

Effects of Nicotine on Responding Maintained by Ethanol in Rats

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Stimuli associated with drugs play an important role in drug consumption. Tobacco and alcohol are two commonly abused drugs that are often used concurrently. The purpose of this experiment was to investigate the effects nicotine might have on the behavior of rats responding for alcohol and alcohol-associated conditioned reinforcers and rats responding for sucrose and sucrose-associated conditioned reinforcers. Nicotine produced higher rates of responding compared to vehicle administration for both sucrose- and alcohol-associated conditioned reinforcers, but not for sucrose or alcohol themselves. In addition, responding for alcohol-associated conditioned reinforcers was higher than responding for sucrose-associated conditioned reinforcers during both nicotine and vehicle administration, although responding for alcohol was higher than responding for sucrose only during vehicle administration and not during nicotine administration. The results extend the finding that nicotine enhances the value of food-associated conditioned reinforcers to alcohol-associated conditioned reinforcers as well. In addition, alcohol-associated conditioned reinforcers may play a greater role in control over behavior than conditioned reinforcers associated with other primary reinforcers in a way that cannot be explained by primary reinforcer efficacy.

INTRODUCTION

Tobacco and alcohol are two widely-used drugs in the United States. The social costs of alcohol and tobacco abuse include personal costs, in the form of disease and mortality, as well as significant economic costs (Harwood, Fountain, & Fountain, 1999; Warner, Hodgson, & Carroll, 1999). The importance of the interactions between tobacco and alcohol use in individuals has been noted, with alcoholism being 10 times more common in smokers than nonsmokers (DiFranza & Guerrera, 1990; Sharpe & Samson, 2002). The focus of the present experiment is on these interactions.

Stimuli associated with drugs play an important role in drug-taking and addiction. For example, smoking a cigarette delivers not only the pharmacological effects of nicotine, but also the taste, smell, and sight of cigarette smoke. Specifically, drug-associated stimuli have been shown to act as conditioned reinforcers; a neutral stimulus, after being paired with a primary reinforcer, becomes a conditioned reinforcer that maintains responding even in the absence of a primary reinforcer (Shahan, 2002; Skinner, 1965). In particular, rats will respond for stimuli that were previously associated with alcohol (Shahan & Jimenez-Gomez, 2006). There is evidence to suggest that one of the principal functions of nicotine, in addition to being a primary reinforcer itself, is to enhance the reinforcing properties of other stimuli (Caggiula et al., 2002). Previous work has shown that nicotine increases responding maintained by food-associated conditioned reinforcers (Raiff & Dallery, 2006). It is possible that such

enhancement will be even greater for drug-associated stimuli.

The so-called observing response procedure has been used recently to study drug-related conditioned reinforcers (Shahan, 2002; Wyckoff, 1952). This procedure is a novel, rigorous, and data-rich approach to study the effect of nicotine on responding for alcohol-associated stimuli. In the observing response procedure, a specific stimulus, known as the S+, is paired with periods when a primary reinforcer (e.g., alcohol) is available, while a second stimulus, known as the S-, is paired with periods of extinction, when no reinforcer is available. Rats are housed in an operant chamber with two levers. Responses on one lever produce unsignaled reinforcement or extinction, i.e. a mixed schedule. Responses on the other lever (observing responses) briefly produce the stimulus associated with reinforcement (S+) or the stimulus associated with extinction (S-), depending on which schedule is in effect. The S+ is typically considered a conditioned reinforcer, since observing is primarily maintained by the S+ alone (Dinsmoor, 1983).

The observing response procedure allows researchers to analyze three relevant responses: observing responses, responses for the primary reinforcer, and responses during extinction. The focus of this experiment was to investigate the effect of nicotine on responding for alcohol as well as alcohol-associated conditioned reinforcers. Based on previous research, we made the following predictions: (a) pretreatment with nicotine would increase responding by conditioned reinforcers, whether the stimuli were paired with sucrose or sucrose plus alcohol; (b) these increases

would be greater for alcohol cues than for sucrose cues; (c) these increases would not be due to general increases in activity; that is, we did not expect to see increases in responding during periods of extinction; and (d) nicotine would not increase responding maintained by sucrose, but it might increase responding maintained by alcohol. This last prediction was more tentative, since studies on the effect of nicotine on alcohol administration have produced mixed results (Sharpe & Samson, 2002).

METHOD

Subjects

Twelve experimentally naïve male rats, subjects 311–322, were used in the experiment (subjects 311, 313, 314, 318, 320, and 322 assigned alcohol as the primary reinforcer, and subjects 312, 315, 316, 317, 319, and 321 assigned sucrose as the primary reinforcer), but one rat from the sucrose group (subject 321) was euthanized owing to a cancerous growth it developed. Therefore, the results presented are only for the remaining eleven rats. The rats were housed in individual cages on a 12-hour light cycle. The rats were maintained at 90% of their free-feeding body weights, fed with standard pelleted rat chow, and received free access to water except for four hours prior to each experimental session.

Apparatus and Materials

Experimental sessions were conducted in four MED Associates (East Fairfield, VT) extra tall operant chambers (Model ENV 007; 30.48 cm long X 24.13 cm wide X 29.21 cm high) contained in separate sound-attenuating cubicles. Back walls, ceilings, and doors of operant chambers were clear polycarbonate, and intelligence panels, sidewalls, grid floors, and drop pans were stainless steel. Intelligence panels were equipped with 2 response levers, which were separated by a food receptacle (5 cm x 5 cm x 3 cm) in which a solenoid operated dipper delivered liquid solutions. The dipper allowed 3 s of access to 0.1 mL of liquid. Three LEDs (red, yellow, and green; 0.8 cm in diameter) were positioned 7 cm above each lever and 0.7 cm apart. The chamber was also equipped with a houselight (28 V) 1.5 cm from the ceiling. MED-PC software and hardware (MED Associates) were used to program experimental sessions and collect and analyze data.

A 5% sucrose solution was used as the primary reinforcer for all rats within the sucrose group except subject 317, which received a 10% sucrose solution in order to maintain sufficient rates of responding. A 95% stock solution of ethanol was used to make a 10% ethanol/5% sucrose solution for all rats in the alcohol group. During nicotine administration, nicotine (Sigma, St. Louis, MO) dissolved in potassium phosphate was used.

Procedure

Experimental sessions were conducted daily at approximately the same time. During lever training, a lever press on either lever would result in 3 s access to the dipper filled with 5% sucrose solution, accompanied by an audible click. In addition, the three LEDs above each lever, which were normally illuminated, were extinguished while the dipper remained accessible.

Discrimination Training

After all rats were reliably pressing levers for sucrose solution, discrimination training began. Each session lasted 30 min. In the discrimination training sessions, only the right lever was active. A multiple variable interval (VI) 30 s-extinction schedule was in effect. During the VI component, a dipper delivery was available, on average, every 30 s. Rats in the sucrose group continued to receive 5% sucrose as the reinforcer, while rats in the alcohol group started with 5% sucrose and began to receive a 2% ethanol/5% sucrose solution on the fourteenth session. The concentration of ethanol was increased from 2% over several sessions in this order: 2%, 4%, 6%, and finally 10% by the seventeenth session. During extinction, lever presses never resulted in a dipper delivery. Stimuli corresponding to both components were present at all times during discrimination training: during the VI component, the houselight was continuously illuminated (S+), while during extinction, the houselight rapidly blinked (S-). The two components alternated during each session, with each component lasting an average of 60 s. In order to prevent responding during the presence of the S-, a DRO (differential reinforcement of other behavior) was implemented; in order for the extinction component to switch to the VI component, 5 s without a lever press was required. Each rat had 55 discrimination training sessions before beginning observing response sessions.

Observing Response

During the observing response sessions, the right lever functioned as it did during the discrimination training sessions. The left lever functioned as the observing response lever; that is, presses on the left lever produced 10 s of either the S+ or S-, depending on which component was in effect. The S+ and S- were not available unless the responses were made on the left lever, unlike the discrimination training sessions. For the first seven sessions, the stimulus presentations were available on a fixed ratio (FR) 1 schedule, in which every response resulted in a stimulus presentation; afterwards, they were available on a VI 15 s schedule. The DRO was removed for the observing response sessions. For the first 18 observing response sessions, no nicotine or vehicle was administered, and the last six sessions during this phase comprise the baseline data for the experiment.

Drug Administration

Following the baseline phase, drug administration began, with subcutaneous injections given immediately prior to experimental sessions. Doses were 0.3 mg/kg for both nicotine and vehicle. Six subjects received nicotine injections for their first six sessions and vehicle injections for their next six sessions, while five received vehicle injections for the first six sessions and nicotine for the next six; this order was counterbalanced across both sucrose and alcohol groups. Two subjects, 316 and 320, received pre-session nicotine on only five days instead of six.

RESULTS

Data Analysis

Data from drug administration sessions were used for analysis. The three types of responses analyzed were observing responses, primary reinforcer-maintained responses, and extinction responses. In addition, response rates for vehicle administration and nicotine administration were compared. This resulted in six different categories of responses (observing responses with vehicle, observing responses with nicotine, primary reinforcer-maintained responses with vehicle, etc.). Mean response rates for sucrose and alcohol groups were calculated for each

category of response. Mean response rates for vehicle sessions and nicotine sessions for each category of response were also calculated. Independent *t*-tests were used to compare mean response rates for each category of response between alcohol and sucrose groups. Paired *t*-tests were used to compare the mean response rates of vehicle sessions and nicotine sessions for each category of response. Alpha was also set at 0.05 for all statistical tests.

Observing Responses

Observing response rates were calculated using responses that occurred only when neither stimulus was present, i.e. during the mixed schedule. Figure 1 shows mean response rates for alcohol and sucrose groups for vehicle and nicotine administration. Observing response rates were significantly higher for the alcohol group compared to the sucrose group during vehicle administration, $t(62) = 2.84$, $p = 0.003$. In addition, these rates were significantly higher for the alcohol group during nicotine administration, $t(62) = 3.35$, $p = 0.001$. Nicotine administration resulted in a statistically significant increase compared to vehicle in observing response rate for both sucrose, $t(28) = 3.31$, $p = 0.001$, and alcohol groups, $t(34) = 4.56$, $p = 0.000$.

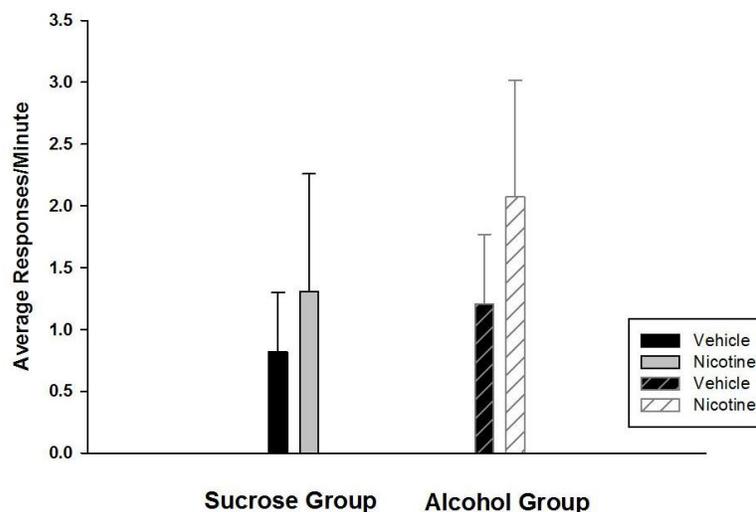


Figure 1: Average response rates for both groups, during both vehicle and nicotine administration, are shown. Error bars represent standard deviation.

Primary Reinforcer-Maintained Responses

Only responses that occurred in the presence of the S+ were used in these calculations. Figure 2 shows mean response rates for the primary reinforcer for both sucrose and alcohol groups during both vehicle and nicotine

administration. Response rates during vehicle administration for the alcohol group were significantly higher than for the sucrose group, $t(62) = 2.39$, $p = 0.010$. The response rates for the alcohol group were not significantly higher than for the sucrose group during

nicotine administration, $t(62) = 1.33$, $p = 0.095$, however. Nicotine administration did not increase responding for either sucrose or alcohol; in fact, there was a small but statistically significant decrease in responding for alcohol during nicotine administration, $t(34) = 2.15$, $p = 0.020$.

Extinction Responses

Only responses that occurred in the presence of the S- were used for these calculations. Figure 3 shows mean

response rates during extinction for both groups, with vehicle and nicotine administration. During vehicle administration, response rates during extinction were significantly higher for the alcohol group than for the sucrose group, $t(62) = 2.50$, $p = 0.007$. They were not significantly different during nicotine administration. Nicotine administration did not significantly increase response rates during extinction for either the sucrose group or the alcohol group.

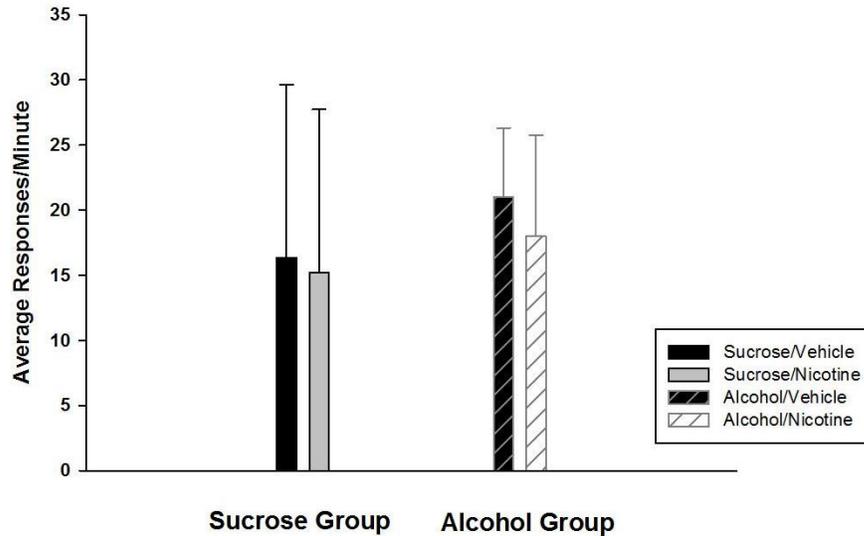


Figure 2. Average response rates for the primary reinforcer are shown. Error bars represent standard deviation.

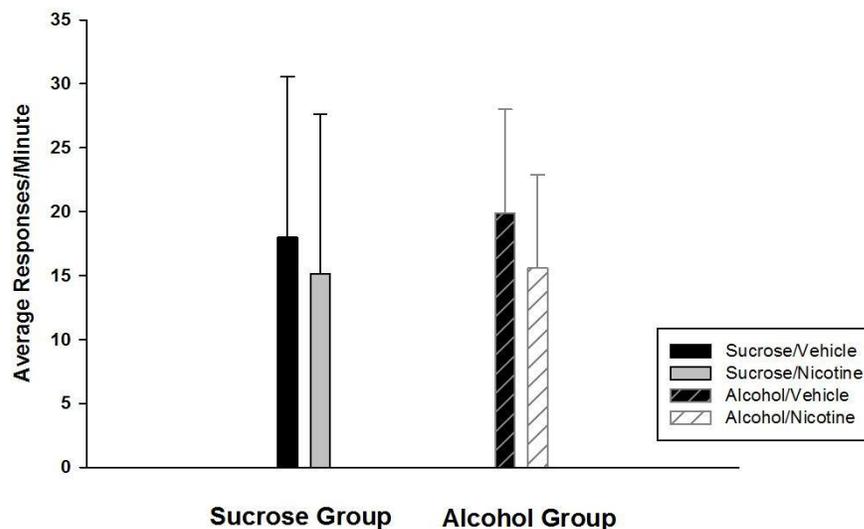


Figure 3. Average response rates during extinction are shown. Error bars represent standard deviation.

DISCUSSION

Both sucrose and alcohol groups showed statistically significant increases in observing response rates with nicotine administration, as predicted. This extends previous findings that nicotine increases responding maintained by sucrose pellet-associated conditioned reinforcers to conditioned reinforcers associated with liquid sucrose solution and alcohol (Raiff & Dallery, 2006). In addition, observing response rates were significantly higher for the alcohol group during both vehicle and nicotine administration, suggesting that alcohol-associated stimuli may play an important role in alcohol consumption. Responding for alcohol itself was significantly higher than for sucrose during vehicle administration, but not during nicotine administration. Therefore, the higher observing response rates during nicotine administration cannot be explained by higher primary reinforcer efficacy.

These findings support the idea that nicotine enhances the value of conditioned reinforcers. Thus, the results of this experiment support the idea that nicotine serves to enhance the reinforcing properties of other stimuli (Caggiula et al., 2002). However, in this experiment, nicotine did not appear to enhance the reinforcing properties of either primary reinforcer. In addition, it did not increase responding during extinction for either group. Therefore, observed increases in observing response rate with nicotine do not appear to be due to general behavioral activation.

Previous work in our laboratory has produced inconclusive findings on the effect of nicotine on responding for primary reinforcers. Raiff and Dallery (2006) found that only chronic administration of nicotine produced increases in responding for sucrose pellets, while acute administration did not. Interestingly, nicotine administration slightly decreased responding maintained by alcohol. Previous studies have produced mixed findings on this topic. Sharpe and Samson also found that nicotine decreased responding for alcohol (2002), while others found that nicotine administration increased responding for alcohol (Blomqvist, Ericson, Johnson, Engel, & Soderpalm, 1996). The strain of rat used may be a factor; we used Long-Evans rats in the present experiment, as did Sharpe and Samson, but Blomqvist et al. used Wistar rats. It should be noted that the lack of an increase in responding for either primary reinforcer during nicotine administration in this experiment might be due to nicotine's anorectic

effect (Bellinger, Cepeda-Benito, & Wellman, 2003). Studying the interactions between nicotine and other non-caloric drugs might shed more light on this issue.

The results of this study have implications for individuals who consume both alcohol and tobacco products. By increasing the value of alcohol cues, nicotine may play a role in relapse in alcoholics, for example. More research on the interaction of nicotine and other drugs, perhaps besides alcohol, should be conducted in order to further our understanding on the concurrent use of multiple drugs and its effects on behavior. In this experiment, only one dose of nicotine and one concentration of alcohol were used; using multiple dosages and concentrations might produce more information on nicotine-alcohol interactions. For example, different concentrations of alcohol might affect results by changing the overall consumption of alcohol by rats.

Evidence for the interaction between nicotine and alcohol exists at the neurobiological level. The nicotinic acetylcholine receptor (nAChR), a ligand-gated ionotropic receptor, is the focus of much research. The nAChR is opened by the neurotransmitter acetylcholine as well as by nicotine. This receptor is found on the mesolimbic dopamine neurons, which are involved in brain reward function and the reinforcing properties of alcohol, and alcohol may serve as a co-agonist to the receptor (Larsson & Engel, 2004). The centrally acting nAChR antagonist, mecamylamine, has been shown to decrease dopamine overflow initiated by alcohol in the nucleus accumbens as well as to decrease alcohol consumption in rats (Larsson & Engel, 2004; Steensland, Simms, Holgate, Richards, & Bartlett, 2007). In addition, varenicline, a partial agonist of the nAChR and a treatment for smoking cessation, has been suggested as a potential treatment for decreasing alcohol consumption, since it was found to selectively decrease alcohol consumption (Kamens, Anderson, & Piccioto, 2010; Steensland et al., 2007).

Although nicotine was not found to increase responding for alcohol in this experiment, the involvement of the nAChR in the neuronal pathways of both nicotine and alcohol suggests an avenue of further research in order to evaluate the interactions between the behavioral effects of both drugs. One such area to investigate is the potential role that nAChRs could play regarding the stimuli associated with nicotine and alcohol. For example, further research might investigate the effect of mecamylamine or varenicline on rats' responding for alcohol-associated cues.

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