Effect of Flavor Variety on Alcohol Self-Administration in Long-Evans Rats

Alcohol abuse has been linked to visceral obesity, a risk factor for type 2 diabetes. Recent evidence suggests that rats are more motivated to binge drink when the delivery of alcohol is variable. Our goal was to explore this finding further by varying the flavor of alcohol within a drinking session. Ten Long-Evans rats were trained to lever press for orally delivered alcohol (10% volume/volume) in a standard operant conditioning apparatus. During baseline conditions, the same flavor of alcohol (cherry or grape) was used throughout the 20-minute session. During experimental conditions, the reinforcer was changed to a different flavor (cherry or grape) half way through the session. The results indicated that more lever pressing (ie, more motivation) occurred in the experimental conditions than in the baseline conditions. These results suggest that self-administering the same flavor throughout a binge-drinking session will decrease motivation to consume alcohol and therefore decrease the health risks associated with heavy alcohol use.

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Introduction

Nationally, 6% of Americans meet the diagnostic criteria for being a heavy drinker (ie, five or more drinks per occasion on five or more days in the past 30 days).[1] The negative consequences of heavy drinking are correlated with an increase in accidental injury, job and family problems, and symptoms of alcohol dependence. Additionally, heavy alcohol consumption in laboratory rats (*Rattus norvegicus*) has been linked with an increase in visceral obesity, a risk factor for type 2 diabetes.[2] Wilson and van Tets' findings suggest that the risk of developing type 2 diabetes can be reduced by decreasing alcohol consumption.

Recently, Murphy and colleagues[3] showed that motivation to self-administer alcohol in rats was enhanced when the delivery of alcohol was variable (ie, unpredictable), vs when it was constant (ie, predictable). Murphy et al's findings suggest that motivation to consume alcohol can be reduced if the properties of the alcohol reinforcer are made constant. The goal of the present study was to extend these findings to the flavor of the alcohol reinforcer. In the constant conditions, rats self-administered the same flavored alcohol throughout a drinking session. In the variety conditions, the flavor of the alcohol was changed halfway through the session. We predicted that by keeping the flavor constant within a drinking session, motivation to consume alcohol would decrease below conditions where the flavor was variable.

Methods

Subjects

Ten experimentally naïve, male Long-Evans rats (Simonsen Laboratories, Gilroy, CA) served as subjects. They were 90 days old at the beginning of the study, and were housed individually in standard laboratory cages. Access to food and water were available *ad libitum* in their home cages. The subjects were exposed to 12:12 hours light/dark cycle (lights off from 7:00 am to 7:00 pm).

Apparatus

The apparatus was a standard two-lever operant conditioning chamber (28.9 cm × 31.8 cm × 29.8 cm). Two 5.1 cm × 5.1 cm openings located 1.9 cm above the floor allowed access to two 0.10 mL dippers. The left dipper hole was located 9.5 cm from the left wall, and the right dipper hole was located 1.3 cm from the right wall. There were two 4.6 cm × 0.2 cm levers. The left lever was located 1.1 cm from the left wall, and the right lever was located 9.4 cm from the right wall of the apparatus. The levers, which required approximately 0.25 N for their operation, were 6.9 cm above the floor and extended 2.2 cm into the enclosure. A light (2.4 cm in diameter) was located 6.4 cm above each lever and 13.3 cm from the ceiling.

Procedure

The subjects were trained to press the lever by a successive-approximations procedure. During this phase, each approximation was reinforced by a 10-second access to a 0.10-mL dipper containing 10% sucrose (weight/volume) diluted in tap water. Subjects remained in the apparatus until they responded at least 100 times for continuous reinforcement. In subsequent sessions, access to reinforcement wase reduced to three seconds and rates of reinforcement were decreased until the

rats responded on a variable-interval 15-second (VI 15-s) schedule during 20-minute sessions. All reinforcers were scheduled according to a 25-interval Fleshler and Hoffman[4] series. Alcohol was introduced, and the concentration of sucrose was reduced, according to a modified version of the sucrose-substitution procedure described by Samson.[5] Alcohol was added in 2.0% (volume/volume) increments over the following eight sessions until the subjects were responding for a 10% sucrose/10% alcohol solution. Thereafter, the concentration of sucrose was reduced in 2.0% increments over the next eight sessions until the subjects were responding for the 10% alcohol solution.

Following the training protocol, rats were placed directly on the baseline procedure. Because both levers were used, the active lever during the first and second halves of the session was counterbalanced across rats. During the first 10-minutes of the session, the active-lever stimulus light was illuminated. Pressing the active lever was reinforced according to a VI 15-second schedule. Reinforcers consisted of 3-second access to a 10% alcohol solution, diluted in grape or cherry Kool-Aid (sugar free). The other stimulus light was not illuminated during this time, and presses on the inactive lever did not have any programmed consequences. During the final 10 minutes of the session, responding on the other lever was reinforced according to the same parameters as above. During the constant conditions, the flavor of the alcohol solution remained the same throughout the session. Two constant conditions were conducted: one for the cherry flavored alcohol and one for the grape flavored alcohol. During the variety conditions, the flavor differed during the last half of the session. For example, if the flavor was cherry during the first 10 minutes of the session, it was changed to the grape flavored alcohol during the second 10 minutes of the session. Each condition was conducted to stability with the requirement that it was in effect for a minimum of 20 sessions. Responding was considered stable when rates of responding during the last five sessions of a condition fell within the range of responding for the entire condition. If this criterion was not met, more sessions were conducted until responding was deemed stable. Excluding reinforcement time, sessions were 20-minutes long and were conducted daily, five times per week.

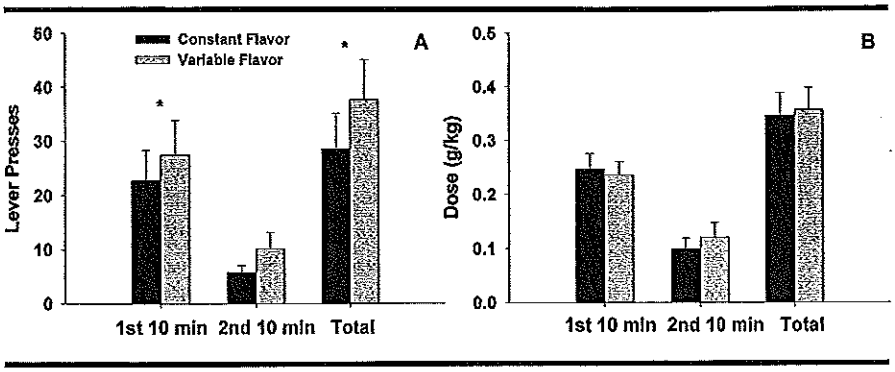

Fig 1. Number of lever presses (Figure 1A) and estimated doses (grams/kilogram) of self-administered alcohol (Figure 1B) for each session half and for the entire session in the constant (black bars) and variety (gray bars) conditions. Each bar represents the mean of all rats (N = 10) during the last five sessions of each condition. Error bars represent ± 1 standard error of the mean. *$P<.05$

Data analysis

The data were averaged over the last five sessions for which each condition was in effect for each rat. To simplify the analyses, aggregates of the constant and variety conditions were created by averaging the two replications together. Within-session changes in alcohol-reinforced responding were determined by dividing the 20-minute session into two 10-minute intervals. Within-session changes in alcohol-reinforced responding, and the amount of alcohol self-administered, were compared across conditions by separate 2 (condition: constant vs variety) × 2 (session half: 1st 10-minute interval vs 2nd 10-minute interval) repeated measures analyses of variance (ANOVA). When necessary, post hoc analyses were conducted with dependent-samples t tests. Results were considered significant when $P<.05$.

RESULTS

The mean number of sessions required to reach stability were 22.3 ± 0.80 and 22.45 ± 0.66 for the constant and variety conditions, respectively. Figure 1 presents the number of alcohol-reinforced lever presses (Figure 1A) and the estimated doses (grams/kilogram) of self-administered alcohol (Figure 1B) during each session half and for the entire session. Each bar is the mean of all rats. A two-way (condition × session half) repeated measures ANOVA was applied to alcohol-reinforced lever pressing. The main effect of condition was significant, $F(1,9)=8.90$, $P<.015$, indicating that more lever presses were emitted in the variety condition than in the constant condition. The main effect of session half was significant, $F(1,9)=9.38$, $P<.013$, indicating that more lever presses were emitted during the first half, than during the second half, of the session. The condition × session half interaction, however, was nonsignificant, $F(1,9)=0.02$, $P>.906$, indicating that the within-session pattern of lever pressing did not differ between the constant and variety conditions. Dependent-samples t tests showed that more lever pressing occurred during the first half of the session in the variety condition (27.46 ± 6.38) than in the

constant condition (22.71 ± 5.66), $t(9)=-3.02$, $P<.007$. The number of lever presses emitted in the constant (5.75 ± 1.29) and variety (10.13 ± 3.04) conditions of the second half of the session approached, but did not reach, statistical significance, $t(9)=-1.67$, $P<.064$. A two-way (condition × session half) repeated measures ANOVA was applied to the dose of self-administered alcohol. The main effect of condition was nonsignificant, $F(1,9)=0.29$, $P>.603$, indicating that the amount of alcohol consumed did not differ between the constant and variety conditions. The main effect of session half, however, was significant, $F(1,9)=24.86$, $P<.001$, indicating that more alcohol was consumed during the first half, than during the second half, of the session. Additionally, the condition × session half interaction was nonsignificant, $F(1,9)=1.46$, $P>.257$, indicating that the within-session pattern of alcohol consumption did not differ between the constant and variety conditions.

Discussion

Our study showed that operant responding for alcohol decreased within experimental sessions even with the programmed conditions of reinforcement were held constant. Previous experiments using alcohol reinforcers observed similar late-session decreases in responding in outbred[6] and alcohol-preferring rats.[7] Additionally, our study showed that operant responding was higher when the flavor of the alcohol reinforcer changed halfway through the session. These results are consistent with past studies that have investigated the role of variety in alcohol motivation. For example, Murphy and colleagues showed alcohol motivation was enhanced when the availability of alcohol was varied from one delivery to the next. These results suggest that self-administering the same flavor throughout a binge-drinking session will decrease motivation to consume alcohol and, therefore, decrease the health risks, such as alcohol dependence and visceral obesity, associated with heavy alcohol use.

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