Effects of taste aversion on alcohol seeking behavior in mice

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Introduction

- Individuals with alcohol use disorders (AUD) have a tendency to drink despite negative consequences. In studies with mice, quinine can be used to model aversion-resistant drinking.[1,2]
- Previous studies have shown that females are more susceptible to alcohol use disorders (AUD) than males. This has been modelled using aversion-resistant drinking in homecages.[2,3]
- **This experiment was designed to study the sex differences in aversion-resistant drinking under an operant conditioning model.**

Methods

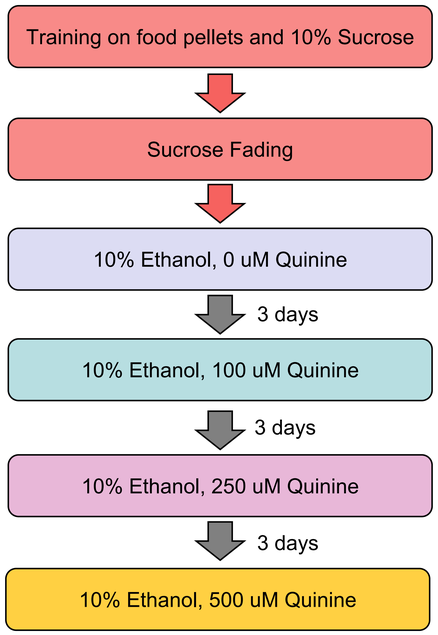
Subjects: C57BL/6J male and female mice were generated from breeding pairs purchased from The Jackson Laboratory, Bar Harbor, ME.

Behavioral Testing: 7 male and 7 female C57BL/6J mice were individually housed. Operant conditioning boxes were used and the mice moved freely for 30 minutes each session. The two "nose pokes" inside of the box acted as a control and a dispenser of a reward. Each mouse used the same operant conditioning box for the duration of the experiment. The mice were initially trained to use the operant box with a food reward and 10% sucrose. After the mice received sufficient responses, the mice passed from this liquid reward, to 10% sucrose/10% ethanol, 5% sucrose/10% ethanol, and 10% ethanol. When each of the mice met the criteria to pass each solution, the quinine sessions began. Three days were spent on 0 uM, 100 uM quinine, 250 uM quinine, and 500 uM quinine in 10% ethanol. Two weeks after the end of operant conditioning, mice were given these same concentrations of quinine in water using a two-bottle choice test in their homecage.
In a separate experiment, 7 male and 7 female C57BL/6J mice were individually housed, food restricted, and used the operant conditioning boxes in the same way as the first group. In this experiment, the only liquid reward available to the mice was 2% sucrose. After 18 sessions, the quinine sessions were done in the same way as the first experiment.

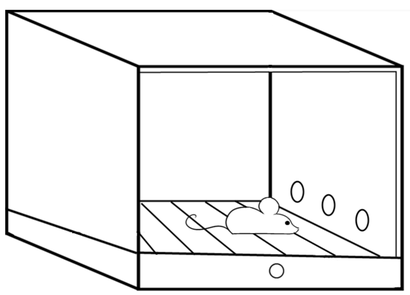
Data Analysis: Data were expressed as amount of ethanol or sucrose consumed per body weight of the mouse, and % preference of the quinine bottle compared to the water bottle. All analyses were conducted in GraphPad Prism (v 8.0) using repeated measures ANOVA and follow-up tests for multiple comparisons, as appropriate. All data are shown as mean ± SEM.

Experimental Design

Timeline



Operant Boxes



Behavioral Training and Quinine Testing

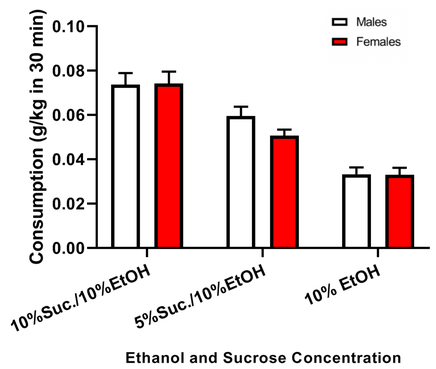


Figure 1. Consumption of liquid rewards during sucrose fading before the introduction of quinine. A repeated measures ANOVA suggests no significant effect of sex between males (n=7) and females (n=7).

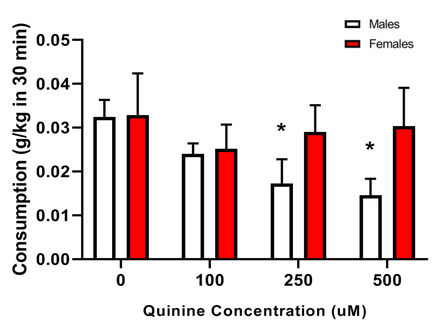


Figure 2. Consumption of 10% EtOH with various concentrations of quinine . A repeated measures ANOVA suggests no significant effect of sex between males (n=7) and females (n=7). * p < .05 when males given 250uM and 500uM quinine were compared to males given 0uM quinine, Dunnett's multiple comparison test

Mice avoid quinine not paired with alcohol

Sucrose and Quinine

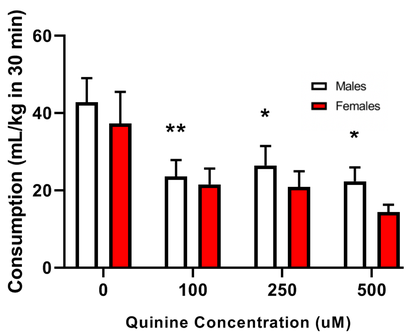


Figure 3. Consumption of 2% Sucrose with various concentrations of quinine . A repeated measures ANOVA suggests no significant effect of sex between males (n=7) and females (n=7). ** p < .01 when males given 100uM quinine. * p < .05 when males given 250uM and 500uM quinine were compared to males given 0uM quinine, Dunnett's multiple comparison test

Water and Quinine

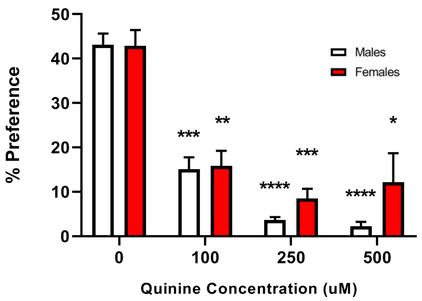


Figure 4. Preference of quinine compared to water. A repeated measures ANOVA suggests no significant effect of sex between males (n=7) and females (n=7). p < .05 when males at 100uM, 250uM, and 500uM were compared to males at 0uM. p < .05 when females at 100uM, 250uM, and 500uM were compared to females at 0uM, Dunnett's multiple comparison test

Future Directions

- Add more animals to the current experiments in order to determine the relationship between females and quinine taste aversion.
- Complete ovariectomies on females to see how sex hormones influence the results found in this experiment.
- Use an alternate method of taste aversion to determine if females exhibit the same results.

Conclusions

- Male mice exhibited aversion to ethanol mixed with 250 uM and 500 uM of quinine, while females did not.
- Males exhibited aversion to sucrose mixed with 100 uM, 250 uM, and 500 uM of quinine, while females did not.
- Both male and female mice showed a preference for the water bottle over bottles containing any concentration of quinine.
- **Female mice exhibited aversion-resistance behavior when quinine was added to ethanol and sucrose. Males only exhibited aversion-resistant behavior at 100 uM of quinine in ethanol.**

References

1. Fulenwider, H., Nennig, S., Price, M., Hafeez, H., and Schank, J. (2019). Sex Differences in Aversion-Resistant Ethanol Intake in Mice. *Alcohol and Alcoholism*, *1-8.*
2. Sneddon, E., White, R., and Radke, A. (2018). Sex Differences in Binge-Like and Aversion-Resistant Alcohol Drinking in C57BL/6J Mice. *Alcohol Clin Exp Res* 43:243–249.
3. Becker, J. and Koob, G. (2016). Sex Differences in Animal Models: Focus on Addiction. *Pharmacol Rev* 68:242–263.

Acknowledgements

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