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Context-Induced Relapse of Conditioned Behavioral Responding to Ethanol Cues in Rats

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Abstract

Background—The environmental context in which drug-conditioned cues are encountered could modulate the capacity of such cues to trigger relapse in abstinent addicts. We explored this hypothesis using a behavioral animal model.

Methods—Rats were trained to discriminate between two auditory stimuli; the first (CS+) was paired with 10% ethanol and the second (CS-) was presented without ethanol. Training occurred in operant conditioning chambers equipped with distinct contextual stimuli and entries into the ethanol delivery port during the stimuli were measured. Behavior was then extinguished by presenting both stimuli without ethanol in a second, different context. Context-dependent renewal of port-entries was tested by presenting the CS+ and CS- without ethanol in the original training context.

Results—At test, port-entries during the CS+ increased significantly compared to extinction levels, while responding during the CS- remained unchanged (n=11). This effect was significantly attenuated when rats received multiple extinction sessions in 3 distinct contexts (n=18), compared to when they received an equivalent number of extinction sessions in a single unique context (n=16). Context-dependent renewal of port-entries was also observed to a CS+ that had been paired with 14% sucrose during training (n=7), but not to a CS+ that was paired with 2% sucrose (n=8).

Conclusions—Environmental contexts can trigger the relapse of behavioral responding to ethanol- and sucrose-predictive cues in rats. For ethanol, this effect can be reduced by extinguishing responses to the ethanol cue in multiple distinct contexts, a manipulation that could increase the efficacy of cue-reactivity treatments for addiction.

Keywords

alcohol; relapse; extinction; Pavlovian discrimination; renewal; cue-reactivity; multiple contexts

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Introduction

Strategies for preventing relapse by drug addicts and alcoholics reflect a rising awareness that drug-conditioned cues, initially neutral stimuli that acquire incentive properties through repeated pairing with a drug's pharmacological effects, can exert considerable control over behavior (1-3). For example, exteroceptive stimuli such as the sight, smell and taste of familiar alcoholic beverages increase subjective reports of craving (4) and elevate physiological measures of reactivity in alcoholics (5-7). Craving and heightened reactivity to alcohol cues can predict future drinking in rehabilitated alcoholics, suggesting that conditioned responses to alcohol cues may trigger alcohol-seeking (8-11).

Such findings prompted the hypothesis that diminishing the associative strength of drug-conditioned cues during rehabilitation might enable addicts to avoid relapse upon encountering cues post-treatment. Unfortunately, cue reactivity treatment, wherein responsiveness to drug cues is extinguished by repeatedly exposing patients to cues in the absence of the drug's pharmacological effects (12-18), appears to have limited effectiveness, particularly for alcoholics (19,20).

This lack of efficacy may be explained by the strong influence of contextual stimuli (i.e., physical characteristics of an environment) present during learning on conditioned behavioral responses. Thus, conditioned responses to cues that predict either aversive or appetitive outcomes can be "renewed" by contexts associated with those outcomes, after extinction is conducted in a second, distinct environment (21-24). The renewal phenomenon indicates that learned associations are not simply "unlearned" during extinction. Rather, new learning occurs that endows conditioned cues with a second meaning. After extinction, behavioral responses to cues are determined by contextual stimuli associated with either the original or the new learning (25,26). This idea is relevant to cue-exposure treatment for addiction, in which conditioned associations between discrete cues and drugs occur in multiple environments, but extinction training is conducted in a single, potentially unrealistic laboratory setting (27).

The influence of environmental contexts on behavior is further illustrated by findings from animal models of drug self-administration, showing that re-exposure to drug-associated contexts after extinction in a different context can renew instrumental responding in rats (28-38). In some of these studies, instrumental responding during self-administration is reinforced by response-contingent drug-delivery *and* a discrete stimulus, making it difficult to determine if subsequent renewal is driven by the drug-conditioned cue per se. Here we sought to test the hypothesis that conditioned behavioral responding to drug-predictive stimuli is modulated by environmental contexts. Hence, we eliminated the response-contingent relationship between behavior and cue/drug delivery by using a procedure in which combined presentations of stimulus and drug occurred in a response-independent manner. Thus, rats were trained in one context to discriminate between a cue paired with 10% ethanol (CS+) and a second cue never paired with ethanol (CS-), as measured by heightened entries during the CS + into the ethanol delivery port. Port-entries were then extinguished in a second context by presenting both stimuli without ethanol. Context-induced renewal of ethanol-seeking was tested by exposing rats to both stimuli without ethanol in the original training context. These findings were compared to renewal using a natural reinforcer, sucrose. Because studies indicate that context-dependent renewal of conditioned fear is reduced if extinction is conducted in multiple contexts as opposed to a single context (37,38), we also determined if renewal to an ethanol-predictive stimulus would be attenuated if rats received extinction in multiple distinct contexts.

Methods and Materials

Subjects

Male, Long Evans rats (Harlan, Indianapolis, IN; 260-280 g on arrival) were individually housed in ventilated polycarbonate chambers (12 hr light/dark cycle, lights on, 7:00 a.m.). Subjects were not food or water restricted at any time. Procedures were approved by the Gallo Center Institutional Animal Care and Use Committee and are in agreement with recommendations in the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council, 1996)

Apparatus

Operant conditioning chambers (Med Associates Inc., St. Albans, VT) were housed within ventilated sound-attenuating cubicles. The chamber right wall featured a central port containing a fluid receptacle, into which fluid was delivered via a 20-ml syringe attached to a pump located outside the sound-attenuating cubicle. Head entries into the port were measured by interruptions of a photo beam. A white chamber light (28V, 100 mA) was located centrally near the ceiling on the left wall. Stimulus presentation (white noise generator and a 5 Hz clicker stimulus) and liquid delivery was controlled by a computer with Med PC IV (Med Associates) software, which also recorded port-entries.

Pre-exposure to ethanol or sucrose in the home-cage

One week after arrival, rats in Experiments 1 and 2a-b were acclimated to the taste and pharmacological effects of ethanol (EtOH) by 24-hr access to EtOH (10% v/v, 95% EtOH in water) and water via 2 bottles on the home cage (16-18 days). To maximize the likelihood that rats would attain significant ethanol blood levels, they were next given limited EtOH access 1-hr/day (water, 23-hrs/day) for 14-18 days. Body weights and liquid volumes were recorded daily. Supplementary Table 1 provides mean EtOH intakes per study

Rats in Experiment 3 were given 24-hr access to water and either 14.2% (14S), which is isocaloric with 10% ethanol (39) or 2% (2S) sucrose (w/v) for 4 days. Mean ($\pm$ SEM) sucrose intake across the last 3 days was 38.21 ± 1.63 and 32.74 ± 1.53 mls for 14S and 2S, respectively.

Experiment 1: Renewal of behavioral responding to an EtOH-predictive stimulus

Discrimination training—Rats ($n=11$) were trained to discriminate between two auditory stimuli across 18 daily 45-minute sessions. Session onset was indicated by illumination of the chamber light 1-minute after placement into the behavioral chambers. Rats received 16 random 10-second presentations each of a white noise or clicker stimulus under the control of separate variable-time 45-second (VT-45) schedules. The onset of one stimulus (CS+) coincided with EtOH (0.2 ml, delivered over the first 6 seconds); the second stimulus (CS-) was presented without EtOH. Ports were checked to ensure rats consumed the EtOH (3.2 mls) delivered.

Training occurred in either of 2 contexts (Context 1, $n=5$; Context 2, $n=6$; see Table 1 for description of contexts) that were distinct across visual, tactile and olfactory domains. The training context (Context A) was kept constant across sessions, and designation of the white noise or clicker stimulus as the CS+ was counterbalanced across context.

Extinction—Rats were placed into the context *not* used during prior discrimination training (Context B) and exposed to the CS+ and CS- in 8 daily sessions as described above. During extinction the pump was activated for 6 seconds but EtOH was not delivered.

Renewal Test—24 hrs after the last extinction session, rats were placed back into Context A and exposed to the CS+ and CS- as described above. The pump was activated for 6 seconds but EtOH was not delivered.

Experiment 2a: Effects of conducting extinction in multiple distinct contexts on renewal of behavioral responding to an EtOH-predictive stimulus

Discrimination training—Naive rats received 17 training sessions as described for Experiment 1, with the following differences: (1) sessions were run Mon-Fri; (2) to facilitate discrimination, a VT-60 schedule of stimulus presentation was implemented, which increased the session duration to 54-minutes but did not alter the total number of CS+ and CS- presentations; and (3) two additional contexts were utilized (Context 3 and Context 4, see Table 1).

Extinction—Rats were divided into two groups. One group (n=9) received 12 extinction sessions in a single context that was different from the self-administration context. The second group (n=10) received 4 extinction sessions in each of 3 different contexts, in a semi-random order, with assignment to a distinct context on each consecutive session (see Table 2). Twelve extinction sessions were required to ensure that both groups had comparable extinction levels before test, and that rats in the multiple context condition received equal exposure to the 3 contexts.

Renewal Test—24 hrs after the last extinction session, rats were placed into their original training contexts and exposed to the CS+ and CS- in the absence of EtOH, as described above.

Experiment 2b: Replication of Experiment 2a

Naive rats received 17 days of training, 12 extinction sessions (single context, n=8; multiple contexts, n=8; see Table 2), and a renewal test as described for Experiment 2a.

Experiment 3: Renewal of behavioral responding to a sucrose-predictive stimulus

Discrimination training—Naive rats were trained in 18 daily sessions as described for Experiment 1, using a VT-60 schedule. Instead of EtOH, one group received 14S (Context 1, n=3; Context 2, n=4) and the second group received 2S (Context 1, n=4; Context 2, n=4).

Extinction & Renewal Testing—Extinction (9 daily sessions) and renewal testing were conducted as described for Experiment 1.

Statistical Analysis

Discrimination training and extinction phases were analyzed separately for each Experiment. For Experiments 1 and 3, repeated-measures analysis of variance (RM-ANOVA) were conducted, with CS (CS+, CS-) and Session as within-subjects factors, and Training Context (Context 1, Context 2) and CS+ type (white noise, clicker) as between-subjects factors. For Experiment 2, the within-subjects factor of Training Context included Contexts 3 and 4, and the between-subjects factors of Group (single vs. multiple extinction contexts), and Replication (Experiment 2a, Experiment 2b) were added. As there were no main effects of Training Context or CS+ type in any experiment, data were collapsed across these variables for further analyses. For Experiment 2 there was no main effect of Replication; therefore, data were collapsed across Experiments 2a and 2b for graphical representation and further analyses.

Comparisons across Phase (training, extinction and renewal test) used data averaged across the last 2 sessions of discrimination training and extinction, and the 1 session of renewal test

data. All significant main effects and interactions were followed by targeted 2-way ANOVAs and t-tests for paired or independent samples. Criterion for significance was $\alpha = 0.05$.

Results

Experiment 1: Renewal of behavioral responding to an EtOH-predictive stimulus

Rats learned to discriminate between an EtOH-predictive stimulus (CS+) and a second, unpaired stimulus (CS-) across 18 sessions (Fig. 1A): they made more port-entries during the CS+ than the CS- [Session, $F(17,170)=9.35$, $p<0.001$; CS, $F(1,10)=37.97$, $p<0.001$; Session $\times$ CS, $F(17,170)=3.20$, $p<0.001$]. Responding during the CS+ was significantly higher than CS- responding on Day 6 and Days 8-18. Mean ($\pm$ SEM) EtOH intake on Day 1 of training was 0.57 ± 0.02 g/kg. Because a constant volume of EtOH was delivered per session (3.2 mls) and rats gained weight during training, EtOH intake on Day 18 was 0.54 ± 0.02 g/kg.

Port-entries during both stimuli diminished across extinction following CS presentations without EtOH in a different context [Session, $F(7,70)=3.07$, $p<0.05$; CS, $F(1,10)=15.40$, $p<0.01$; Session $\times$ CS, $F(7,70)=2.10$, $p=NS$]; significant discrimination was observed on Days 19-21, 23, and 24, but not the final 2 sessions (Fig. 1A).

Port-entries during the 10-sec interval before each CS (pre-CS; Fig. 1B) did not differ across Session [$F(17,170)=1.36$, $p=NS$], CS [$F(1,10)=0.01$, $p=NS$] or in the interaction [Session $\times$ CS, $F(17,170)=0.86$, $p=NS$] during discrimination training, but did decrease across extinction [Session, $F(7,70)=3.42$, $p<0.05$; CS, $F(1,10)=1.15$, $p=NS$; Session $\times$ CS interaction, $F(17,170)=0.63$, $p=NS$].

Normalized port-entries (Fig. 1C) were calculated by subtracting pre-CS responding from responding during the corresponding CS to achieve a behavioral measure that accounted for changes in baseline. ANOVA confirmed that rats discriminated between the CS+ and CS- across training [Session, $F(17,170)=9.74$, $p<0.001$; CS, $F(1,10)=33.49$, $p<0.001$; Session $\times$ CS, $F(17,170)=1.71$, $p<0.05$], with significant discrimination on Days 12-18.

Across extinction (Fig. 1C), responding during the CS+ was elevated compared to the CS- [CS, $F(1,10)=13.74$, $p<0.01$], with no main effect of Session [$F(7,70)=0.20$, $p=NS$] or significant Session $\times$ CS interaction [$F(7,70)=1.53$, $p=NS$]. However, normalized port-entries were significantly lower at the end of extinction compared to training [Fig. 2; Phase, $F(1,10)=102.66$, $p<0.001$; CS, $F(1,10)=34.66$, $p<0.001$; Phase $\times$ CS, $F(1,10)=8.40$, $p<0.05$] for each stimulus (CS+, $p<0.001$; CS-, $p<0.05$).

Fig. 2 presents the primary data of interest, showing responding at the end of discrimination training, the end of extinction, and during the renewal test. Compared to extinction, placing rats back into the prior training context and presenting both stimuli without EtOH elicited a specific increase in port-entries during the EtOH-predictive CS+ [Phase, $F(1,10)=6.19$, $p<0.05$; CS, $F(1,10)=24.85$, $p<0.01$; Phase $\times$ CS, $F(1,10)=11.01$, $p<0.01$]. CS+ responding was significantly higher at test than during extinction ($p<0.05$), while CS- responding at test was no different from extinction ($p>0.05$), but significantly reduced compared to discrimination training ($p<0.001$). There was also a small but significant renewal in pre-CS responding (Supplementary Fig. 1A) and total port-entries (Supplementary Fig. 1B), that does not account for the enhanced responding during the CS+, but that may reflect direct conditioning to the context.

Experiment 2a-b: Effects of conducting extinction in multiple distinct contexts on renewal of behavioral responding to an EtOH-predictive stimulus

Fig. 3A, which represents combined data from Experiments 2a and 2b, depicts port-entries at the end of training and across extinction in either a single context or multiple contexts. There was no difference between Single Context and Multiple Context groups [Group, $F(1,32)=0.15$, $p=NS$] in discrimination between the CS+ and the CS- [CS, $F(1,32)=130.66$, $p<0.0001$] during training [Session, $F(16,512)=15.66$, $p<0.0001$; Session $\times$ CS [$F(16,512)=8.76$, $p<0.0001$; Session $\times$ CS $\times$ Group, $F(16,512)=0.53$, $p=NS$]. EtOH intake across the last 2 days of training was 0.54 ± 0.01 g/kg.

Port-entries during the CS+ decreased for rats given extinction sessions in a single different context [Fig. 3A, left panel; Session, $F(11,165)=6.71$, $p<0.0001$; CS, $F(1,15)=84.01$, $p<0.0001$; Session $\times$ CS, $F(11,165)=5.05$, $p<0.0001$] or in 3 different contexts [Fig. 3A, right panel; Session, $F(11,187)=6.20$, $p<0.0001$; CS, $F(1,17)=34.55$, $p<0.0001$; Session $\times$ CS, $F(11,187)=7.13$, $p<0.0001$].

Following extinction, placement into the prior discrimination training context caused a selective renewal of port-entries during the CS+ in rats that underwent extinction in a single context, but not in rats extinguished in multiple contexts [Fig 3B; Phase, $F(1,32)=11.11$, $p<0.01$; CS, $F(1,32)=45.54$, $p<0.0001$; Group, $F(1,32)=3.41$, $p=0.07$; Phase $\times$ CS, $F(1,32)=11.48$, $p<0.01$; Phase $\times$ Group, $F(1,32)=7.43$, $p<0.05$; CS $\times$ Group, $F(1,32)=9.84$, $p<0.01$; Phase $\times$ CS $\times$ Group, $F(1,32)=5.46$, $p<0.05$]. CS+ responding was higher at test compared to extinction for rats extinguished in a single context ($p<0.01$), but not for rats extinguished in multiple contexts ($p>0.05$). There was no renewal of pre-CS responding (Supplementary Figs. 2A and 2B) or total port-entries for either group (Supplementary Fig 2C).

Experiment 3: Renewal of behavioral responding to a sucrose-predictive stimulus

Rats trained with 14S (Fig. 4A) learned to discriminate between the sucrose-predictive CS+ and the unpaired CS- [Session, $F(17,102)=4.42$, $p<0.0001$; CS, $F(1,6)=11.63$, $p<0.05$; Session $\times$ CS, $F(17,102)=3.18$, $p<0.0001$]. Normalized port-entries during both stimuli were initially equally high (days 1-10); however, CS- responding was significantly lower than CS+ responding from Days 12-18.

Port-entries diminished across extinction (Fig. 4A) following CS presentations without 14S in a different context [Session, $F(8,48)=6.68$, $p<0.0001$; CS, $F(1,6)=20.23$, $p<0.01$; Session $\times$ CS, $F(8,48)=3.64$, $p<0.01$]. Significant discrimination was observed on extinction days 19, 24, 25 and 27.

Fig. 4B depicts the outcome from the renewal test. Normalized port-entries at the end of extinction were significantly lower compared to training [Phase, $F(1,6)=25.29$, $p<0.01$; CS, $F(1,6)=41.27$, $p<0.01$; Phase $\times$ CS, $F(1,6)=13.93$, $p<0.01$], with a reduction in CS+ responding ($p<0.001$) but no change in CS- responding ($p>0.05$). Port-entries during the CS+ were renewed upon placement into the prior training context after extinction [Phase, $F(1,6)=13.74$, $p<0.01$; CS, $F(1,6)=34.35$, $p<0.01$; Phase $\times$ CS, $F(1,6)=19.04$, $p<0.001$]; CS+ responding at test was significantly higher than extinction ($p<0.01$). CS- responding at test was no different from extinction ($p>0.05$), but significantly lower than training ($p<0.05$). There was no renewal in preCS responding (Supplementary Fig. 3A) or total port-entries (Supplementary Fig. 3C).

Rats trained with 2S (Fig. 4C) rapidly learned to discriminate between the sucrose-predictive CS+ and the unpaired CS- [Session, $F(17,119)=5.13$, $p<0.001$; CS, $F(1,7)=10.85$, $p<0.05$; Session $\times$ CS, $F(17,119)=2.13$, $p<0.01$]. Normalized port-entries were higher during the CS+ than the CS- on days 2, 4, 9-11, and 14-18.

During extinction without 2S in a different context (Fig. 4C), CS+ responding remained elevated [CS, $F(1,7)=30.14$, $p<0.01$], with no main effect of Session [$F(8,56)=1.46$, $p=NS$] or CS $\times$ Session interaction [$F(8,56)=1.39$, $p=NS$]. However, ANOVA on normalized port-entries at the end of training and the end of extinction (Fig. 4D) indicated a significant reduction in responding during both stimuli in extinction [Phase, $F(1,7)=67.57$, $p<0.001$; CS, $F(1,7)=20.89$, $p<0.01$; Phase $\times$ CS, $F(1,7)=0.45$, $p=NS$]. This decrease was verified by paired-samples t-tests (CS+, $p<0.001$; CS-, $p<0.001$).

When placed into the prior training context, rats trained with 2S did not show renewal of port-entries (Fig. 4D). Although they still discriminated between both stimuli [CS, $F(1,7)=39.84$, $p<0.001$] there was no main effect of Phase [$F(1,7)=1.71$, $p=NS$] or significant Phase $\times$ CS interaction [$F(1,7)=2.81$, $p=NS$]. Paired-sample t-tests indicated no significant differences across extinction and test for either stimulus ($p>0.05$ for CS+ and CS-). There was no renewal of pre-CS responding (Supplementary Fig. 3B) or total port-entries (Supplementary Fig. 3C).

Discussion

Current research on relapse prevention is fueled in part by the hypothesis that conditioned responses to cues can facilitate drug-seeking behaviors that ultimately cause relapse. Thus, it is of value to investigate the precise conditions under which conditioned responses to such cues are generated. The present data indicate that behavioral responding to cues that predict drug reinforcers are modulated by the environmental context in which those cues occur. Specifically, conditioned responding during an ethanol-predictive stimulus was selectively renewed by placement into an ethanol-associated context following extinction in a different context. Renewal was also observed to a stimulus that predicted sucrose delivery, although the effect depended on sucrose concentration. Importantly, conducting extinction in multiple contexts prevented subsequent renewal when the ethanol stimulus was presented in an ethanol-associated environment.

The present behavioral model was designed to test the influence of environmental contexts on ethanol-seeking, defined as goal-directed behavioral responding evoked by ethanol-predictive cues. The selective renewal of CS+ responding at test with no change in CS-responding provides evidence that a specific CS+-outcome association was conditioned. Our findings are in general agreement with previous studies in which renewal was observed to a CS paired with food pellets (23,40), and extend this research by providing a CS- control to demonstrate specificity, and by demonstrating this renewal effect with the abused drug, ethanol. The present procedure therefore provides new and specific insight into how environmental contexts influence goal-directed actions triggered by cues that signal ethanol.

This procedure differs from appetitive conditioning paradigms that assess the renewal of Pavlovian conditioned behavioral responses (e.g., head-jerk, orientation) to discrete cues (23), which are often spatially and physically distinct from behaviors made to obtain the reinforcer (41), and which reflect attention towards the CS rather than the unconditioned stimulus or goal. In our procedure, port-entry, the goal-directed response, is the proximate behavior to the act of ethanol consumption in a chain of cue-triggered behavioral responses. Thus, measuring port-entries may provide valuable insight into the conditioning factors that contribute to cue-triggered ethanol-seeking.

In this manner, our procedure also diverges from self-administration models that examine the renewal of instrumental responding, which, in the case of ethanol self-administration, involves acquisition of an operant response (nose-poke or lever-press) that is then followed by movement to the port to consume ethanol, with or without an intervening ethanol-predictive cue; renewal is measured as an increase in operant responding (32-34). Likewise, in studies of

instrumental response renewal for other drugs of abuse, such as cocaine, the behavior measured is the operant response, as there is no subsequent port entry behavior when drug is administered intravenously (28-31,36,42). In all of these operant procedures, the reinforcer is presented in a response-contingent manner. Here, the delivery of ethanol or sucrose was not dependent on port-entries. However, procurement of these liquids did necessitate port-entries, our behavioral measure of interest. Because ethanol-predictive cues can develop Pavlovian incentive properties following similar conditioning within a Pavlovian-Instrumental transfer paradigm (43), we favor the hypothesis that Pavlovian conditioning mechanisms contribute to the expression of ethanol-seeking behavior measured as conditioned port entries; however, instrumental contingencies could also contribute to this process.

Behavioral discrimination observed during training in rats that received ethanol likely resulted from the development of a positive association between the CS+ and the pharmacological effects of ethanol. However, the taste and smell of ethanol could have acquired conditioned incentive properties during ethanol pre-exposure in the home-cage, which produced reasonable concentrations of blood ethanol (Supplementary Fig. 4). Behavioral discrimination may therefore reflect an association between the CS+ and the conditioned reinforcing properties of the taste and/or smell of ethanol. While both explanations are pertinent to the main goals of this study, the question of which is most applicable here could be addressed in future experiments by measuring blood ethanol concentrations during discriminative training.

In Experiment 2, renewal to an ethanol CS+ was inhibited after extinction in multiple different contexts, compared to extinction in a single different context. This finding, the first to demonstrate the effectiveness of this behavioral manipulation in an appetitive conditioning paradigm, strongly suggests that extinguishing conditioned behavioral responses in multiple environments may enhance the efficacy of cue-reactivity treatments for addiction. The mechanisms underlying this effect require further investigation. Exposure to the discriminative stimuli without ethanol in 3 distinct contexts may have enhanced extinction learning, thereby facilitating the recall of a cue-no ethanol association at test. Alternatively, increasing the total number of contextual stimuli present throughout extinction may have engendered generalization across contexts. Exposure to multiple contexts per se may also have produced nonspecific effects that contributed to the attenuation of renewal. Although the results of this study were replicated, the experimental design did not match groups for overall exposure to novel contexts, an important consideration for future experiments. The design of such a study is complicated by the possible presence of direct excitatory conditioning between contexts and ethanol (unpublished observations, Chaudhri and Janak).

While our results are largely consistent with the literature (37,38,44), two studies found that conducting extinction in multiple contexts was not effective at preventing renewal of conditioned fear (45,46). In those studies extinction occurred during one session in each of 3 distinct contexts, whereas in the present study 4 extinction sessions were conducted in each of 3 contexts. Therefore, repeated, nonconsecutive exposures to each extinction environment may have enhanced the effectiveness of the present procedure in reducing context-dependent renewal. Alternatively, this discrepancy could reflect differences in fear-conditioned and appetitive-conditioned response extinction.

Behavioral and neural mechanisms that regulate feeding in humans may be similar to those that influence relapse in drug users (47). For example, discrete food-conditioned cues can trigger craving and activate brain regions similar to those that are stimulated by drug-conditioned cues (48). As expected by the overlap in brain circuitry, conditioning for both drug and natural rewards can be observed within most animal models of appetitive conditioning. Therefore, we expected to observe renewal for sucrose. Discriminative responding was attained for sucrose at two concentrations, with similar patterns of discrimination (Figs. 1C & 4C) and

total port entries (Supplementary Fig. 5) for 10% ethanol and 2S. Surprisingly however, significant renewal was only observed for ethanol or the isocaloric 14S. Although this finding may imply that renewal was driven by the caloric content of the reinforcers, differences in the pattern of discrimination learning for 14S (Fig. 4A) and 10% ethanol (Fig. 1C) indicate that behavioral responding was not solely influenced by calories. Indeed, evidence suggests that alcohol preference and intake is regulated by its pharmacological, rather than food-like, properties (39,49). While the influence of conditioned stimuli is modulated by context for both ethanol and sucrose, recent work suggests that conditioning with drug reinforcers may produce greater strength and/or longevity in conditioned reward-seeking behavior than conditioning with natural reinforcers (50), including for ethanol vs. sucrose in an operant renewal model (34). Therefore, subsequent research should assess differences in renewal across repeated testing for ethanol- and sucrose-predictive stimuli.

In summary, we have shown that Pavlovian-conditioned discriminative stimuli impact ethanol- and sucrose-seeking (see also, 43,51), and that environmental contexts within which relationships between these stimuli and reinforcers are formed become important modulators of subsequent behavior. Given that repeated drug use, as well as food intake, often occurs against distinct and recognizable backdrops, it is important to consider the influences of contextual stimuli on conditioned associations involving these reinforcers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Discrimination training and extinction with a 10% ethanol reinforcer. Data represent Mean ($\pm$ S.E.M.) port-entries during the CS+ (filled circles) and CS- (open circles) in daily discrimination training sessions with ethanol (Context A, sessions 1-18) and extinction sessions without ethanol (Context B, sessions 19-26). **(A)** Port-entries during each 10-sec stimulus **(B)** Port-entries during the 10-sec interval before each stimulus (pre-CS) **(C)** Normalized port-entries during each CS, obtained by subtracting pre-CS responding from responding during the corresponding 10-sec CS. * $p < 0.05$, CS+ vs. corresponding CS-.

Figure 2.

Renewal of conditioned responding for ethanol. Mean ($\pm$ S.E.M.) normalized port-entries made during the CS+ (filled bars) and CS- (open bars) in discrimination training sessions with ethanol in Context A, extinction sessions in Context B, and renewal testing in Context A. Data are averaged over the last 2 sessions for discrimination training and extinction, and are from the single session renewal test. * $p < 0.05$, ** $p < 0.001$, CS+ vs. corresponding CS-; # $p < 0.05$, CS+ training vs. CS+ extinction; ^ $p < 0.05$, CS+ extinction vs. CS+ test.

Figure 3.

Extinction in multiple contexts attenuates renewal of conditioned responding for ethanol. **(A)** Mean ($\pm$ S.E.M.) normalized port-entries made during the CS+ (filled) and CS- (open) in discrimination training (DT) sessions with ethanol (Bars; averaged across the last 2 sessions) and extinction sessions without ethanol (symbols) when extinction occurred in a single context (left panel) or multiple contexts (right panel). * $p < 0.05$, ** $p < 0.0001$, CS+ vs. corresponding CS-. **(B)** Mean ($\pm$ S.E.M.) normalized port-entries for extinction (averaged across the last 2 sessions) and 1 renewal test session in the original training context without ethanol. * $p < 0.01$, CS+ vs. corresponding CS-; ^ $p < 0.01$, CS+ extinction vs. CS+ test; # $p < 0.01$, CS+ single context vs. CS+ multiple contexts at test.

Figure 4.

Renewal of conditioned responding for 14.2%, but not 2%, sucrose. (A & C) Mean ($\pm$ S.E.M.) normalized port entries across session made during the CS+ (filled circles) and CS- (open circles) for rats trained with either 14.2% sucrose (A; 14S) or 2% sucrose (C; 2S).

Discrimination training was conducted with sucrose in Context A (sessions 1-18) and extinction sessions without sucrose in Context B (sessions 19-27). * $p < 0.05$, CS+ vs. corresponding CS- (B & D) Mean ($\pm$ S.E.M.) normalized port-entry data across the last 2 days of discrimination training, the last 2 days of extinction, and from the single session renewal test (CS+ filled bars; CS- open bars; Mean) for rats trained with 14S (B) or 2S (D) * $p < 0.05$, ** $p < 0.01$ CS+ vs. corresponding CS-; # $p < 0.001$, CS+ training vs. CS+ extinction; ^ $p < 0.01$, CS+ extinction vs. CS+ test.

Table 1

Description of the physical components of each context.

	Visual stimulus	Tactile stimulus	Olfactory stimulus
Context	Walls [*]	Floor ⁺	Odor
1	Clear Plexiglas	Wire mesh insert	Strawberry air freshener taped to outside of front chamber wall
2	Black chamber walls	Smooth semi-transparent Plexiglas insert	Single spray of 30% acetic acid solution misted into bedding beneath chamber floor
3	White chamber walls	Stainless steel bar floor	Exp 2a: ½ a pine scented air freshener placed on the floor of the sound attenuating cubicle Exp 2b: 4 drops of lemon essential oil placed into bedding beneath chamber floor
4	Black and white vertical striped chamber walls	Textured semi-transparent Plexiglas insert	4 drops of peppermint extract on a cotton-pad, placed on the floor of the sound attenuating cubicle

^{*} Walls were modified using paper to cover the clear Plexiglas.

⁺ Floors were modified using an insert that was placed over the existing stainless steel bar floor.

Table 2
Assignment of training, extinction, and renewal test contexts for subjects in Experiment 2a and 2b.

Group	Discrimination training context	Extinction context for each session	Renewal context	Exp 2a: number of rats	Exp 2b: number of rats
Group 1 (n=16) Extinction in a single context	1	2,2,2,2,2,2,2,2,2,2,2,2,2,2,2,2	1	n=2	n=1
	2	1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1	2	n=2	n=2
	3	4,4,4,4,4,4,4,4,4,4,4,4,4,4,4,4	3	n=2	n=2
	4	3,3,3,3,3,3,3,3,3,3,3,3,3,3,3,3	4	n=3	n=2
Group 2 (n=18) Extinction in multiple contexts	1	2,3,4, 3,2,4, 2,4,3, 4,2,3	1	n=3	n=2
	2	1,4,3, 4,1,3, 1,3,4, 3,1,4	2	n=3	n=2
	3	4,2,1, 2,4,1, 4,1,2, 1,4,2	3	n=2	n=2
	4	3,1,2, 1,3,2, 3,2,1, 2,3,1	4	n=2	n=2