Aversion-resistant alcohol drinking in male and female mice

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Introduction

- Human and animal research suggests that females are more susceptible to key phases in the addiction process such as initiation, binging, and relapse[1].
- Aversion-resistant ("compulsive") drinking in mice can be demonstrated using a bitter tasting substance and mixing It with ethanol to see if the mice will continue to drink despite the aversive taste[2].
- **Here, we tested for differences in escalation of ethanol drinking and aversion-resistant ethanol drinking in male and female mice.**

Methods

Subjects: C57BL/6J male and female mice were generated from breeding pairs purchased from The Jackson Laboratory, Bar Harbor, ME.

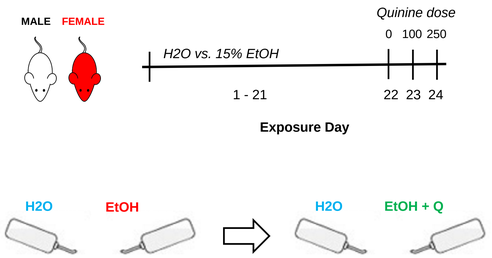
Behavioral Testing: 24 mice (12 male, 12 female) were socially housed in groups of two, given standard care on a reverse light/dark cycle (lights off at 7:00am). Mice were moved into individual housing when they were most active, which was approximately 3 hours into their dark cycle. In these individual housing, each mouse was given two bottles, one containing water, the other containing 15% ethanol (EtOH). After two hours had passed, the mice were returned to their social housing and bottles were weighed. After three weeks of the same procedure (drinking 5 days/week), quinine was added to the bottles of 15% ethanol. The first day of quinine addition included a concentration of 100µM and the second day added a concentration of 250µM.

A control group of mice (9 male, 10 female) were also socially housed, but placed in individual housing during the active period. Unlike the experimental group, these mice were given a bottle of water and a bottle of 15% ethanol with 100µM quinine every day, instead of just at the end of testing. Otherwise, these mice were cared for, housed, and under the same light/dark cycle as the experimental group.

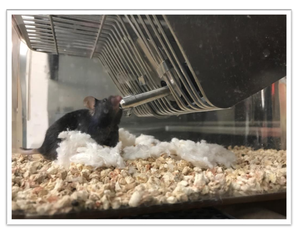
Data analysis: Data were expressed as mL of fluid consumed per kg of body weight for each individual mouse and averaged across groups (male and female). Preference data were calculated as [(Vol EtOH)/(Vol EtOH+Vol H2O)]x100. All analyses were conducted in GraphPad Prism (v. 7.0) using repeated measures ANOVA and follow-up tests for multiple comparisons, as appropriate. All data are shown as mean ± SEM.

Experimental design

Experimental timeline



Two-bottle limited access protocol



"Drinking in the dark"

Escalation and aversion-resistant drinking

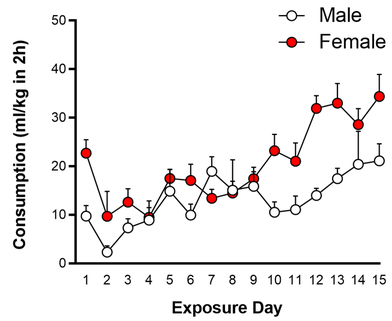


Figure 1. Consumption in 2 hours of 15% EtOH per day of exposure. A repeated measures ANOVA suggests a significant main effect of Day, $p < .0001$; a significant main effect of Sex between female ($n = 11$) and male ($n = 12$), $p < .001$; and a significant interaction, $p < .05$.

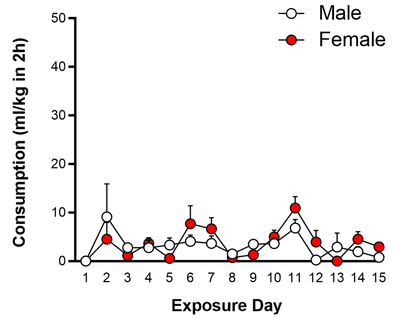


Figure 2. Consumption in 2 hours of H_2O per day of exposure between female ($n = 11$) and male ($n = 12$).

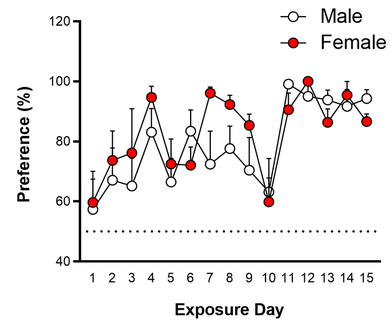


Figure 3. Percent preference of 15% EtOH vs. H_2O per day per 2 hours of exposure between female ($n = 11$) and male ($n = 12$).

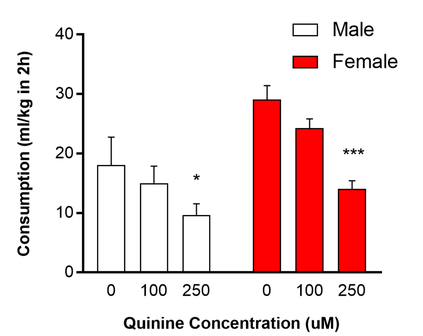


Figure 4. Quinine dose-response between female ($n = 11$) and male ($n = 12$). Consumption in 2 hours of 15% EtOH with increasing quinine concentration (i.e., 0µM, 100µM, 250µM). *$p < .05$, ***$p < .001$ vs. 0µM, Holm-Sidak corrected T-test.

Effects of quinine on escalation of drinking

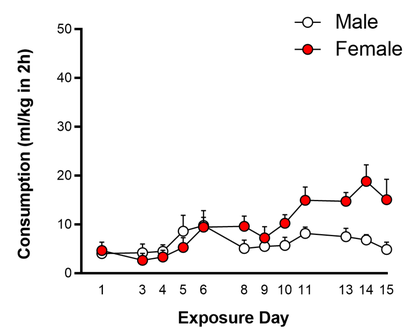


Figure 5. Consumption in 2 hours of 15% EtOH+100µM Quinine per day of exposure. A repeated measures ANOVA suggests a significant main effect of Day, $p < .0001$; a significant main effect of Sex between female ($n = 10$) and male ($n = 9$), $p < .01$; and a significant interaction, $p < .01$.

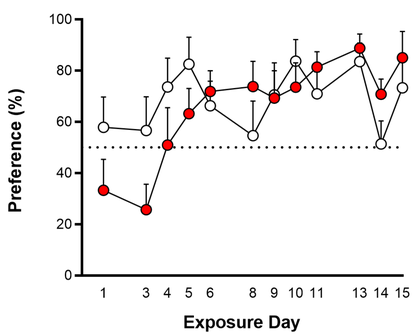


Figure 6. Percent preference of 15% EtOH+100µM Quinine vs. H_2O per day per 2 hours of exposure between female ($n = 10$) and male ($n = 9$). A repeated measures ANOVA suggests a significant main effect of Day, $p < .001$.

Future directions

- Examine additional factors that could affect aversion-resistant alcohol drinking, such as age, housing conditions, and exposure to stress.
- Determine the time course over which aversion-resistant drinking develops and whether this inflexible behavior emerges sooner in females vs. males.
- Explore differences in taste reactivity and the aversive properties of quinine in females vs. males.
- Investigate sex differences in other models of inflexible behavior.

Conclusions

- Female C57Bl/6J mice consumed more 15% EtOH than males in the "drinking in the dark" procedure.
- Female and male mice did not differ in aversion-resistant intake of EtOH+Quinine (100µM).
- Quinine reduced levels of EtOH consumption in both sexes but prevented escalation in males only.
- **We confirmed an increased vulnerability for ethanol consumption in females. More work will be needed to validate use of the aversion-resistant drinking model in female mice.**

References

1. Anker, J.J., Carroll, M.E. (2010). Females Are More Vulnerable to Drug Abuse than Males: Evidence from Preclinical Studies and the Role of Ovarian Hormones. Biological Basis of Sex Differences in Psychopharmacology. Current Topics in Behavioral Neurosciences, 8, 73-96.
2. Lesscher, H.M., Kerkhof, L.W., Vanderschuren, L.J. (2010). Inflexible and Indifferent Alcohol Drinking in Male Mice. Alcohol Clin Exp Res, 34, 1219-1225.
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Acknowledgements

Research supported by the Department of Psychology at Miami University. Thanks to Bridget Dames, Drew Schleicher, Josh Setters, Olivia Ramsey, and Elena Penhos for help with data collection.