

Examining the effects of caffeine and saccharin on acute ethanol intake in male C57BL/6J mice

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

Behavioral Neuroscience Laboratory, Department of Psychology, Bridgewater College, Bridgewater, VA, USA

INTRODUCTION

- Caffeine is the most consumed psychoactive drug, with upwards of 87% of Americans consuming it daily (FDA).
- Alcohol is another commonly consumed psychoactive drug in society. One in six US adults report 4 episodes of binge drinking per month (CDC).
- Caffeine is often consumed as an ingredient in beverages and food; with its addition to so many products, public health concerns have grown. Over 30% of adults age 18-28 report mixing caffeine and alcohol at least once within the past year.
- This combination was believed to increase rates of alcohol poisoning and eventually led to the FDA limiting caffeinated alcoholic beverage production.
- Despite being considered a reinforcer in human studies, research has been inconclusive, inconsistent, or weak in establishing caffeine as a reinforcer in animals. Similar findings exist for alcohol.
- Studies have shown that caffeine may act as a reinforcement enhancer, potentiating the reinforcing effects of various drug and non-drug reinforcers. Research is limited and inconsistent in evaluating caffeine's reinforcement enhancing effects on alcohol self reinforcement.
- The goal of this study is to better understand the reinforcement enhancing effects of caffeine by observing its effect on alcohol intake. Also, the potentiating effects of 0.2% saccharin on alcohol consumption will be observed.

METHODS

Subjects: 17 drug-naive C57BL/6J mice were individually housed in a room that was controlled for temperature (22°C) and humidity (50%). Mice were kept on a reverse 12 hour light-dark cycle from 7AM-7PM. Mice were food restricted, receiving between 3.8-4.2 grams of food per day to maintain 85% free bodyweight. Water was available ad libitum.

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:

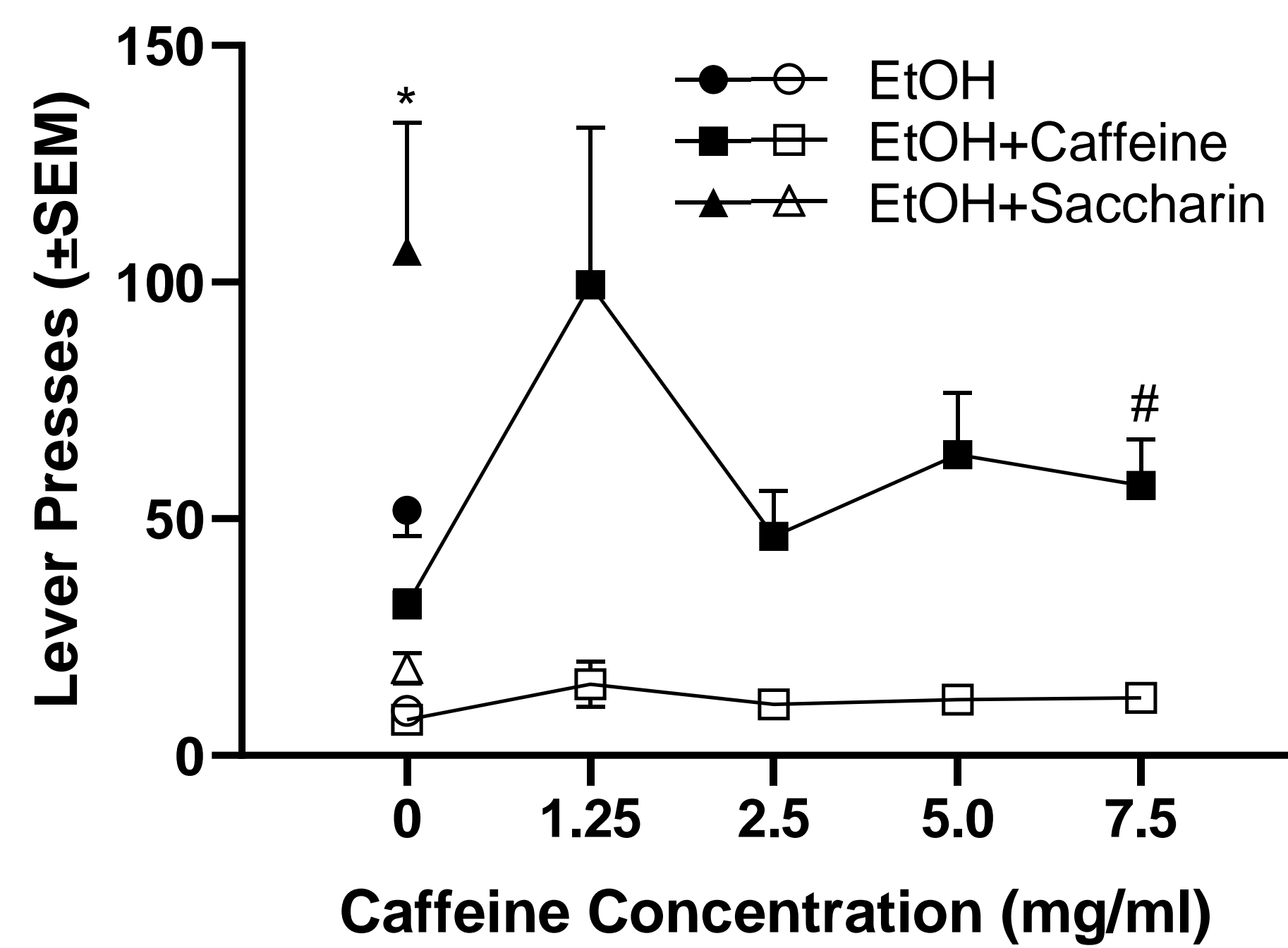
Ethanol: Ethanol solutions (50%) were diluted to 5% (v/v) concentrations with tap water. Solutions were used for two weeks.

Ethanol+Caffeine: 5% ethanol solutions were mixed with varying concentrations (0, 1.25, 2.5, 5, 7.5 mg/ml) of caffeine anhydrous (Sigma, St.Louis, MO).

Ethanol+Saccharin: Saccharin (Sigma, St. Louis, MO) was dissolved in 5% to a concentration of 0.2% w/v.

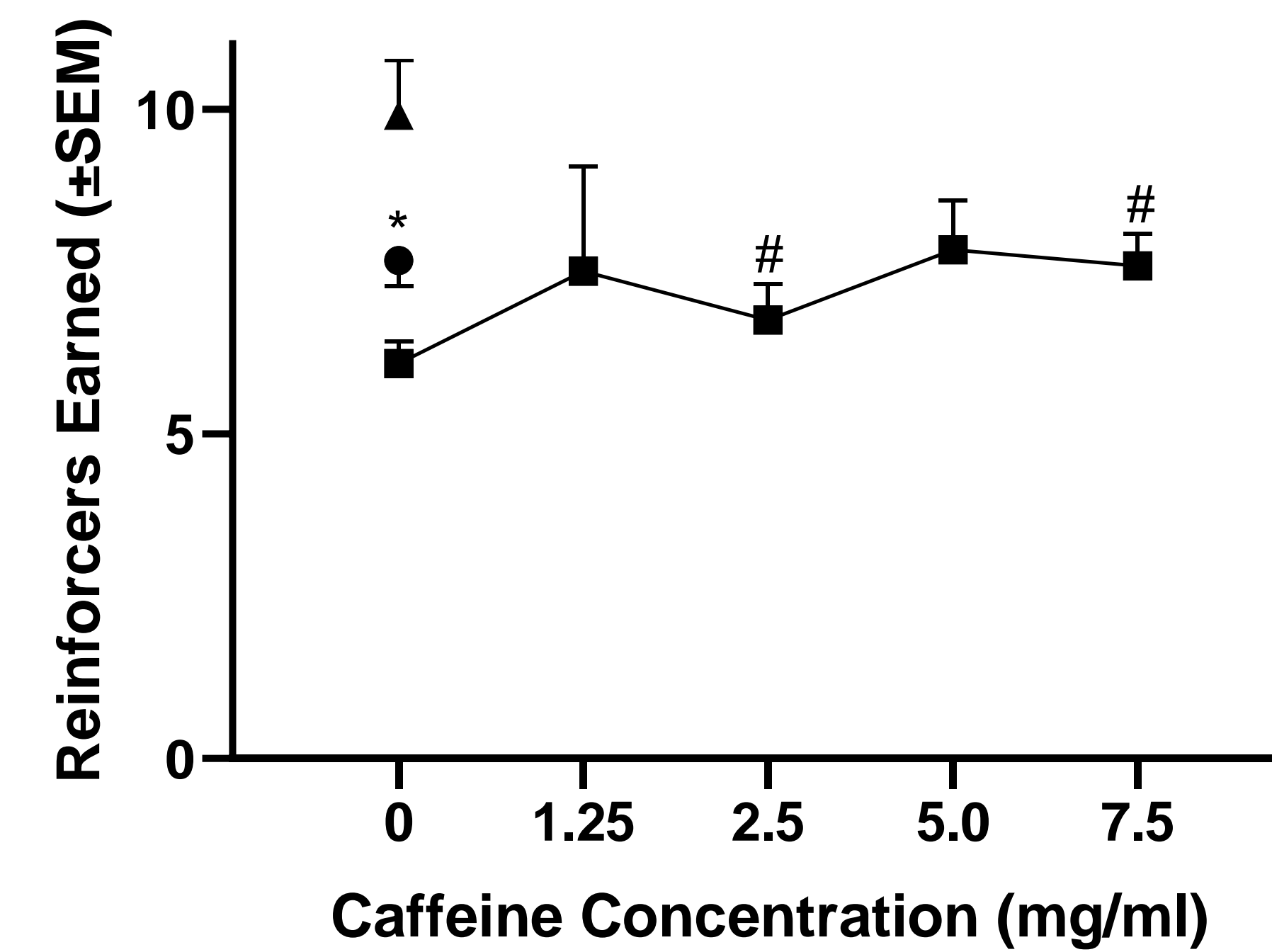
RESULTS

A Lever Presses Dose Response

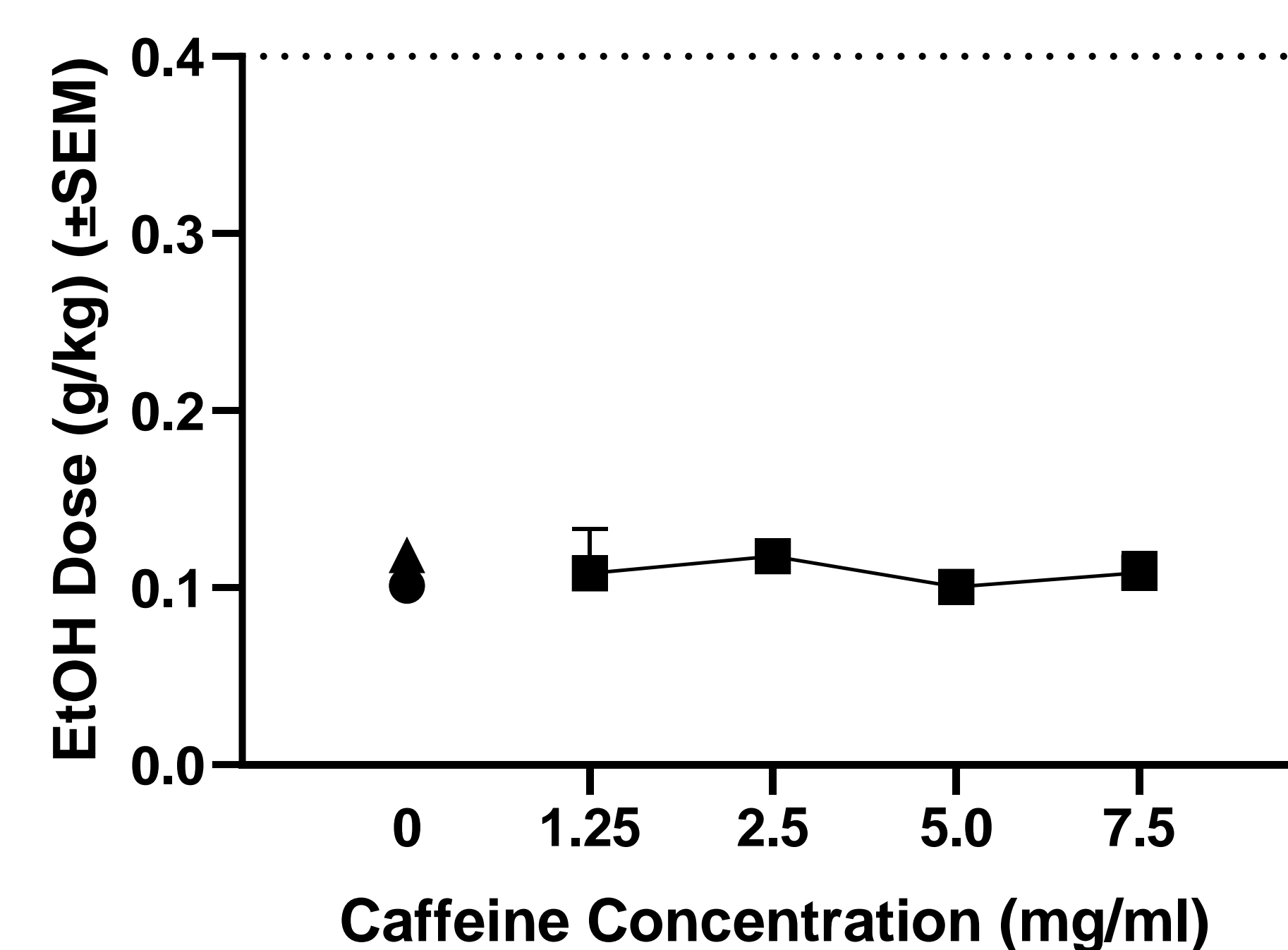


Top Figures: Effects of EtOH (n=6), EtOH+Caffeine (n=6), and EtOH+Saccharin (n=5) as a reinforcer on active lever presses, inactive lever presses, and reinforcers earned. Each symbol represents the group average of lever presses over the final two days of testing at the designated caffeine concentrations for EtOH+Caffeine group and a representative two-day average for the EtOH and EtOH+Saccharin groups. Error bars represent the standard error of the mean. The combination of ethanol and saccharin increases lever pressing and reinforcers earned compared to ethanol or ethanol and caffeine. **Figure A:** A 3 (Reinforcer) x 5 (Caffeine Concentration) Mixed ANOVA on active lever presses revealed a main effect of Reinforcer and a significant Caffeine Concentration x Reinforcer interaction. Pairwise comparisons revealed significantly higher active lever presses for the EtOH+Saccharin group compared to EtOH ($p < .05$) as marked by the asterisks. Additionally, the octothorp (#) denotes significantly higher active lever presses for the EtOH+Saccharin compared to the EtOH+Caffeine group at 7.5 mg/ml ($p < .05$). No main effects or interactions were found for inactive lever presses. **Figure B:** A 3 (Reinforcer) x 5 (Caffeine Concentration) Mixed ANOVA on reinforcers earned revealed a main effect of Reinforcer, Caffeine Concentration, and a significant Caffeine Concentration x Reinforcer interaction ($p < .05$). Pairwise comparisons revealed higher reinforcers earned in the EtOH group compared to the EtOH+caffeine group ($p < .05$) as noted by the asterisks. Additionally, octothorps (#) denote significantly lower reinforcers earned for the EtOH+Caffeine groups at 2.5 and 7.5 mg/ml when compared to the EtOH+saccharin group ($p < .05$).

B Reinforcers Earned Dose Response

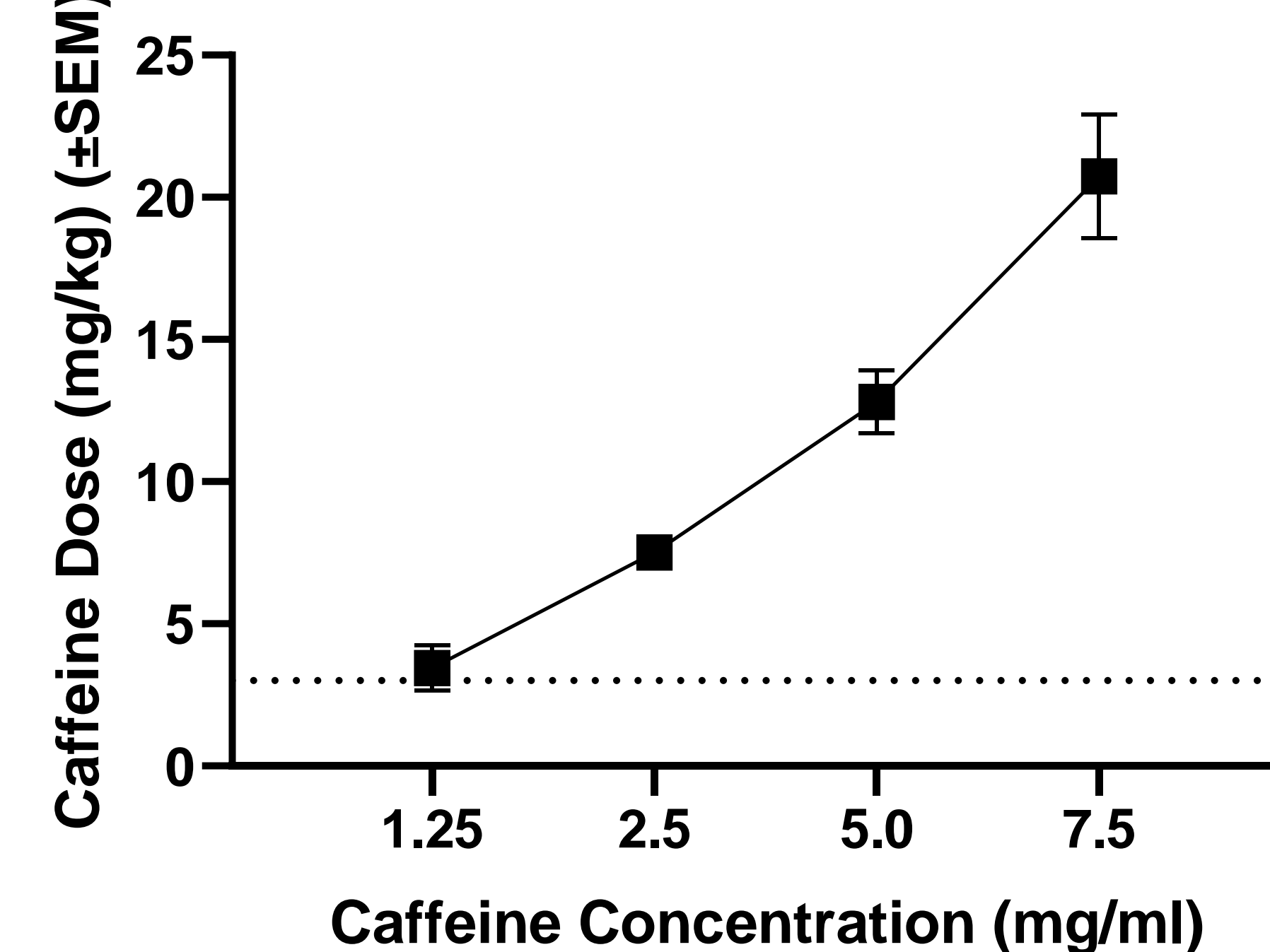


C Ethanol Dose Response



Bottom Figures: Ethanol and caffeine doses earned. **Figure C:** Effects of EtOH (n=6), EtOH+Caffeine (n=6), and EtOH+Saccharin (n=5) on total ethanol dose consumed. The dotted line represents the threshold dose for ethanol's psychoactive effects. Each point represents the group average of lever presses over the final two days of testing at the designated caffeine concentrations for EtOH+Caffeine group and a representative two-day average for the EtOH and EtOH+Saccharin groups. Error bars represent the standard error of the mean. It appears that no group was able to experience the psychoactive effects of ethanol. This is likely due to the limited access to low concentrations of ethanol solutions and a limited time frame (1 hour). **Figure D:** Caffeine dose earned by the EtOH+Caffeine group at all caffeine concentrations. Each symbol represents the group average of lever presses over the final two days of testing at the designated caffeine concentrations. Error bars represent the standard error of the mean. The dotted line represents the threshold dose for the psychoactive effects of caffeine. On average, mice in the EtOH+Caffeine group experienced above-threshold doses of caffeine at each concentration available. Mice experienced low to moderate-high doses of caffeine as caffeine-concentrations increased.

D Caffeine Dose Response



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 training, mice were randomly assigned to one of three reinforcer conditions. For the remainder of the study the mice lever pressed for access to a 0.01 ml solution of ethanol, ethanol+caffeine, or ethanol+saccharin. Caffeine concentrations changed after five sessions throughout the study in order to yield a dose-response curve. The order of exposure to caffeine concentrations was randomly assigned. Sessions were conducted every other day Monday-Friday to avoid tolerance. During the testing, mice responded on a Progressive Ratio (PR) schedule during a 1-hour session. A Progressive Ratio schedule increases the amount of lever presses necessary to earn a reinforcer upon completion of the previous contingency. This measures motivation to obtain said reinforcer. Active lever presses, inactive lever presses, and number of reinforcers earned were noted after each session.

CONCLUSIONS

- Ethanol alone and Ethanol+caffeine are less motivating than EtOH+Saccharin
- Caffeine functions as an oral reinforcer
 - EtOH+Caffeine averaging above 20 mg/kg
- Limited results on ethanol's reinforcing effects
 - Mice only experienced sub-threshold doses

FUTURE DIRECTIONS

- Exploring higher concentrations of ethanol in this operant paradigm.
- Examining binge-drinking behaviors using free access paradigms with higher concentrations of ethanol (10%).
 - Drinking-in-the-dark paradigm
 - Saccharin+EtOH
 - Caffeine+EtOH
 - EtOH alone

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