over water, stage in the estrus cycle (for females only), and sex. After determining a relationship between binge history and abnormal behavior during withdrawal, mice were examined for social behavior following acamprosate, a pharmacotherapy treatment for AUD, or SGE-516, a synthetic neuroactive steroid.

## Research Question

**Is there a sex difference in the impact of drinking history or stress history on behavior during withdrawal from alcohol?**

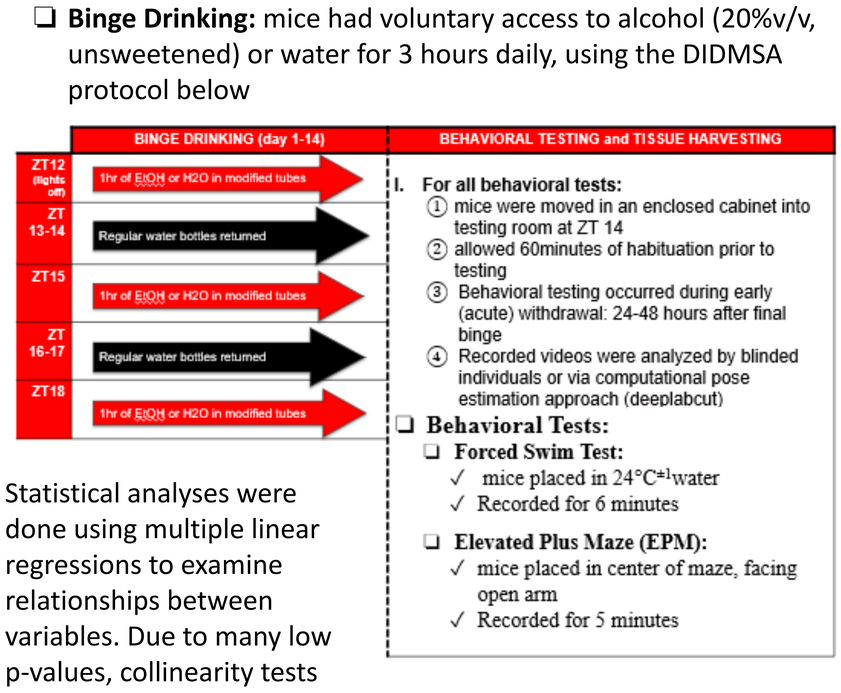
## Methods

***Subjects***

- Female C57BL/6J adults (PD $63^{\pm 3}$) were purchased from JAX and singly on a reverse 12/12 light/dark schedule for at least 1week
- Estrous smears were taken for 10 days prior to initiation of experiment to ensure normal cycling
- Animals had *ad libitum* access to food
- Animals had *ad libitum* access to water except during the 3 hours of DIDMSA binge drinking during which alcohol (20% v/v, unsweetened) or water was provided in modified 10mL graduated drinking tubes fitted with ball bearings.
- n= 8-16 mice/solution for behavioral tests

***Procedure***

❑ **Binge Drinking:** mice had voluntary access to alcohol (20%v/v, unsweetened) or water for 3 hours daily, using the DIDMSA protocol below




Statistical analyses were done using multiple linear regressions to examine relationships between variables. Due to many low p-values, collinearity tests were run to ensure the validity of the model.

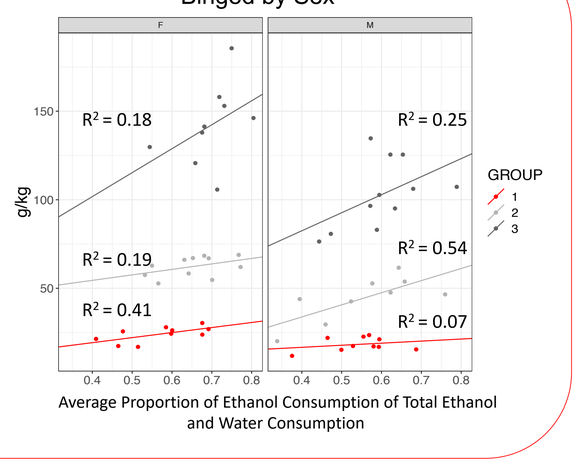
## Results

**Does biological sex moderate impact that history of binge drinking has on alcohol preference?**

**Figure 1:**

- Mice in group 1 binged for three days, group 2 for seven days, and group 3 for 14 days.
- Overall, female mice exhibited higher rates of ethanol consumption and preference for ethanol over water for each group.
- Running a multiple linear regression revealed that female mice drank 15.8 g/kg more than the males with a p value of .0001
- Female mice preferred ethanol 7.3% percent more than males with a p value of .005 . Mice in group 3 drank 9% more than mice in group 1 with a p value of 0.004

1. Ethanol Preference vs. Total Ethanol Binged by Sex

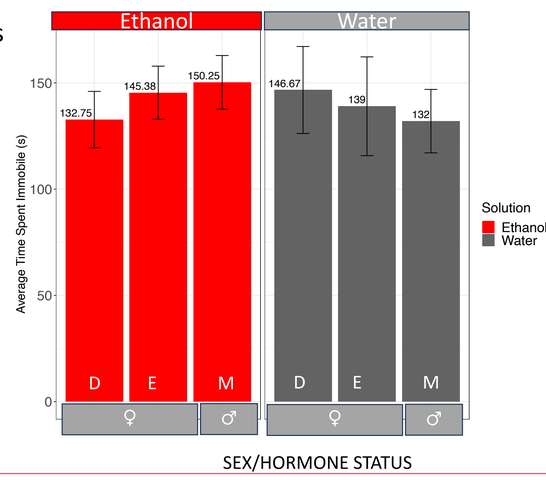



**Does biological sex moderate impact that history of binge drinking has on behavior during withdrawal?**

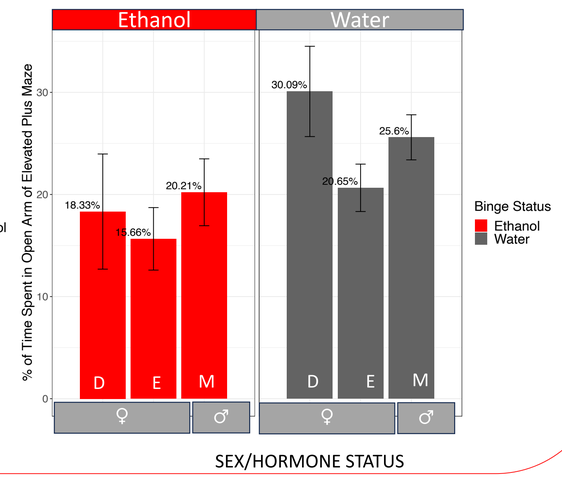
**Figures 2 & 3:**

- Solution and Binge Status are equivalent
- For the FST, only diestrus females showed an increase in average time spent immobile after binging water, not ethanol, while for the EPM, diestrus females showed the largest increase in time spent in the open arm.
- For the rest of the hormone statuses, percent spent in the open arm increased marginally and average time immobile decreased for those who binged on water.

2. Average Time Spent Immobile by Hormone Status in a Forced Swim Test

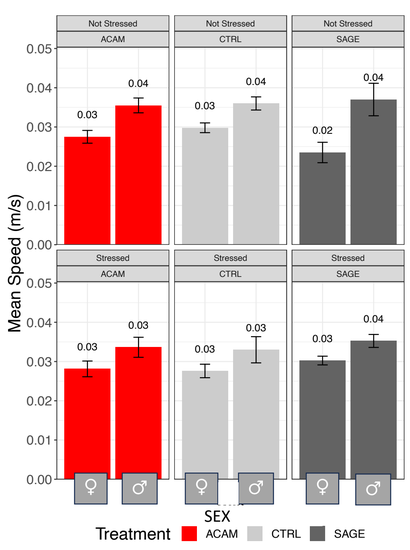



3. Percent of Time Spent in Open Arm of EPM by

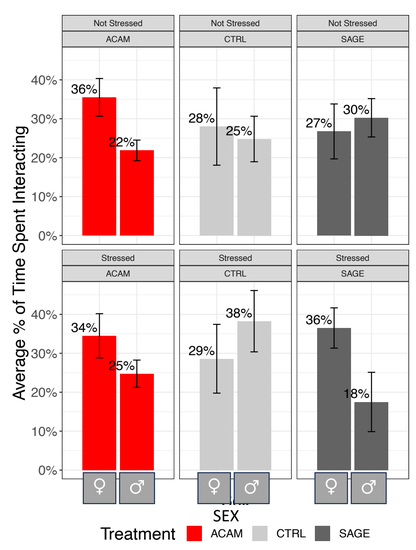



**Can treatment moderate the effects that binge drinking and biological sex have on behaviors during withdrawal?**

4. Average Speed by Sex, Stress History and Treatment




5. Percent of Time Spent Interacting by Sex, Stress History, and Treatment




Time spent interacting refers to the duration the mouse spent near the jail containing the other mouse in the test.

6. Example Trajectory Plot from Social Interaction Test

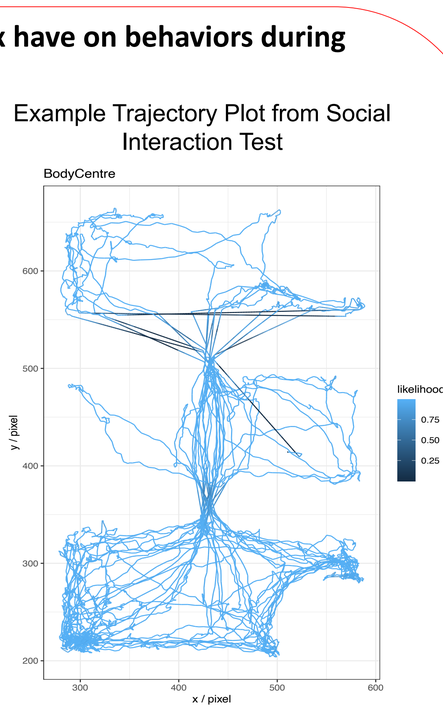



**Figure 6:** This is a trajectory plot created using markerless-pose estimation software DeepLabCut to provide a visualization for the social interaction test.

**Figures 4 & 5:** *All mice binged ethanol prior to testing*

- There was little statistical significance between stress history and treatment with the average speed or time spent being social.
- However, the effect of sex on these variables was marginally significant, as % time interacting of females was 7% higher than male mice with a p value of 0.058.

## Discussion

- Biological sex moderates effect of binge drinking history on later preference for alcohol. In particular, females with any drinking history show enhanced preference for alcohol when compared to their water drinking controls.
- Both biological sex and hormone status moderates the impact that binge drinking history has on anxiety-like behavior during withdrawal. Diestrus females show almost twice as much anxiety-like behavior during withdrawal when compared to their water drinking control group.
- Lastly, biological sex also impacts efficacy of treatment following a history of chronic stress. Using computational pose estimation protocol we get a richer data set showing changes in interaction between our experimental mice and a sex-matched juvenile. Only males show an impact of social stress on later social interaction.

## References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