

Sex and Age Influence Binge Alcohol Drinking and Anxiety-like Behavior

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INTRODUCTION

- Only 10% of people who drink alcohol will go on to develop an Alcohol Use Disorder (AUD)
- Sex and age of first drink are shown to moderate the relationship between binge drinking and the development of an AUD in clinical populations.
- Binge alcohol may cause maladaptive plasticity in GABAergic signaling in systems that control stress and affect leading to behavioral changes that increase one's vulnerability to developing an AUD.
- Changes in these systems may vary between sex and across age and may underlie the unique progression from risky drinking to AUD.
- We used a mouse-model of binge drinking to test our proposal

METHODS

Subjects:

- Female and male C57BL/6J adults (PD >70) and adolescents (PD28) were housed at Wesleyan University on a reverse 12/12 light/dark schedule (n=5-8 mice/solution/age/sex)
- Adults were singly housed, adolescents were socially housed
- Animals had *ad libitum* access to food
- Animals had *ad libitum* access to water except during the 3 hours of binge drinking during which alcohol (20% v/v, unsweetened) replaced the usual water source

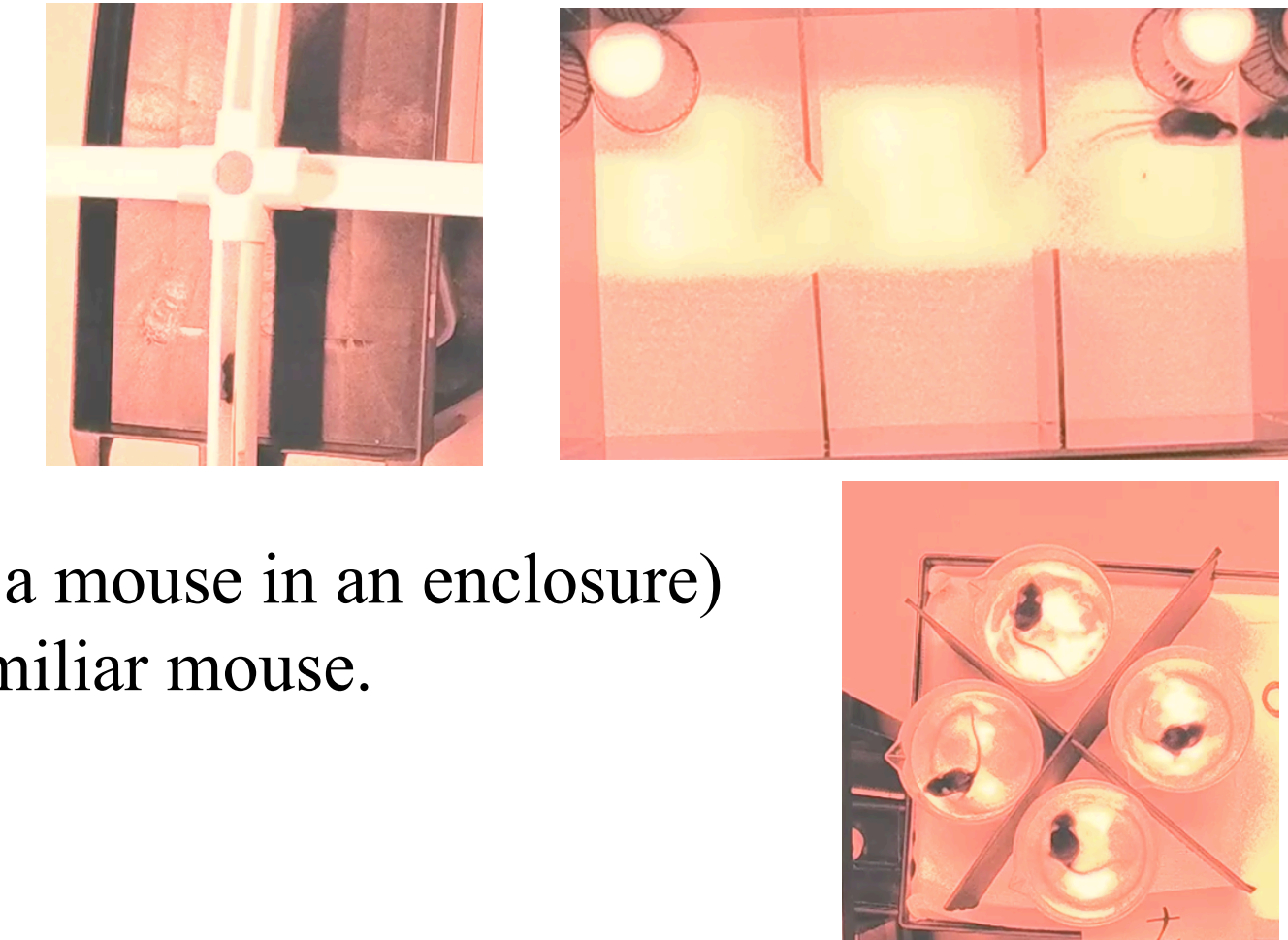
Treatment:

Binge Drinking: mice had voluntary access to alcohol (20%v/v, unsweetened) for 1 hour, 3 times daily for 14 days.

Withdrawal: Protracted withdrawal tests began 12 days after last ethanol access period.

Behavioral Tests:

- Elevated Plus Maze (EPM):**
 - ✓ Mice placed in center of maze, facing open arm
 - ✓ Recorded for 5 minutes
- Social Interaction Test:**
 - ✓ Mice placed in center of testing arena (one section has an empty jar and one section has a mouse in an enclosure)
 - ✓ Recorded for 5 minutes w/o other mouse (baseline activity) and 5 minutes with an unfamiliar mouse.
- Forced Swim Test:**
 - ✓ Mice placed in 24°C±1 water
 - ✓ Recorded for 6 minutes



RESULTS

I. Age alters binge drinking behavior for females

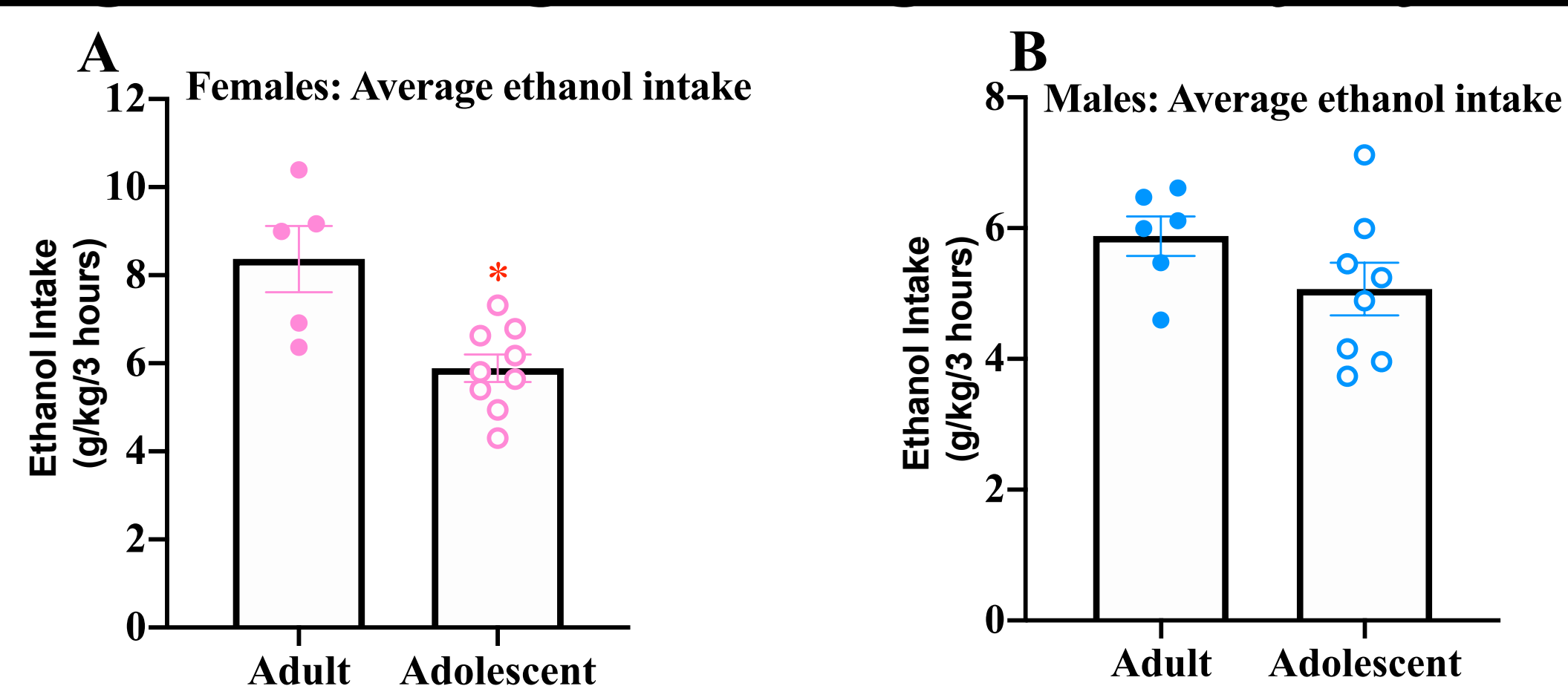


Figure 1: Average ethanol intake across 2 weeks of access. Mice had limited access to alcohol each day and a score representing their average intake across the 14 day was calculated. **A.** Females show a significant effect of age on average intake, with adolescents consuming less than adults. **B.** Males do not show a significant effect of age on average intake.

II. Age alters the expression of sex differences in binge drinking

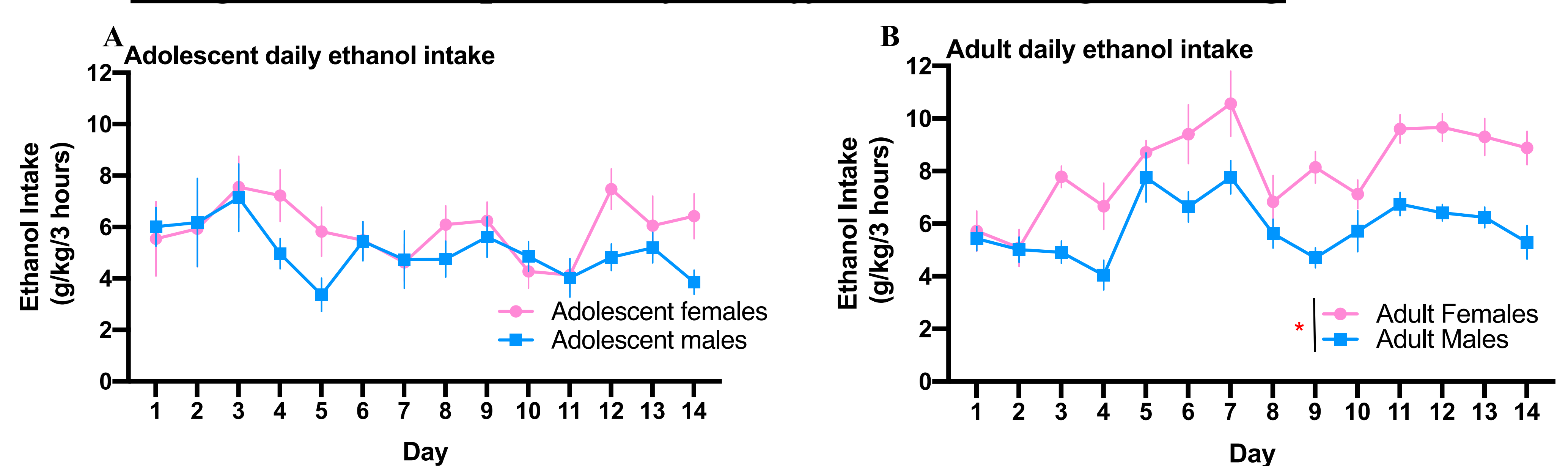


Figure 2: Daily ethanol intake across 14-day access period. **A.** Adolescents show no effect of sex on binge drinking behavior. **B.** We found a significant main effect of sex for adults, with females drinking more alcohol than males ($p < 0.05$).

III. Binge drinking did not enhance social anxiety

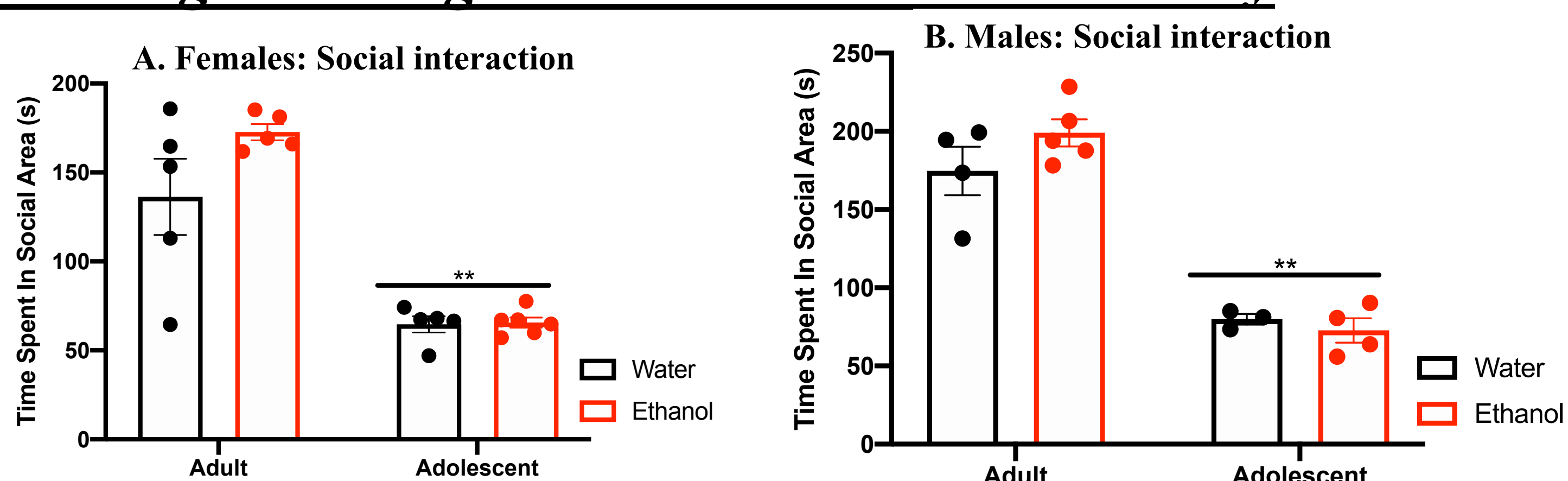


Figure 3: Total time spent on the social side of a 3-sided social interaction apparatus. We calculated the amount of time each mouse spent in the social area during the social interaction test. **A.** There was no significant effect of alcohol exposure on social interaction during protracted withdrawal for females, though there was an overall effect of age, as adolescent females spent less time in the social area of the apparatus. **B.** Similarly, for males, we found no difference between the water and ethanol groups for either age, but did find that adolescents in general spent less time in the social area compared to adults ($p < 0.01$).

IV. Age alters anxiety-like behavior for females

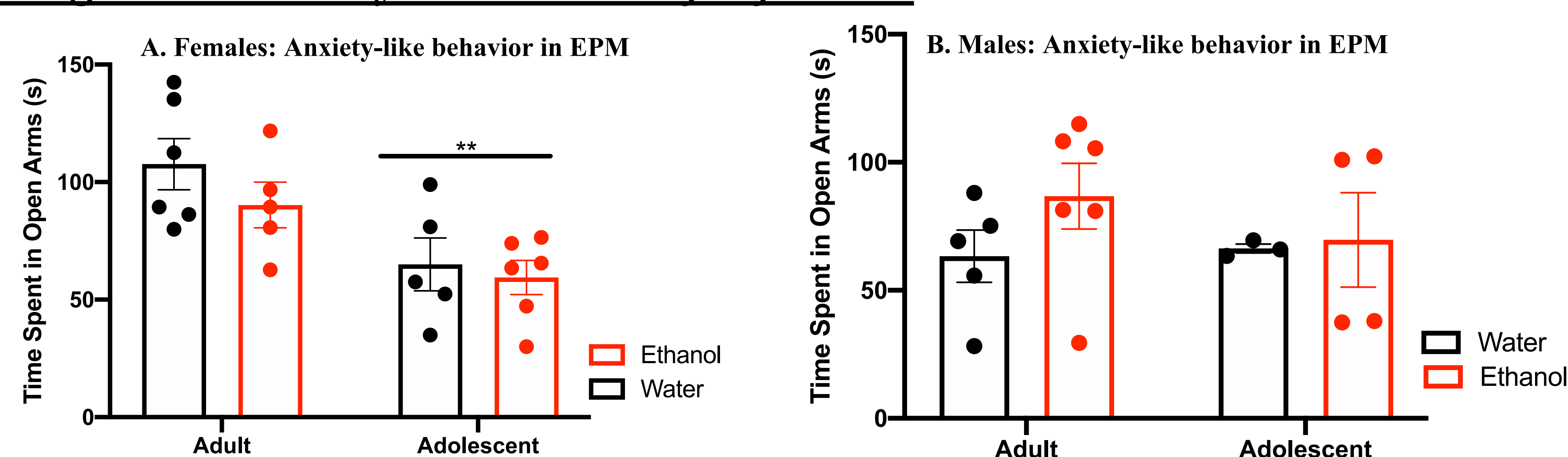


Figure 4: Total time spent in anxiogenic open arms of the elevated plus maze. We calculated the time that each mouse spent in the open arms during the elevated-plus maze. **A.** Females showed no effect of alcohol exposure on anxiety-like behavior during protracted withdrawal, though there was an overall effect of age, as adolescent females spent less time in the open arms of the maze. **B.** Males do not show this compared to adult females.

V. Binge drinking did not enhance depressive-like behavior

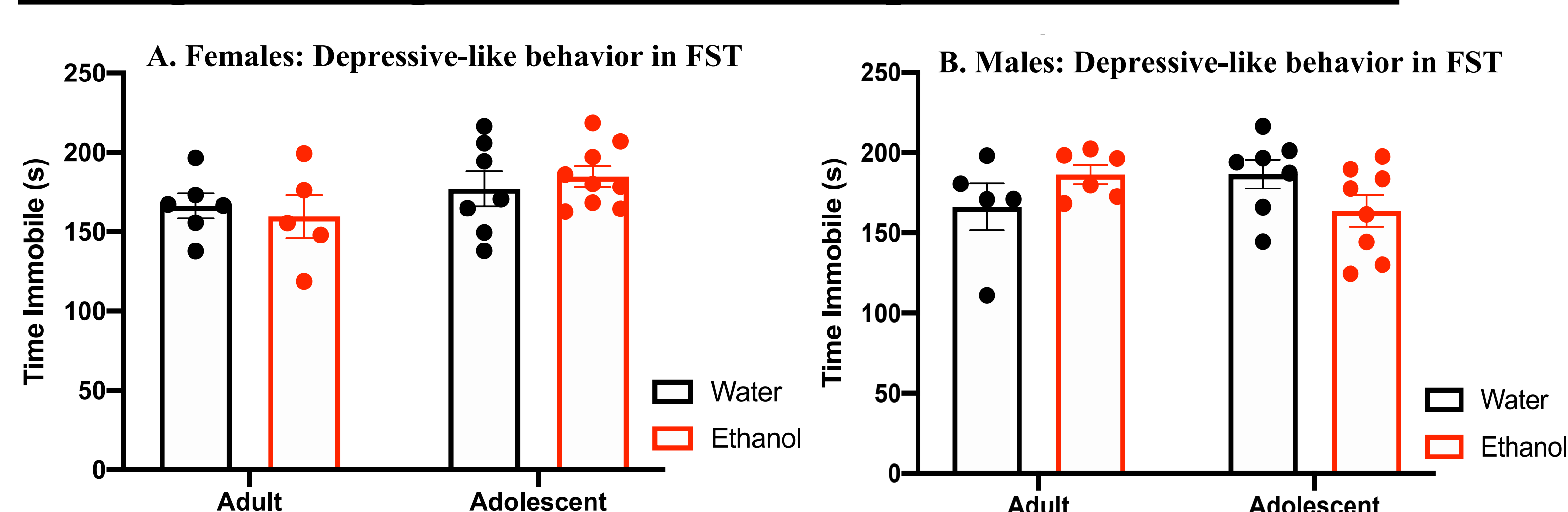


Figure 5: Total time immobile in the forced swim task. We calculated the time each mouse spent immobile during the forced swim test. **A.** Females showed no effect of age or treatment on this behavior. **B.** Males displayed similar behavior across age and treatment groups.

VI. GABAergic plasticity and binge drinking

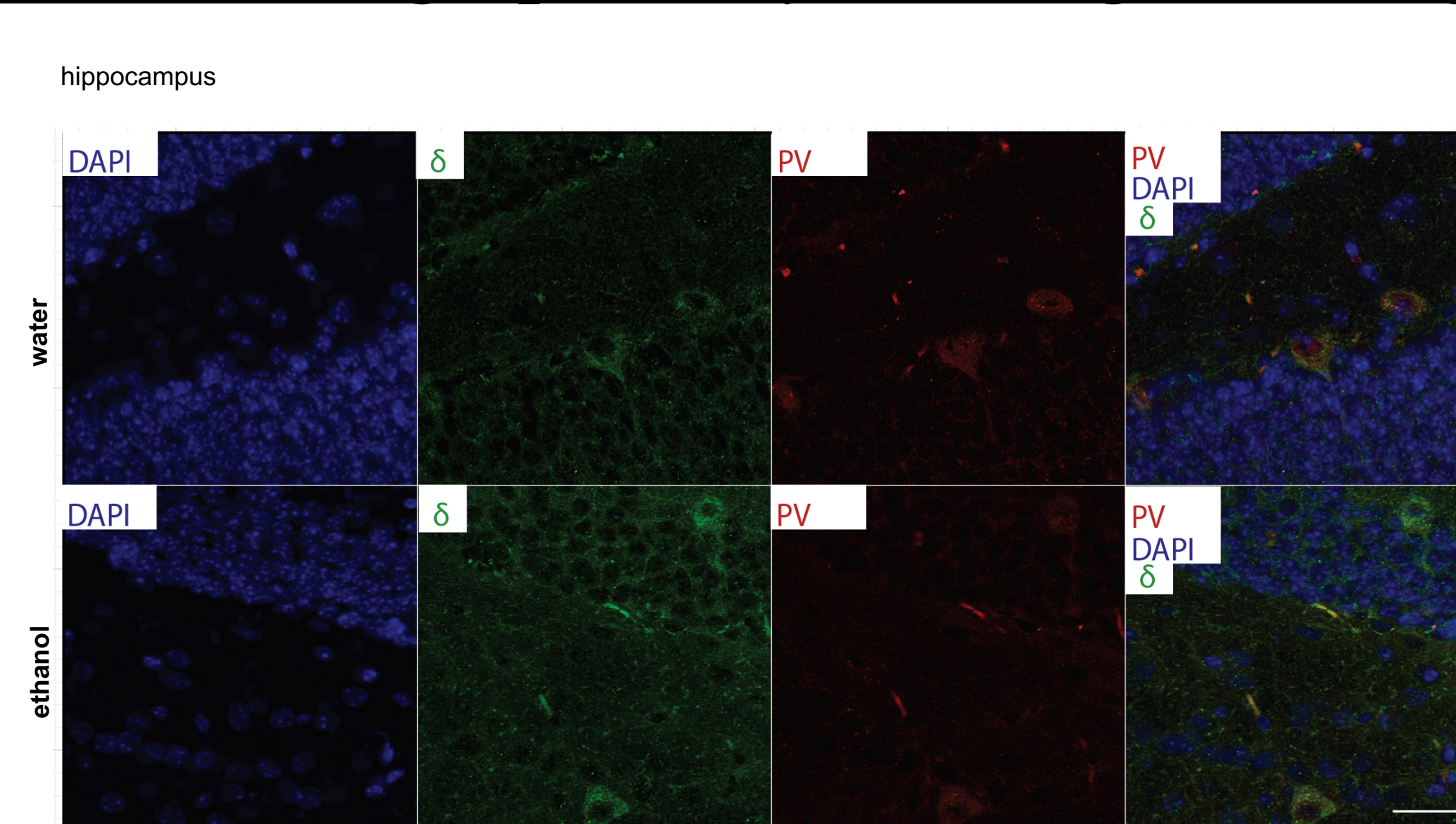


Figure 6. Published findings showing enhanced GABA(A) receptor expression on parvalbumin interneurons during early withdrawal from binge drinking. Future work will determine whether changes in GABAergic signaling in the hippocampus noted during early withdrawal (Melón et al., 2019) are reversed following long-term abstinence.

SUMMARY AND CONCLUSIONS

- These data suggest that sex difference in binge drinking behavior is more prominent in adults than in adolescents.
- Previous work showing higher intakes among adolescents may be influenced by stress associated with isolation housing.
- We did not replicate prior work showing long lasting effects of binge drinking during protracted withdrawal. Variability in drinking behavior may have affected these outcomes.
- Future studies will use the tissue we harvested from the mice and perform IHC. We want to compare changes in levels of proteins important to GABA signaling across groups to determine whether this resetting of behavior during protracted withdrawal is associated with a reversal of GABAergic signaling changes seen during acute withdrawal from alcohol.