Saccharin Potentiates Oral Ethanol Self-Administration in Male C57BL/6J Mice.

Kynah Walston, Kelsi Listman, Alexia Will, Kaytie Perez, Erin Voeghtly, Melia Teixeira, Rebekah Vaughan, & Dr. Curtis Bradley

Bridgewater College Behavioural Neuroscience Lab, Bridgewater College, Bridgewater, VA

Introduction

- Alcohol (Ethyl alcohol, or ethanol) is one of the most consumed psychoactive substances in society. Over 20% of US adults report binge drinking within the past month. Furthermore, over 5% of US adults are diagnosed with Alcohol Use Disorder (NIAAA).
- Most drugs are tested for their abuse potential in society using a self-administration paradigm. This model allows animals to work or voluntarily consume a drug for a short period of time.
- Ethanol self-administration models are historically difficult to establish.
- Reliable ethanol self-administration is commonly established through selective breeding or prolonged sucrose-fading procedures.
- These nuanced methods for establishing ethanol self-administration bring into question the ability of ethanol to function as a reinforcer.
- Oral ethanol self-administration typically uses a diluted ethanol solution without any flavorants or gustatory reinforcers. The inability to mask the flavor of alcohol may impact the willingness of animals to drink the alcohol solution.
- The taste of ethanol may be a limiting factor in ethanol self-administration research and could explain the difference between rodent self-administration and human alcohol consumption.
- To better understand the divide between animal research and real-world alcohol consumption, a model is needed that mimics typical human alcohol consumption.
- Alcohol is commonly consumed with gustatory reinforcers including sweeteners such as sucrose and saccharin.
- Saccharin, a noncaloric artificial sweetener, functions as a mild reinforcer in rodents.

Objective

The current study investigated the relationship between saccharin, ethanol reinforcement, and ethanol reinforcement enhancement. We hypothesized that saccharin would enhance ethanol consumption.

Method

Subjects:

Eighteen, non-naive male C57BL/6J mice that have been exposed to EtOH and caffeine in prior research were used. The mice were individually housed in a temperature-controlled room maintained on a reversed 12:12 hour light: dark cycle. The mice were food restricted (3.8-4.2g/day) and allowed free access to water in their home cage.

Apparatus

Six standard operant chambers were equipped with two levers, a liquid dipper, and house light. One lever was inactive, while the other dispensed a reinforcer solution when pressed on a Progressive Ratio (PR) schedule of reinforcement. The solutions were presented in an .01 ml cup. Operant chambers were enclosed in a sound proof chamber.

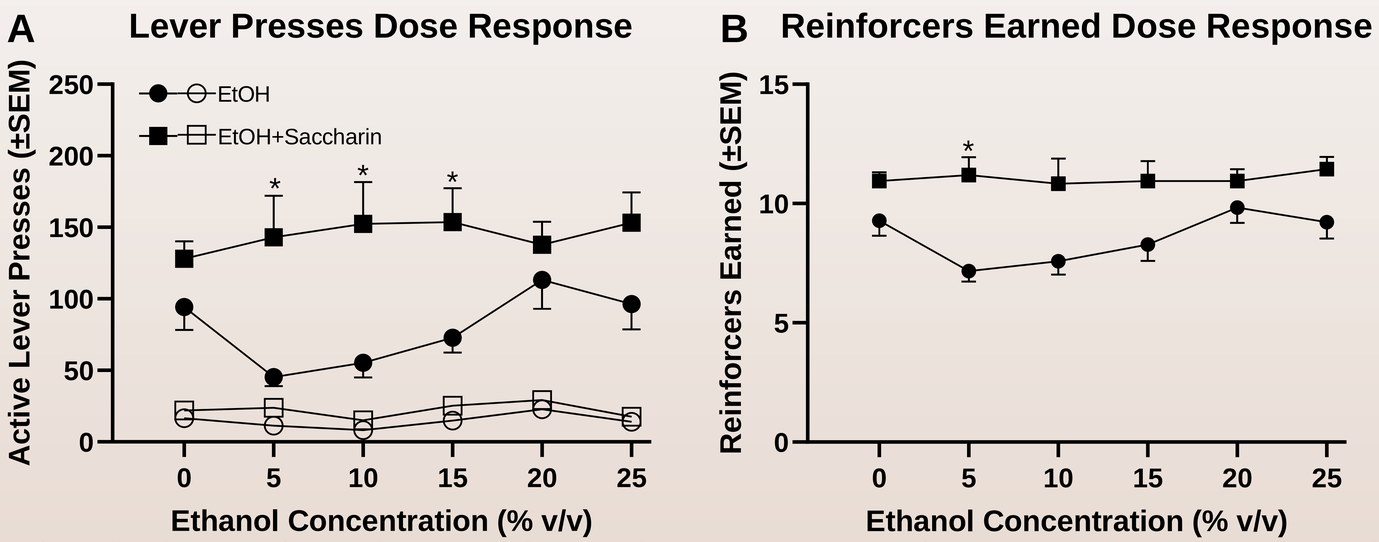
Drugs and Solutions:

Ethanol: Ethanol solutions (50%) were diluted to assigned concentrations (0, 5, 10, 15, 20, & 25%) via tap water. Solutions were used for two weeks.

Saccharin+Ethanol: Saccharin solutions were diluted to 0.2% w/v with tap water and assigned ethanol concentrations.

Eighteen male mice were separated into two reinforcer groups: ethanol and ethanol+saccharin. During 1-hr sessions, mice could lever press on a Progressive Ratio schedule of reinforcement for access to a designated reinforcer. Sessions continued for five days at each ethanol concentration (0%, 5%, 10%, 15%, and 20%).

Results



Top Figures: Effects of EtOH (n=9) and EtOH+Saccharin (n=9) as a reinforcer on active lever presses, inactive lever presses, and reinforcers earned. Each point represents the group average of lever presses over the final two days of testing at the designated ethanol concentrations. Error bars represent the standard error of the mean. **Figure A:** The combination of ethanol and saccharin increases active lever presses at lower concentrations compared to ethanol alone. A 2 (Reinforcer) x 6 (Ethanol Concentration) Mixed ANOVA revealed a significant main effect of reinforcer group and a significant interaction of reinforcer type and ethanol concentration. As noted by the asterisks, pairwise comparisons revealed the EtOH+Saccharin group responded more on the active lever compared to the EtOH group at 5, 10, and 15% ethanol concentrations ($p<.05$). A 2 (Reinforcer) x 6 (Ethanol Concentration) Mixed ANOVA revealed a significant main effect of reinforcer group ($p=.0005$) on inactive lever presses. **Figure B:** Saccharin potentiates reinforcers earned. A 2 (Reinforcer) x 6 (Ethanol Concentration) Mixed ANOVA revealed a significant main effect of reinforcer group and a significant interaction of reinforcer type and ethanol concentration. As noted by the asterisks, a planned pair-wise comparisons revealed group differences at the 5% ethanol concentration ($p<.05$).

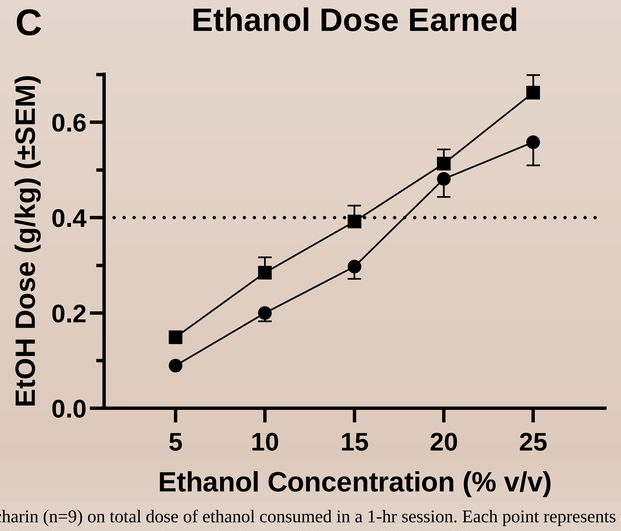


Figure C: Effects of EtOH (n=9) and EtOH+Saccharin (n=9) on total dose of ethanol consumed in a 1-hr session. Each point represents the group average of ethanol dose over the final two days of testing at the designated ethanol concentrations. Error bars represent the standard error of the mean. The dotted line represents the possible threshold dose of alcohol necessary for a psychoactive effect (Hindmarch, 1980).

Procedure

Training. All mice were shaped to lever press for dipper presentations of 0.2% saccharin solution under a fixed ratio (FR1) reinforcement schedule. Training continued until they earned 20 reinforcers within one hour session.

Experiment. After shaping, mice were assigned to 2 groups separated by reinforcer solution type: ethanol and saccharin (EtOH+Saccharin) and Ethanol alone (EtOH). During each one-hour session, the mice were placed in operant chambers with their assigned reinforcer and ethanol concentration. Mice responded on a Progressive Ratio (PR) schedule of reinforcement. Sessions were conducted every other day Monday through Friday.

Each of the six alcohol concentrations was maintained for 5 days for a total of 30 test days. The order of ethanol concentrations was randomly assigned. Active lever presses, inactive lever presses, reinforcers earned, and doses were recorded and analyzed for group differences.

Conclusions

- Saccharin potentiates active lever pressing regardless of ethanol concentration.
- Saccharin motivates ethanol self-administration at sub-threshold doses.
  - Drug sensitization via saccharin?
- High concentrations of ethanol motivate self-administration regardless of presence of saccharin.
- Water just as reinforcing as ethanol? Potential for conditioned reinforcement effect.

Future Directions

Finding upper limits of ethanol concentration for full dose-response curve.

Sex Differences? Replication with female C57BL/6J mice.

Conduct 0% ethanol trials at beginning and end of study to elucidate potential conditioned reinforcement effects.

Binge alcohol consumption. Testing free access to +10% ethanol in home cage for extended periods