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Ethanol-Paired Stimuli Can Increase Reinforced Ethanol Responding

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Abstract

While ethanol-paired stimuli are frequently postulated to increase drinking motivation and thus increase ethanol responding and precipitate relapse, no study has demonstrated increases in ethanol-reinforced responding following presentation of an ethanol-paired stimulus that had not previously been part of a contingent relationship. Previous studies have shown that food-paired stimuli can increase food responding that is at low rates and increase food consumption in food-sated rats. In Experiment 1, we show that an ethanol-paired stimulus can increase ethanol responding that is at low levels late in the experimental session, presumably due to satiation. However, these increases may have resulted from either associative or non-associative mechanisms. In Experiment 2, we compared the effects of an ethanol-paired stimulus to those of the same stimulus in a Truly-Random-Control group. In a Truly-Random-Control, the stimulus and ethanol each are presented on independent random schedules, and thus any differences between the effects of the stimulus in the experimental and control groups is likely attributable to the association between the stimulus and ethanol. The stimulus increased ethanol-reinforced responding in both the experimental and control groups, but these increases were greater in the experimental than the control group. Thus, both stimulus-change and the pairing of the stimulus with ethanol may result in increases in ethanol-reinforced responding.

Keywords

alcoholism; alcohol; ethanol self-administration; operant behavior; Pavlovian conditioning; craving; relapse; Lewis rat; Long-Evans Hooded rat

Introduction

Alcohol- or drug-paired Conditioned Stimuli (CSs) are widely thought to be key culprits in precipitating relapse (Perry, Zbukvic, Kim, & Lawrence, 2014; Wikler, 1948). These CSs can maintain responding and elicit approach (Cunningham & Patel, 2007; Davis & Smith, 1976; Leri et al., 2009; Peters & De Vries, 2014; Srey, Maddux, & Chaudhri, 2015; Uslander,

Acerbo, Jones, & Robinson, 2006; but see Kearns & Weiss, 2004), and thus, facilitate approach toward locations where seeking and consumption have previously occurred (see Tomie, 1995). Situating individuals in locations where seeking and consumption of alcohol and drugs had previously occurred likely sets the occasion for (serves a discriminative function for) further alcohol- and drug-seeking, and consumption.

Stimuli paired with alcohol or drug delivery can also be used in second-order schedules in which completion of one schedule results in stimulus presentation and serves as a unit of responding that is reinforced under another schedule. When such stimuli are presented contingently under second-order schedules, responding is increased over when these stimuli are not presented (Goldberg, Schindler, & Lamb, 1990; Katz, 2016; Schindler, Panlilio, & Goldberg, 2002). Further, again delivering these stimuli, contingent upon meeting the schedule requirements, reinvigorates responding that has decreased to low levels due to extinction (Kelleher & Goldberg, 1977). Non-contingent delivery of drug-paired stimuli can also facilitate responding that has decreased to low levels due to extinction (de Wit & Stewart, 1981). The informational role of paired stimuli appears to be at least partially (if not wholly) responsible for such effects (see discussion in Williams, 1994, and for a more general discussion of information and conditioned reinforcement, see Shahan & Cunningham, 2015).

Thus, alcohol- or drug-paired stimuli may increase alcohol- or drug-seeking, or consumption by providing information about alcohol or drug availability, and also by causing the individual to approach a location where alcohol or drugs are more easily obtained. Additionally, alcohol- or drug-paired CSs are thought to increase motivation for alcohol or drugs, and, thus, increase alcohol or drug seeking and consumption (Koob, Stinus, Le Moal, & Bloom, 1989; Stewart, de Wit, & Eikelboom, 1984; Wikler, 1948). Evidence for and against such a role has been obtained using Pavlovian-Instrumental-Transfer (PIT) procedures in which the instrumental relationship between the response and ethanol or drug delivery is trained separately from the relationship between stimulus presentation and ethanol or drug delivery (see Lamb, Schindler, & Pinkston, 2016 for a review). Subsequently, when ethanol or a drug is no longer delivered, the effects of the ethanol- or drug-paired stimuli upon responding are observed. Increases are taken as evidence of increased motivation.

Increases in responding can frequently be observed when responding is in extinction (Corbit & Janak, 2007, 2016; Glasner, Overmier, & Balleine, 2005; Krank, 2003; Krank, O'Neill, Squarey, & Jacob, 2008; Lamb, Ginsburg, & Schindler, 2016, experiment 3; LeBlanc, Ostlund, & Maidment, 2012; Milton et al., 2012; but see Lamb, Schindler, et al., 2016, experiment 1; Kruzich, Congleton, & See, 2001; LeBlanc et al., 2012). However, these increases may be attributable to the CS signaling the potential availability of drug or ethanol, i.e., the informational value of the CS. Two studies have examined CS effects on alcohol- or cocaine-reinforced responding in which the informational value of the CS in changing the rate of responding is presumably less, and thus changes in responding might be more likely to reflect motivational effects of the CS. Both of these studies failed to find increases in responding following CS presentations (Krank, 2003; LeBlanc et al., 2012). However, this might be due to responding already being at high levels when the CS effects were tested,

precluding (or at least making difficult to observe) any further increases. Consistent with this, studies using a food-paired CS have shown that the food-paired CS can increase low rates (but not necessarily high rates) of food-reinforced behavior (e.g., Lovibond, 1983). Additionally, studies using food-paired CSs also have shown that these CSs can increase eating in food-sated rats (Holland, Petrovich, & Gallagher, 2002; Weingarten, 1983), suggesting that CS-elicited increases in ethanol responding might be observable late in experimental sessions when responding has decreased, presumably due to satiation.

In Experiment 1 of this paper, we report the effects of an ethanol-paired CS on ethanol-reinforced responding occurring late in a session in rats that had previously participated in an experiment comparing whether PIT differed between Lewis and Long-Evans Hooded rats, when responding was in extinction. This earlier experiment reported by Lamb and co-workers (Lamb, Ginsburg, Greig, & Schindler, 2019) was conducted to examine whether differences in the likelihood of detecting PIT in previous experiments was the result of the strain used. Experiment 1 showed that this stimulus could increase ethanol-reinforced responding and did so similarly in Hooded and Lewis rats. Because the purpose of the original experiment was to compare the effects of the stimulus in Lewis and Hooded rats, the appropriate control groups to show whether the effects observed were the result of pairing with ethanol were not included. Thus, in Experiment 2, we compared the effects of an ethanol-paired stimulus in an experimental group to the effects of the same stimulus in a Truly-Random-Control (TRC) group in Lewis rats. In a TRC, the stimulus and the US with which it is paired in the experimental group are each presented on independent random schedules. Thus, the stimulus predicts neither the presentation of the US nor the absence of the presentation of the US in the TRC group. In this way, the TRC controls for all the possible non-associative factors that might result in the stimulus producing changes in responding, and avoids the stimulus acquiring potential inhibitory properties as might be seen using an explicitly unpaired control group. Thus, the TRC is the appropriate control for assessing CS effects upon responding (Rescorla, 1967).

Methods

Experiment 1

All procedures conducted on the rats were approved by the local Institutional Animal Care and Use Committee and were in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals (2011). Twelve Hooded rats and 15 Lewis rats that participated in Experiment 2 of Lamb et al., 2019 were used in this experiment. Rats had previously been induced to drink ethanol using post-prandial drinking and taught to respond for ethanol under an RI 30-second schedule of ethanol presentation. Rats had also previously had 2-minute presentations of the light above the ethanol-response lever paired with ethanol delivery under an RT schedule of ethanol delivery, with the ethanol-response lever removed from the experimental chamber. After these pairing sessions, rats again responded for ethanol under the RI schedule for a single session, and on the following day, the rats underwent a test session in which the responding was in extinction and the light was occasionally presented. These results were reported in Lamb et al. (2019).

Subsequently, rats responded again for ethanol with responses resulting in 3-second 0.1-mL dipper presentations of 8% (w/v) ethanol in filtered water under an RI 30-second schedule for 1 hour, during which an 8-kHz tone sounded (80 dB). This continued for eight sessions, the last five of which are reported here. This was followed by five sessions in which the light above the ethanol-response lever that had been paired with ethanol delivery was lit during the period of 35–37 and 53–55 minutes into the experimental session. Subsequent to these five sessions, the light no longer was lit during the session.

Responding during minutes 35–37 and 53–55 (the target periods) and during minutes 33–35 and 51–53 (the pre-target periods) as well as the differences between these are reported.

We analyzed these data and total session data using mixed effects regressions (*xtmixed* command in STATA version 14) with rat strain (Lewis coded as 0 and Hooded as 1), and phase (0, 1, & 2) used as indicator variables, and sequential sessions within a phase coded as a continuous variable (1, 2, 3, 4, and 5). Rat strain was treated as a between-rat variable, while phase and session were treated as within-rat variables.

Experiment 2

Subjects—All procedures conducted on the rats were approved by the local Institutional Animal Care and Use Committee and were in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals (2011). Thirty-two male Lewis rats weighing between 260 and 285 grams were purchased from Envigo. Only Lewis rats were used in Experiment 2, as the results of Lamb et al., 2019 showed that the magnitude of PIT during extinction did not depend upon whether the experiment was conducted in Lewis or Hooded rats, and the results of Experiment 1 showed that this was also the case when responding was reinforced. Rats were individually housed, and for approximately 2 weeks were allowed unrestricted access to food and water. After this, food was restricted to 12–15 g per day, but water was freely available except as noted below.

Rats were induced to drink ethanol solutions using post-prandial drinking as described below. Because one set of eight experimental chambers was used for these experiments, rats were trained to respond for ethanol in four shifts of eight rats as described below. Twenty-four rats made 15 or more responses in at least five sequential sessions and were randomized to either the experimental or control groups. Randomization occurred according to the following rules: (1) as rats were run in shifts, randomization was stratified by shift; (2) randomization was also stratified within shifts by the amount of ethanol earned in the week before randomization; and then (3) within each strata, rats were assigned to one condition or the other by a coin flip until half were assigned to one group (if an odd number, then half minus 0.5 were assigned to one group and half plus 0.5 to the other), after which the remaining rat(s) were assigned to the other condition.

Apparatus—Experiment 2 occurred in the same eight commercially available operant chambers as Experiment 1 (ENV-008, Med-Associates, Georgia, Vermont, United States), with two levers arranged on either side of one wall with a light above each and a houselight above the chamber on the opposite wall. A trough was positioned between the levers where rats could access food pellets (45 mg chow flavored, BioServ, Frenchtown, New Jersey,

United States) and solutions (via a 0.1-mL dipper mechanism) when they were delivered. Chambers were also equipped with a speaker connected to a tone generator (ANL-926, Med-Associates, Georgia, Vermont). Stimuli presentation, reinforcement delivery, and response recording were controlled using a program written and executed with commercially available software (Med-PC IV, Med-Associates, Georgia, Vermont). Operant chambers were housed in ventilated, light- and sound-attenuating cubicles (ENV-022V, Med-Associates, Georgia, Vermont). Pink noise was generated in the room housing operant equipment to further isolate tones within the operant chamber.

Induction of ethanol drinking—As in Experiment 1, at mid-day, rats were fed their daily food ration. Two hours prior, drinking water was removed and 1 hour before feeding, another bottle was placed in the cage and remained available for 2 hours (i.e., until 1 hour after food was presented). For the first 3 days, this bottle contained no ethanol; after this, the ethanol concentration was 2% for 5 days. Subsequently, every third day after this, the ethanol concentration was increased by increments of 2%, i.e., 4%, then 6%, and then 8% (w/v), after which the concentration was raised to 12% for 3 days. The concentration was then returned to 8% for 9 days.

Ethanol self-administration

Self-administration sessions lasted 1 hour. During these, an 8-kHZ, 80-dB tone sounded. For the initial session, 3-second presentations of a 0.1-mL dipper filled with 8% (w/v) ethanol occurred under a Random Time (RT) 10-second schedule. Subsequently, ethanol was delivered for each lever press (3 sessions), then under a Random Interval (RI) 5-second schedule (4 sessions), then a RI 10-second (3 sessions), and finally under a RI 30-second schedule (14 sessions). Then the rats began either Pavlovian Conditioning or the Truly Random Control condition.

Pavlovian Conditioning and Truly Random Control

Ten sessions were conducted during this phase. During these sessions, the response levers were removed from the experimental chambers. Pavlovian Conditioning sessions were 60 minutes in duration. Every third minute, there was a 50% probability of turning on the cue light located over the position where the ethanol lever had been for 2 minutes. While this light was lit, 4-second presentations of ethanol occurred under an RT 25-second schedule (with the RT schedule not operating during the dipper presentation and the probability query window set to 1 second). During the Truly Random Control condition, light presentations occurred under the same schedule, but ethanol deliveries occurred under a RT 80-second schedule (with the RT schedule operating during dipper presentations, but the query window set to 5 seconds). This resulted in the same pattern of light presentations in both conditions, but in the Pavlovian Conditioning condition all ethanol presentations occurred during the 2-minute light presentations, while in the Truly Random Control condition a similar number of ethanol presentations were delivered during the session, but these deliveries were distributed throughout the experimental session without regard to whether the light was on or not.

Test sessions

Following Pavlovian Conditioning or exposure to the Truly Random Control condition, rats responded again for ethanol with responses resulting in 3-second 0.1-mL dipper presentations of 8% (w/v) ethanol in reverse-osmosis filtered water under an RI 30-second schedule for 1 hour, during which an 8-kHz tone sounded (80 dB). This continued for 5 sessions and was followed by 5 sessions in which the light above the ethanol-response lever that had been paired with ethanol delivery was lit during the period 35–37 and 53–55 minutes into the experimental session. Subsequent to these 5 sessions in which the light was lit, the light no longer was lit during the session.

Data were analyzed similarly to the data in Experiment 1, except that instead of strain as a factor, experimental group was included as a factor.

Results

Experiment 1

Previously reported training results—As we have previously reported (Lamb et al., 2019), post-prandial drinking induced substantial drinking in both strains. On the last day of drinking induction, Hooded rats drank an average of 2.3 g/kg ethanol as compared to 1.3 g/kg in the Lewis rats, and this difference was statistically significant [$t(25) = 7.2$, $p < 0.0001$]. Pavlovian Conditioning was effective and similar in both strains with change scores (rate of magazine entries during the CS minus the rate of magazine entries during its absence) significantly greater than zero during all 10 training sessions in both strains. Repeated-measures ANOVA on rates during the CS compared to rates in its absence across sessions indicated a significant effect for CS [$F(1,475) = 727.1$, $p < 0.0001$], and for strain indicating the greater propensity of the Hooded rats to make magazine entries [$F(1,473) = 9.9$, $p = 0.004$], but neither a strain by CS interaction nor a 3-way interaction among strain, session, and CS was significant, indicating that magazine entries were increased during CS presentations similarly in both strains across sessions.

Test results

We conducted an ABA design experiment. In the first A phase, the light was not presented in either the two 2-minute target periods nor in the two 2-minute pre-target periods preceding the target periods. During the B phase, the light was presented during the target periods, and finally during the second A phase, the light was again not presented. First, we analyzed responding that occurred in pre-target periods across phases (Fig. 1A). There was no evidence that responding in the pre-target differed between phases (compared to the first A phase: B phase: $z = -0.5$, $p = 0.60$; second A phase: $z = 0.1$, $p = 0.91$), changed across sessions within a phase (sessions: $z = 0.9$, $p = 0.39$; B phase by session: $z = 0.6$, $p = 0.58$; Second A phase by session: $z = -0.5$, $p = 0.64$), or that the effects of phase or sessions within a phase depended upon rat strain (B phase by Strain: $z = -1.1$, $p = 0.29$; Second A phase by Strain: $z = -0.3$, $p = 0.74$; Strain by sessions: $z = -1.5$, $p = 0.13$; B phase by Strain by sessions: $z = 1.4$, $p = 0.16$; Second A phase by Strain by sessions: $z = 1.0$, $p = 0.32$). However, Hooded rats tended to make about 2.8 more responses than Lewis rats during the pre-target periods ($z = 2.1$, $p = 0.03$). As responding in the pre-target period was relatively

constant, but differed by a fixed amount between strains, we examined the potential effects of light presentations using change scores (responding in the target period minus responding in the pre-target period).

We analyzed target period change scores (Fig. 1B) similarly to how we had analyzed responding in the pre-target period using mixed effects regression. This analysis strongly supported the notion that target period change scores increased by about 8.2 responses in the phase the light was presented ($z = 5.6$, $p < 0.001$) and provided evidence that this effect decreased by about 0.9 responses per session ($z = -2.0$, $p = 0.04$). In the following phase, when the light was no longer presented, change scores returned to their original level ($z = 0.2$, $p = 0.84$), which was not reliably different from zero (1.1, 95%CL: -1.0 to 3.1), and change scores did not systematically increase or decrease across sessions in phases when the light was not presented ($z = -1.0$, $p = 0.31$; and $z = -0.1$, $p = 0.93$). This analysis provided no indication that rat strain modified any of these effects (all relevant $z < |1.03|$ and all $p > 0.30$).

In addition, we also examined whether CS-elicited increases in responding were accompanied by increases in the number of dipper presentations earned in a manner similar to the way we examined changes in responding. Surprisingly, the number of dipper presentations earned in the pre-target period increased by about 1.7 presentations during the B phase that the CS was presented, compared to the preceding A phase ($z = 3.0$, $p = 0.003$), an effect that tended to be greater in the Lewis than in the Hooded rats ($z = 1.8$, $p = 0.07$). As the number of dipper presentations earned in the target period did not differ between the first A phase and the B phase when the CS was presented ($z = 0.1$, $p = 0.90$), the difference between these values became more negative by the amount of the increase observed in pre-target responding when the first A phase and the B phase are compared ($z = 2.5$, $p = 0.01$).

In summary, presentations of the ethanol-paired CS increased ethanol-reinforced responding late in experimental sessions when responding is normally at low rates. While these effects declined over time, these effects were still reliably observed on the fifth and last session (t test change score = 0, $t(26) = 3.8$, $p = 0.0008$). In addition, while the Hooded rats responded more than the Lewis rats, the effects of CS presentations were similar in Hooded and Lewis rats.

We also examined whether exposure to the ethanol-paired light changed the number of responses made (Fig. 1C) or dipper presentations (Fig. 1D) earned across the entire session. We used a similar mixed effects regression strategy to analyze these data. Several observations can be made about these data. The most consistent one is that Hooded rats responded more and earned more dipper presentations than Lewis rats (31.9 more responses, $z = 2.1$, $p = 0.04$; and 13.3 more dipper presentations, $z = 2.6$, $p = 0.01$). This resulted in Lewis rats earning an average of 0.80 (SD = 0.29) g/kg of ethanol and Hooded rats earning an average of 1.14 (SD = 0.30) g/kg during each experimental session. Responding and dipper presentations tended to be greatest at the beginning and end of each 5-session phase, an effect that was more pronounced in the Hooded rats, and likely reflected the Monday to Friday routine of how the experiments were conducted. This effect was statistically significant for responding (strain by sessions $z = 2.1$, $p = 0.04$), but not for dipper

presentations ($z = 1.1$, $p = 0.28$). There also appeared to be a secular trend for this U-shaped pattern to become more like a sideways L across phases (significant Second A phase by sessions interactions and Second A phase main effects), e.g., on Monday and Friday of the first A phase, responding was 11.4 and 12.4 responses above the grand mean, while on Wednesday it was 15.7 responses below the grand mean, which contrasts with values of -4.7, -13.5, and 25.1 on Monday, Wednesday, and Friday in the second A phase; values for the B phase were intermediate between these values. Importantly, there was no evidence that CS presentations changed the overall number of responses or dipper presentations that occurred (responses: all effects related to the B Phase, $p > 0.09$; dipper presentations: all effects related to the B phase, $p > 0.30$). This analysis provides no evidence that the CS presentations substantially affected responses made or dipper presentations earned outside of the limited period the CS was lit.

Experiment 2

While Experiment 1 clearly showed that a light paired with ethanol delivery could increase late-session responding in both Lewis and Hooded rats, the experiment as designed does not provide definitive information about whether this was a result of the ethanol pairing or simply stimulus presentation. In Experiment 2, we assessed the extent to which this effect was a result of pairing the light with ethanol. We did this by comparing the effects of light presentation in two groups. In the first group, the light was paired with ethanol in the same way as in Experiment 1. In the second group, the light was presented according to the same schedule used in Experiment 1, but ethanol was presented on an independent random schedule that presented ethanol at the same overall rate of ethanol presentation as in Experiment 1, but so that light and ethanol presentations were uncorrelated. Thus, the light should acquire neither inhibitory nor excitatory properties (Rescorla, 1967).

Training results

Post-prandial drinking induced robust and similar drinking in the experimental and control groups (see Fig. 2A). No t -test comparing drinking in each session was significant (p values > 0.16). Drinking in the last session averaged 1.10 (SD: 0.47) g/kg in the control group and 1.33 (SD: 0.47) g/kg in the experimental group.

During Pavlovian Training or Control sessions, magazine entries occurred at a higher rate during the light than in its absence in the experimental group, but not in the control group (Fig. 2B). This is demonstrated statistically by a regression analysis of the rate of magazine entries in which the effect of light presentation was not significant ($z = 0.45$, $p = 0.65$), but the interaction of the effect of the light presentation with the experimental group was significant ($z = 4.72$, $p < 0.001$). There was also a trend toward a lower rate of magazine entries when the light was not lit in the experimental group compared to the control group ($z = -1.67$, $p < 0.10$). These effects are illustrated in Fig. 2B, in which the rates of magazine entries in the periods when the light was lit and when the light was not lit are plotted. These results are consistent with effective conditioning to the light in the experimental group but not the control group, but likely also reflect the timing of dipper presentations in the two groups.

Test results

In this between-group experiment utilizing an ABA design, differences between the two groups during the B phase, but not the two A phases, would support the hypothesis that an ethanol-paired CS can increase ethanol-reinforced responding through associative mechanisms. In the B phase, but not the two A phases, a light was presented during the target periods. In the experimental group, the light had been paired with ethanol presentations. In the control group, the light was presented under the same schedule as in the experimental group, but ethanol deliveries occurred randomly throughout the session at a rate equivalent to the overall rate in the experimental group. Thus, in the experimental group, light presentations predicted ethanol delivery, but in the control group, light and ethanol presentations are uncorrelated.

We first analyzed rates of responding in the pre-target period in the two groups (Fig. 3A). The only reliable between-group difference in the rate of pre-target period responding was that the experimental group responded more during the first session of the second A phase than the control group. This is reflected in a significant second A phase by session-by-group interaction ($z = -1.99, p < 0.05$), and significant session by second A phase ($z = 2.39, p = 0.02$) and second A phase by group ($z = 2.64, p < 0.01$) interactions and second A phase effects ($z = -2.65, p < 0.01$). To account for these effects, the correlation between pre-target and target responding, and to parallel the analysis of the first experiment, we used change scores (the difference between target and pre-target responding) in our subsequent analysis.

Analysis of the change scores indicates that light presentations increased responding in both groups and did so to a greater extent in the experimental group than in the control group (Fig. 3B). In particular, rats made an average of 7.2 more responses in the target period compared to the pre-target period when the light was presented, compared to the preceding A phase in the control group ($z = 3.76, p < 0.01$). On average, the experimental group made an additional 5.4 responses more than the control group ($z = 1.99, p < 0.05$). There was no indication that these effects declined over time (while there was an effect of session: $z = 2.03, p = 0.05$, there was not a session by B phase interaction: $z = -1.35, p = 0.18$, nor was there a group by B phase by session interaction: $z = -0.5, p = 0.96$).

In addition, we also examined whether CS-elicited increases in responding were accompanied by increases in the number of dipper presentations earned in a manner similar to how we examined changes in responding. The number of dipper presentations earned in the pre-target period was similar across the A and B phases, did not depend on group assignment, and did not change across sessions within a phase. Crucially, the first A phase to B phase comparison with group assignment interaction was not significant ($z = 1.4, p = 0.17$). While there was a trend for the number of dipper presentations earned during target periods to be greater in the B phase than the first A phase ($z = 1.8, p = 0.07$), this effect did not depend on group assignment ($z = 0.4, p = 0.73$). This resulted in a similar trend for the change score in the number of dipper presentations earned during the B phase to be greater than in the first A phase ($z = 1.8, p = 0.07$, which again did not depend upon group assignment: $z = 1.3, p = 0.18$).

We also examined the total number of responses made in each experimental session (Fig. 3C) and the total number of dipper presentations earned in each session (Fig. 3D). Rats responded on average 106 times in each experimental session, with this value being relatively constant across sessions, phases, and group assignment. Similarly, rats earned on average 43 dipper presentations in each experimental session, with this value being relatively constant across sessions, phases, and group assignment. This resulted in the control rats earning an average of 0.94 (SD: 0.20) g/kg of ethanol and the experimental rats earning 0.96 g/kg of ethanol (SD: 0.22).

Discussion

Ethanol-reinforced responding increased if a light that had been paired with ethanol delivery was presented late in the experimental sessions, when responding was normally at low levels. Some of this effect is the result of that pairing and some of this effect is likely the result of non-associative mechanisms. This increase in responding under these conditions was not accompanied by an increased number of earned ethanol deliveries. These findings can be compared and contrasted with those obtained in the two other available studies that examined the effects of a cocaine- or an ethanol-paired stimulus using a PIT procedure on reinforced responding, and found that the paired stimulus did not increase responding. The possible implications of these findings are also considered.

LeBlanc et al. (2012) studied the effects of a cocaine-paired stimulus on cocaine-reinforced responding and found no increases in cocaine-reinforced responding using a PIT procedure. Notably, however, LeBlanc et al. (2012) also did not find increases in previously cocaine-reinforced behavior in a parallel group that was tested in extinction, and it was only after combining the two groups and partially extinguishing the CS-US relationship that they were able to obtain PIT in extinction. They attribute the first failure to observe PIT to CS-elicited CRs interfering with the CS-elicited increases in responding. Thus, the report by LeBlanc et al. (2012) cannot be taken as strong evidence against the ability of a drug-paired CS to increase drug-maintained responding, given their failure to observe CS-induced increases in behavior under extinction conditions. Krank (2003) did observe CS-induced increases in ethanol responding that was in extinction, but failed to observe subsequent increases in reinforced ethanol responding in this group. We previously postulated that this might have resulted from some extinction of the response increasing properties of the CS from the previous trial in extinction (Lamb, Schindler, et al., 2016), but our Experiment 1 showing increases after similar exposure to extinction makes this explanation less tenable. However, one should note that in the Krank (2003) study, while change scores for the rats for whom the stimulus had been paired with ethanol delivery were not greater than zero (i.e., the rate of responding during the CS was similar to the rate of responding in the period that preceded it), change scores for the paired rats were greater than change scores for either rats not previously exposed to the stimulus or rats for whom the stimulus was explicitly unpaired. Additionally, CS presentations occurred throughout the short test session during which responding was relatively high. Therefore, increases in responding may have been difficult to observe. These factors make the Krank (2003) study, like the LeBlanc et al. (2012) study, only weak evidence against the ability of drug-paired CSs to increase drug-reinforced responding. Thus, while our experiments show that under some conditions an ethanol-paired

stimulus can increase responding for ethanol, there exist other conditions under which drug-paired stimuli less reliably increase drug-reinforced behavior.

The results of Experiment 2 indicated that while some of the increase in responding seen following presentation of the ethanol-paired stimulus was due to associative mechanisms, part of this effect is also likely due to non-associative mechanisms. Stimulus presentation may cause dishabituation and reinvigorate responding by this non-associative mechanism (McSweeney & Swindell, 1999). Most previous studies have tried to control for non-associative factors by comparing changes in responding from immediately before light presentation to during light presentation, between a group in which the light was explicitly paired with ethanol presentation and another group in which the light presentation is explicitly unpaired with ethanol presentation. The problem with this comparison is that the stimulus now predicts the absence of ethanol delivery and may become inhibitory (see Rescorla, 1967). This problem can be avoided by presenting the stimulus and ethanol on independent random schedules as was done in Experiment 2, in which case the stimulus no longer predicts either ethanol delivery or its absence (Rescorla, 1967). Such Truly Random Controls (TRC) have only rarely been used, and in the three studies in which a TRC has been used, one study found increases in ethanol responding that was in extinction while two did not (Lamb, Ginsburg, et al., 2016; Kruzich et al., 2001). Experiment 2 extends the conditions under which an ethanol-paired stimulus has been demonstrated to increase ethanol responding, and also demonstrates the importance of including the appropriate control conditions, as stimuli can have clear and large effects upon ethanol responding independent of their association with ethanol. This finding lends support to McSweeney's argument for the important role of habituation in controlling the level of responding (see McSweeney & Swindell, 1999).

Thus, the increases observed following presentation of an ethanol-paired stimulus in this study of ethanol-reinforced responding and in other studies of responding for ethanol that is in extinction may result from associations that result from pairing the stimulus with ethanol. However, it is equally likely these increases may result from non-associative mechanisms such as dishabituation. Additionally, there are other associative mechanisms beyond increases in motivation, by which an ethanol-paired stimulus might increase ethanol responding. For example, the stimulus might maintain responding and elicit an approach toward it, which, if the stimulus is located near the means for obtaining alcohol or drug, increases the probability of future use because these means set the occasion for future drug use (see Krank, 2003 for a discussion of this, also see Tomie, 1995). Alternatively, the CS may have motivational effects that non-specifically increase the frequency of a variety of behavior (Corbit & Janak, 2007; but see Lamb, Ginsburg, et al., 2016 and Lamb, Schindler, et al., 2016 for an alternative interpretation of these effects). Future studies aimed at dissecting the mechanisms by which stimuli increase the likelihood of drug or alcohol use would aid our understanding of the factors that control use. Finally, it is important to note that this increase in responding during the CS was not accompanied by an increase in responding outside of the time when the CS was presented, nor did this increase in responding result in an increase in the number of ethanol deliveries earned either during times when the CS was presented or outside of this period. When the level of ethanol seeking is well controlled by other factors (such as in these experiments), the increase in

responding by the CS is limited to the period of CS presentation, suggesting that drug-paired CS may be more likely to precipitate relapse early in recovery than later in recovery when other factors exert less effective control over ethanol seeking, and this seeking is likely to be more effective in increasing ethanol consumption (see Ginsburg & Lamb 2013a, b).

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References

- Corbit LH, & Janak PH (2007). Ethanol-associated cues produce general pavlovian-instrumental transfer. *Alcoholism: Clinical and Experimental Research*, 31, 766–774. doi:10.1111/j.1530-0277.2007.00359.x
- Corbit LH, & Janak PH (2016). Changes in the Influence of Alcohol-Paired Stimuli on Alcohol Seeking across Extended Training. *Frontiers in Psychiatry*, 7, 169. doi:10.3389/fpsy.2016.00169 [PubMed: 27777560]
- Cunningham CL, & Patel P (2007). Rapid induction of Pavlovian approach to an ethanol-paired visual cue in mice. *Psychopharmacology (Berl)*, 192, 231–241. doi:10.1007/s00213-007-0704-4 [PubMed: 17265074]
- Davis WM, & Smith SG (1976). Role of conditioned reinforcers in the initiation, maintenance and extinction of drug-seeking behavior. *The Pavlovian Journal of Biological Sciences*, 11, 222–236. doi:10.1007/bf03000316
- de Wit H, & Stewart J (1981). Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology (Berl)*, 75, 134–143. doi:10.1007/bf00432175 [PubMed: 6798603]
- Ginsburg BC, & Lamb RJ (2013a). A history of alternative reinforcement reduces stimulus generalization of ethanol-seeking in a rat recovery model. *Drug and Alcohol Dependence*, 129, 94–101. doi:10.1016/j.drugalcdep.2012.09.017 [PubMed: 23122598]
- Ginsburg BC, & Lamb RJ (2013b). Shifts in discriminative control with increasing periods of recovery in the rat. *Alcoholism: Clinical and Experimental Research*, 37, 1033–1039. doi:10.1111/acer.12048
- Glasner SV, Overmier JB, & Balleine BW (2005). The role of Pavlovian cues in alcohol seeking in dependent and nondependent rats. *Journal of Studies on Alcohol*, 66, 53–61. doi:10.15288/jsa.2005.66.53 [PubMed: 15830903]
- Goldberg SR, Schindler CW, & Lamb RJ (1990). Second-order schedules and the analysis of human drug-seeking behavior. *Drug Development Research*, 20, 217–229.
- Holland PC, Petrovich GD, & Gallagher M (2002). The effects of amygdala lesions on conditioned stimulus-potentiated eating in rats. *Physiology & Behavior*, 76, 117–129. doi:10.1016/s0031-9384(02)00688-1 [PubMed: 12175595]
- Katz JL (2016). Contributions to drug abuse research of Steven R. Goldberg's behavioral analysis of stimulus-stimulus contingencies. *Psychopharmacology (Berl)*, 233, 1921–1932. doi:10.1007/s00213-015-4149-x [PubMed: 26564234]
- Kearns DN, & Weiss SJ (2004). Sign-tracking (autoshaping) in rats: a comparison of cocaine and food as unconditioned stimuli. *Learning & Behavior*, 32, 463–476. doi:10.3758/bf03196042 [PubMed: 15825887]
- Kelleher RT, & Goldberg SR (1977). Fixed-interval responding under second-order schedules of food presentation or cocaine injection. *Journal of the Experimental Analysis of Behavior*, 28, 221–231. doi:10.1901/jeab.1977.28-221 [PubMed: 411869]
- Koob GF, Stinus L, Le Moal M, & Bloom FE (1989). Opponent process theory of motivation: neurobiological evidence from studies of opiate dependence. *Neuroscience and Biobehavioral Reviews*, 13, 135–140. doi:10.1016/s0149-7634(89)80022-3 [PubMed: 2682399]

- Krank MD (2003). Pavlovian conditioning with ethanol: sign-tracking (autoshaping), conditioned incentive, and ethanol self-administration. *Alcoholism: Clinical and Experimental Research*, 27, 1592–1598. doi:10.1097/01.Alc.0000092060.09228.De
- Krank MD, O'Neill S, Squarey K, & Jacob J (2008). Goal- and signal-directed incentive: conditioned approach, seeking, and consumption established with unsweetened alcohol in rats. *Psychopharmacology (Berl)*, 196, 397–405. doi:10.1007/s00213-007-0971-0 [PubMed: 17965977]
- Kruzich PJ, Congleton KM, & See RE (2001). Conditioned reinstatement of drug-seeking behavior with a discrete compound stimulus classically conditioned with intravenous cocaine. *Behavioral Neuroscience*, 115, 1086–1092. doi:10.1037//0735-7044.115.5.1086 [PubMed: 11584921]
- Lamb RJ, Ginsburg BC, Greig A, & Schindler CW (2019). Effects of rat strain and method of inducing ethanol drinking on Pavlovian-Instrumental-Transfer with ethanol-paired conditioned stimuli. *Alcohol*, 79, 47–57. doi:10.1016/j.alcohol.2019.01.003 [PubMed: 30641121]
- Lamb RJ, Ginsburg BC, & Schindler CW (2016). Effects of an ethanol-paired CS on responding for ethanol and food: Comparisons with a stimulus in a Truly-Random-Control group and to a food-paired CS on responding for food. *Alcohol*, 57, 15–27. doi:10.1016/j.alcohol.2016.10.009 [PubMed: 27916139]
- Lamb RJ, Schindler CW, & Pinkston JW (2016). Conditioned stimuli's role in relapse: preclinical research on Pavlovian-Instrumental-Transfer. *Psychopharmacology (Berl)*, 233, 1933–1944. doi:10.1007/s00213-016-4216-y [PubMed: 26800688]
- LeBlanc KH, Ostlund SB, & Maidment NT (2012). Pavlovian-to-instrumental transfer in cocaine seeking rats. *Behavioral Neuroscience*, 126, 681–689. doi:10.1037/a0029534 [PubMed: 22866668]
- Leri F, Zhou Y, Goddard B, Levy A, Jacklin D, & Kreek MJ (2009). Steady-state methadone blocks cocaine seeking and cocaine-induced gene expression alterations in the rat brain. *European Neuropsychopharmacology*, 19, 238–249. doi:10.1016/j.euroneuro.2008.09.004 [PubMed: 18990547]
- Lovibond PF (1983). Facilitation of instrumental behavior by a Pavlovian appetitive conditioned stimulus. *Journal of Experimental Psychology*, 9, 225–247. [PubMed: 6153052]
- McSweeney FK, & Swindell S (1999). General-process theories of motivation revisited: The role of habituation. *Psychological Bulletin*, 125, 437–457.
- Milton AL, Schramm MJ, Wawrzynski JR, Gore F, Oikonomou-Mpegeti F, Wang NQ, et al. (2012). Antagonism at NMDA receptors, but not beta-adrenergic receptors, disrupts the reconsolidation of pavlovian conditioned approach and instrumental transfer for ethanol-associated conditioned stimuli. *Psychopharmacology (Berl)*, 219, 751–761. doi:10.1007/s00213-011-2399-9 [PubMed: 21766171]
- National Research Council. (2011). *Guide for the Care and Use of Laboratory Animals*, 8th edition. Washington, DC: National Academies Press.
- Perry CJ, Zbukvic I, Kim JH, & Lawrence AJ (2014). Role of cues and contexts on drug-seeking behaviour. *British Journal of Pharmacology*, 171, 4636–4672. doi:10.1111/bph.12735 [PubMed: 24749941]
- Peters J, & De Vries TJ (2014). Pavlovian conditioned approach, extinction, and spontaneous recovery to an audiovisual cue paired with an intravenous heroin infusion. *Psychopharmacology (Berl)*, 231, 447–453. doi:10.1007/s00213-013-3258-7 [PubMed: 24026484]
- Rescorla RA (1967). Pavlovian conditioning and its proper control procedures. *Psychological Review*, 74, 71–80. doi:10.1037/h0024109 [PubMed: 5341445]
- Schindler CW, Panlilio LV, & Goldberg SR (2002). Second-order schedules of drug self-administration in animals. *Psychopharmacology (Berl)*, 163, 327–344. doi:10.1007/s00213-002-1157-4 [PubMed: 12373434]
- Shahan TA, & Cunningham P (2015). Conditioned reinforcement and information theory reconsidered. *Journal of the Experimental Analysis of Behavior*, 103, 405–418. doi:10.1002/jeab.142 [PubMed: 25766452]
- Srey CS, Maddux JM, & Chaudhri N (2015). The attribution of incentive salience to Pavlovian alcohol cues: a shift from goal-tracking to sign-tracking. *Frontiers in Behavioral Neuroscience*, 9, 54. doi:10.3389/fnbeh.2015.00054 [PubMed: 25784867]

- Stewart J, de Wit H, & Eikelboom R (1984). Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. *Psychological Review*, 91, 251–268. [PubMed: 6571424]
- Tomie A (1995). Cam: An animal learning model of excessive and compulsive implement assisted drug-taking in humans. *Clinical Psychology Review*, 15, 145–167.
- Uslaner JM, Acerbo MJ, Jones SA, & Robinson TE (2006). The attribution of incentive salience to a stimulus that signals an intravenous injection of cocaine. *Behavioural Brain Research*, 169, 320–324. doi:10.1016/j.bbr.2006.02.001 [PubMed: 16527365]
- Weingarten HP (1983). Conditioned cues elicit feeding in sated rats: a role for learning in meal initiation. *Science*, 220, 431–433. doi:10.1126/science.6836286 [PubMed: 6836286]
- Wikler A (1948). Recent progress in research on the neurophysiologic basis of morphine addiction. *The American Journal of Psychiatry*, 105, 329–338. doi:10.1176/ajp.105.5.329 [PubMed: 18890902]
- Williams BA (1994). Conditioned reinforcement: Experimental and theoretical issues. *The Behavior Analyst*, 17, 261–285. doi:10.1007/bf03392675 [PubMed: 22478192]

Highlights

- Alcohol Paired Conditioned Stimuli are thought to increase alcohol seeking.
- However, this has been difficult to demonstrate when alcohol seeking actually results in alcohol delivery.
- Here we show that an alcohol-paired conditioned stimulus increases responding late in a session when that responding is at low levels and results in alcohol delivery.

Fig. 1.

This figure illustrates the effects of the ethanol-paired CS in Lewis and Hooded rats from Experiment 1. Hooded rats generally responded more than Lewis rats, but the ethanol-paired CS increased responding similarly in both strains, an effect that was limited to the period the CS was presented. Panel A shows the mean number of responses made by Lewis (circles) and Hooded (triangles) rats during the pre-target periods (minutes 33–35 and minutes 53–55) of the 1-hour experimental sessions across 15 sessions. During the middle five sessions, a light paired with ethanol delivery would be on during the target period (minutes 35–37 and minutes 55–57 of the experimental sessions), i.e., in the periods immediately following that for which mean responses are plotted in Panel A. In Panel B, the mean of the difference between responses in Target and Pre-Target periods are plotted. In panel C, the mean total number of responses in each session is plotted, and in panel D, the mean number of dipper presentations earned is plotted.

Fig. 2.

This figure shows that post-prandial drinking induced similar ethanol consumption in the experimental and control groups of Experiment 2, and that during Pavlovian conditioning or the Truly Random Control (TRC) condition, magazine entries were greater during the light presentation in the experimental group in which the light was paired with ethanol delivery than in the TRC condition. Panel A shows the median g/kg ethanol consumed by Experimental (filled points) and Control (open points) rats in sequential 2-hour post-prandial drinking sessions across sequential sessions of changing ethanol concentrations. In Panel B, the mean rate of magazine entries across sequential Pavlovian Training (filled points) or Control (open points) sessions is plotted for both the period when the light was lit (circles) and when it was not lit (triangles).

Fig. 3.

This figure shows that the ethanol-paired CS increased ethanol-reinforced responding in the experimental group of Experiment 2, and the same stimulus also increased responding in the Truly Random Control (TRC) condition, though to a lesser extent, and that these effects were limited to the period in which the stimulus was presented. Panel A shows the mean number of responses made by Experimental (filled circles) and Control (open circles) rats during the pre-target periods (minutes 33–35 and minutes 53–55) of the 1-hour experimental sessions across 15 sessions. During the middle five sessions, a light that had been paired with ethanol delivery in the experimental group would be on during the target period (minutes 35–37 and minutes 55–57 of the experimental sessions), i.e., in the periods immediately following that for which mean responses are plotted in Panel A. In Panel B, the mean of the difference between responses in Target and Pre-Target periods are plotted. In panel C, the mean total number of responses in each session is plotted, and in panel D, the mean number of dipper presentations earned is plotted.