Video Article

A Procedure to Observe Context-induced Renewal of Pavlovian-conditioned Alcohol-seeking Behavior in Rats

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Abstract

Environmental contexts in which drugs of abuse are consumed can trigger craving, a subjective Pavlovian-conditioned response that can facilitate drug-seeking behavior and prompt relapse in abstinent drug users. We have developed a procedure to study the behavioral and neural processes that mediate the impact of context on alcohol-seeking behavior in rats. Following acclimation to the taste and pharmacological effects of 15% ethanol in the home cage, male Long-Evans rats receive Pavlovian discrimination training (PDT) in conditioning chambers. In each daily (Mon-Fri) PDT session, 16 trials each of two different 10 sec auditory conditioned stimuli occur. During one stimulus, the CS+, 0.2 ml of 15% ethanol is delivered into a fluid port for oral consumption. The second stimulus, the CS-, is not paired with ethanol. Across sessions, entries into the fluid port during the CS+ increase, whereas entries during the CS- stabilize at a lower level, indicating that a predictive association between the CS+ and ethanol is acquired. During PDT each chamber is equipped with a specific configuration of visual, olfactory and tactile contextual stimuli. Following PDT, extinction training is conducted in the same chamber that is now equipped with a different configuration of contextual stimuli. The CS+ and CS- are presented as before, but ethanol is withheld, which causes a gradual decline in port entries during the CS+. At test, rats are placed back into the PDT context and presented with the CS+ and CS- as before, but without ethanol. This manipulation triggers a robust and selective increase in the number of port entries made during the alcohol predictive CS+, with no change in responding during the CS-. This effect, referred to as context-induced renewal, illustrates the powerful capacity of contexts associated with alcohol consumption to stimulate alcohol-seeking behavior in response to Pavlovian alcohol cues.

Video Link

The video component of this article can be found at https://www.jove.com/video/51898/

Introduction

Remaining sober is a considerable challenge faced by individuals suffering from alcohol abuse disorders. Abstinence is a time of vulnerability to the behavioral, psychological and physiological impact of environmental stimuli that routinely accompany alcohol use, which can, through Pavlovian conditioning, become associated with inebriation[1,2]. Exposure to alcohol predictive cues can elicit conditioned responses such as alcohol craving, which may promote alcohol-seeking behaviors that facilitate relapse[3,4].

The stereotypical sequences of behavior that lead to alcohol consumption can cause certain types of stimuli to be routinely experienced immediately before the pharmacological effects of alcohol intake. For example, the sight, smell and taste of alcohol are sensory properties of alcohol that reliably precede intoxication. In addition to such cues, which are referred to as 'discrete' or 'proximal' cues, environmental contexts in which drugs are regularly used can also provoke craving[5,6]. Exposure to physical locations in which drugs have previously been used may therefore be a critical trigger for relapse [7].

Animal models have been developed to study the neural mechanisms that mediate the impact of drug associated contexts on drug-seeking behavior[8-13]. The procedure described herein allows for the investigation of how contexts associated with alcohol consumption can modulate alcohol-seeking behavior that is elicited by a discrete, alcohol predictive Pavlovian cue.

Pavlovian discrimination training is conducted in a specific environmental context, where rats are trained to behaviorally distinguish between two auditory conditioned stimuli, a CS+ that is paired with alcohol, and a CS- that is not. Extinction sessions are then conducted in a different context, where responding to the CS+ diminishes as a result of alcohol being withheld. Subsequently, reexposure to the alcohol associated, Pavlovian training context triggers a selective increase in alcohol-seeking behavior elicited by the CS+, with no change in responding to the CS-. This result, which we have consistently replicated[9,14-16], extends findings from instrumental conditioning procedures in which drug contexts have been found to stimulate the renewal of operant responses associated with drug delivery[10,13].

Protocol

All procedures are approved by the Animal Research Ethics Committee at Concordia University and concur with recommendations from the Canadian Council on Animal Care.

1. Animals

1. Obtain male Long-Evans rats (220 – 240 g on arrival).
2. Maintain rats in a temperature (21 °C) and humidity (44%) controlled animal care facility, on a 12 hr light-dark cycle with lights on at 7:00 am.
3. Pair-house rats upon arrival. After 3 days, house rats individually in plastic shoebox cages with beta-chip bedding, a nylon bone, and free access to standard rat chow and water. Use cage lids that have double grommets to allow for the subsequent placement of a second bottle, in addition to the water bottle. NOTE: Rats may be individually housed as soon as they arrive in the animal care facility.
4. Begin to handle rats daily. NOTE: As a guide, one week of handling is usually sufficient to acclimate them to being picked up and handled by the experimenter.

2. Intermittent Ethanol Access in the Home Cage

NOTE: Conduct intermittent ethanol access in the home cage to ensure that rats will drink physiologically relevant quantities of ethanol before the behavioral training phase of the experiment begins [17-19]. Initiate this procedure during the light phase, although it could be initiated at any time during the light or dark cycle.

1. Prepare two bottles for each rat.
    1. Use a 100 ml plastic graduated cylinder as the ethanol bottle and a 473 ml standard plastic water bottle. Use a relatively small capacity ethanol bottle, filled almost to maximum volume capacity, to minimize loss of ethanol due to leakage and evaporation.
    2. Fill one bottle with 15% ethanol, made by diluting 95% ethanol in tap water. NOTE: Other ethanol concentrations, such as 10% or 20%, may be used in place of 15% ethanol.
    3. Fill the second bottle with tap water.
    4. Insert a rubber stopper, with a sipper tube that contains a ball bearing to minimize leakage, into each bottle.

2. Set up two control cages, treated identically to the home cages with the exception that the control cages do not contain rats or bedding.
    1. Prepare ethanol and water bottles for the control cages as specified in steps 2.1.1 – 2.1.4.
    2. Use the measures obtained from the control cages to control for spillage and/or evaporation that may occur over the course of the ethanol access session (as described in steps 2.8.1 – 2.8.2).

3. Weigh all the ethanol and water bottles. Record all weights.
4. Weigh all the rats. Record all weights.
5. Place the ethanol bottle and the water bottle on each home cage and control cage at the same time. Leave both bottles on the cages for 24 hr.
6. At the end of the 24 hr period, remove both bottles from the cages at the same time.
7. Weigh all the ethanol and water bottles. Record all weights.
8. To determine how much ethanol and water was consumed during the 24 hr period, subtract the weight of each bottle when it was removed from the home cage from the weight of the bottle when it was placed on the home cage 24 hr earlier for each rat.
    1. Do the same for the two control cages, and then calculate the average amount of spillage for each fluid.
    2. Subtract the average amount of ethanol and water spillage, respectively, from the corresponding ethanol and water consumption measures for each rat.
    3. Use these spillage controlled difference measures to calculate ethanol consumption in g/kg, consumption of water, and ethanol preference.

9. To calculate ethanol consumption (g/kg), first convert the weight (g) of ethanol solution consumed into volume (ml) based on the density of the total solution (ethanol + water components), according to the formula Volume (ml) = Weight (g)/Density (g/ml). NOTE: The density of the solution will vary as a function of ethanol concentration.
    1. Next, multiply the volume (ml) of ethanol solution consumed by the density (g/ml) of ethanol scaled for the specific percentage solution used, to yield the weight (g) of ethanol consumed.
    2. Divide the weight (g) of ethanol consumed by the rat weight (kg) to obtain the g/kg measure of ethanol consumption.

10. To calculate ethanol preference, express ethanol intake (ml) as a percentage of total fluid (ethanol + water, ml) intake.
11. Return the water bottle only back to the rat's home cage. For the next 24 hr, give the rats access to water but not ethanol. Instead of an ethanol bottle, place a pre-weighed second water bottle on the home cage.
12. At the end of the 24 hr period, remove both water bottles from the cage at the same time.
13. Weigh both sets of water bottles. Record all weights.
14. Subtract the weight of each bottle when it was removed from the home cage from the weight of the bottle when it was placed on the home cage 24 hr earlier, subtracting out the average spillage measure obtained from control cages as described in steps 2.8.1 – 2.8.2.
15. Use these spillage-controlled difference measures to calculate consumption of water.
16. Begin the cycle again by providing both ethanol and water bottles to rats, as described in steps 2.1 through 2.14.
    1. Repeat this cycle for multiple (about 12) sessions to induce high and stable levels of ethanol consumption. See **Figure 1** for representative results of ethanol consumption (g/kg) in the home cage.

2. Alternate the spatial position of the ethanol and water bottle on the cage lid across sessions to prevent the development of a side preference.
3. Make a fresh ethanol solution once per week to ensure accurate and stable ethanol concentration across sessions. Minimize the impact of evaporation of ethanol over prolonged periods of time using this approach.

3. Apparatus

1. House each conditioning chamber inside a ventilated, sound-attenuating cubicle.
2. Construct each conditioning chamber of a stainless steel metal rod floor, a clear polycarbonate ceiling, clear polycarbonate back wall and front door, and aluminum metal panel sidewalls.
    1. Install a fluid port centrally near the base of the right metal sidewall. Equip the entrance to the fluid port with an infrared photocell to detect entries into the port.
    2. Connect polyethylene tubing from the back of the fluid port to a 20 ml syringe containing ethanol using a blunt-tipped needle. Place the syringe into a syringe pump located outside the sound-attenuating cubicle. For Pavlovian discrimination training sessions, load ethanol into the syringe before placing it into the pump. Prime the tubing by manually advancing the pump, and then wipe away excess ethanol from the fluid port.
    3. On the left sidewall, install a white houselight centrally near the ceiling of the chamber.
    4. Install a clicker (2 Hz cycle) and a white noise generator with speaker in the upper left corner of the left sidewall.
3. Outfit each conditioning chamber with a specific configuration of visual, olfactory, and tactile stimuli to create a distinctive context.
    1. Use two different contexts, designated Context 1 and Context 2. Configure half of the conditioning chambers as Context 1 and the other half as Context 2.
    2. To create Context 1, place black cardboard panels over the ceiling and front and back walls of the chamber, spray lemon odor (10% v/v, made by diluting lemon oil in tap water) into a Petri dish and place it in the center of a metal waste tray under the floor of the chamber, and insert a clear polycarbonate floor panel over the metal rod floor. Line the waste tray with absorbent paper towel or bench liner.
    3. To create Context 2, leave the ceiling and front and back walls of the chamber uncovered, so that they are clear, spray almond odor (10% v/v, made by diluting benzaldehyde in tap water) into a Petri dish and place it centrally in a metal waste tray under the floor of the chamber, and insert a perforated metal floor panel over the metal rod floor. Line the waste tray with absorbent paper towel or bench liner.

4. Habituation

Habituate rats to the behavioral training room and each of the two contexts within conditioning chambers. For this phase of the experiment, do not load ethanol into the 20 ml syringes.

1. Habituate rats to the process of being transported from the animal care facility to the behavioral training room in the laboratory in two short habituation sessions.
    1. In the first session, load the rats in their home cages onto a wheeled cart in the animal care facility, and transport them to the behavioral training room in the laboratory. Illuminate the room lights, turn on the fans in each sound attenuating cubicle, exit the room, close the door, and let the rats adjust to this new environment for 20 min, while remaining in their home cages on the cart. At the end of this 20 min period, return the rats to the animal care facility.
    2. In the second session, bring the rats to the behavioral training room in the laboratory. As before, leave the lights and cubicle fans on in the room. Remain in the room, with the door closed to minimize distractions, and handle and weigh each rat in the behavioral training room. Record the weights, and then return the rats to the animal care facility.
2. Habituate rats to the conditioning chambers in two short habituation sessions.
    1. Transport the rats to the behavioral training room, record each rat's weight, and load each rat into its designated conditioning chamber.
    2. In the first habituation session set up half the chambers as Context 1 and the remainder as Context 2.
    3. In the second habituation session set up each chamber with the context that was not used in the previous session.
    4. Conduct habituation sessions in a counterbalanced fashion, such that half of the rats are first exposed to Context 1, followed by Context 2, and the other half are first exposed to Context 2, followed by Context 1. Ideally, conduct both habituation sessions in the same day, although they could be spread across two days if necessary.
    5. For habituation sessions, use a computer program to illuminate the houselight 1 min after the program has been issued. Record the total number of fluid port entries made during a 20 min session. At the end of the session, program the houselight to turn off. Do not present any ethanol or cues during habituation sessions.
3. Remove rats from the chambers, return them to their home cages, and transport them back to the animal care facility.
4. Clean the equipment (*e.g.*, floor panels, waste trays, Petri dishes). See step 5.7 for further details.

5. Pavlovian Discrimination Training (PDT)

Train rats to behaviorally discriminate between one auditory cue that is paired with ethanol and a second auditory cue that is not. For this phase of the experiment, fill the 20 ml syringes with 15% ethanol.

1. Outfit each conditioning chamber with a specific configuration of visual, olfactory, and tactile stimuli to create a distinctive context, as described in steps 3.3.2 and 3.3.3

1. Assign half of the rats to Context 1 and the other half to Context 2 in a counterbalanced fashion, based on ethanol consumption and preference averaged across the last 2 sessions of the home cage ethanol access procedure. Refer to the context used during PDT as Context A. NOTE: Each rat should only experience one context in this phase of training.
2. Make sure that the olfactory stimulus is the last contextual stimulus to be added to each conditioning chamber. Close the doors of the chamber and the sound-attenuating cubicle to contain the olfactory stimulus and minimize the comingling of different odors.

2. Bring the rats into the behavioral training room, record each rat's weight, and load each rat into its designated conditioning chamber.
3. Initiate the computer program that controls the following sequence of events during each PDT session.
    1. At 5 min after the program has been issued, illuminate the houselight.
    2. Present the white noise stimulus and the clicker stimulus 16 times each. Designate one of these auditory stimuli to be the CS+ and the other to be the CS-, in a counterbalanced fashion across both contexts.
    3. Make each CS+ and CS- trial last 10 sec.
    4. Deliver 0.2 ml ethanol into the fluid port during the last 6 sec of the CS+. Do not deliver any ethanol during the CS-.
    5. Make each PDT session last 58 min on average. Present the CS+ and the CS- each on a variable time 67 sec schedule.
    6. Record fluid port entries during the 10 sec CS interval (the 10 sec during CS presentation), the 10 sec pre-CS interval (the 10 sec immediately prior to CS onset), and the 10 sec post-CS interval (the 10 sec immediately following CS offset) for both CS+ and CS- presentations, as well as total fluid port entries for the entire session. Also record the duration of each port entry made during these intervals.
    7. At the end of the session, turn the houselight off.

4. Remove rats from the chambers, return them to their home cages, and transport them back to the animal care facility.
5. Check the fluid ports to ensure that rats consumed the 3.2 ml of ethanol delivered in each PDT session.
6. Flush the ethanol out of the polyethylene tubing with a 60 ml syringe containing a small amount of water. Then, pass 60 ml of air through the tubing using a second, empty syringe, to make sure it is dry.
7. Clean the equipment. For daily cleaning, wash the floor panels, waste trays, and Petri dishes. Once a week, clean the entire conditioning chamber. Do not use scented cleaning products to avoid introducing unwanted odors into the experimental apparatus.
8. Continue PDT sessions daily until rats show stable discrimination in their port entry responses to the CS+ vs. CS-. NOTE: Port entries made in response to the CS+ should increase over sessions, whereas port entries made in response to the CS- should remain low. The exact number of PDT sessions necessary to achieve discriminated conditioned responding can vary, but, as a guide, plan on approximately 20 sessions.

6. Extinction

1. Outfit each conditioning chamber with visual, olfactory, and tactile stimuli to create a context that is distinct from the one the rats experienced during PDT sessions. For example, if Context 1 was used in PDT, configure the chamber as Context 2 for extinction sessions. Refer to the context used during extinction as Context B.
2. Conduct extinction sessions identically to PDT sessions, but do not deliver any ethanol during the CS+.
    1. Use the same computer program that was used during PDT to present the CS+ and CS-.
    2. To maintain consistency across phases of the experiment, load an empty syringe into the syringe pump, and leave the pump turned on. Make sure the polyethylene tubing does not contain ethanol before extinction sessions.

3. Continue extinction sessions daily until rats no longer show discriminated responding between the CS+ and CS-. NOTE: Port entries made in response to the CS+ should gradually decline across extinction sessions, until there is no difference between port entries made in response to the CS+ and port entries made in response to the CS-. The exact number of extinction sessions necessary to extinguish conditioned responding can vary, but as a guide, plan on approximately 8 sessions.
4. Clean the equipment at the end of each session, as described in step 5.7.

7. Context-induced Renewal Test

1. Equip each conditioning chamber with the visual, olfactory, and tactile stimuli to create the context that was experienced during PDT sessions. For example, if a given rat experienced Context 1 in PDT sessions, configure his chamber as Context 1 in the test session. Refer to the context used during the renewal test as Context A.
2. Conduct the context-induced renewal test identically to PDT sessions, but do not deliver any ethanol during the renewal test. Present the CS+ and CS- as before during PDT sessions, but withhold the ethanol.
    1. Use the same computer program that was used during PDT to present the CS+ and CS-.
    2. To maintain consistency across phases, load an empty syringe into the syringe pump, and leave the pump turned on. Make sure the polyethylene tubing does not contain ethanol before the test session.

3. Observe context-induced renewal of Pavlovian-conditioned alcohol-seeking behavior. NOTE: This renewal effect will be evident by a selective increase in the number of port entries made in response to the CS+, with no change in port entry behavior made in response to the CS-, relative to CS+ and CS- responding observed at the end of extinction training.
4. Clean the equipment at the end of the session, as described in step 5.7.

Representative Results

Intermittent ethanol access in the home cage: Ethanol consumption (g/kg) and ethanol preference increase across sessions of intermittent ethanol access in the home cage, typically reaching stable levels within 8 – 12 sessions (**Figure 1**). In the data set shown in **Figure 1**, stable ethanol consumption averaged across the last two sessions of home cage ethanol access ranged from 1.33 – 6.44 g/kg for individual rats. The

home cage ethanol consumption phase of the experiment can be stopped once the g/kg consumption has plateaued, and rats are maintained at this stable level of drinking for a number of sessions.

To analyze data from this phase, conduct separate repeated-measures analysis of variance (ANOVA) across the within-subject factor of session for ethanol consumption (g/kg) and ethanol preference.

Schematic of context-induced renewal procedure: Each phase of the experimental procedure (PDT, extinction, renewal test) is illustrated in **Figure 2**. See the figure caption for further details.

Response measures and data analysis: In all behavioral phases described below, port entry responses made during the CS+ and CS- are normalized with reference to pre-CS baseline port entry responses. To create the normalized CS measures, subtract the number of port entries made during the pre-CS baseline interval (the 10 sec immediately prior to CS onset) from the number of port entries made during the presentations of the CS+ and CS-. The normalized CS+ and normalized CS- measures serve as indicators of behavior elicited specifically by CS presentations, elevated above baseline levels.

Conduct separate repeated-measures ANOVA with the repeated within-subject factors of session and CS type (CS+ vs. CS-) for the PDT and extinction phases. For comparisons across phase (PDT, extinction, renewal test), use data averaged across the last two sessions of PDT and extinction and the one session of renewal test data in repeated-measures ANOVA. To follow up on significant main effects and interactions, use *post hoc* t-tests for independent or paired samples that are corrected for multiple comparisons. Criterion for significance is set at $\alpha = 0.05$.

PDT: Port entry responses to the CS+ increase across PDT sessions, whereas port entry responses to the CS- remain low and stable, overall. Note that port entry responses to the CS- may increase slightly in the early sessions of PDT training; however, port entries made in response to the CS- stabilize at a much lower level than those made in response to the CS+. Successful discrimination is evidenced by a statistically significant elevation in port entries during the CS+ compared to the CS- (**Figure 3**). This result indicates that Pavlovian-conditioned alcohol-seeking behavior has been established to the CS+, but not the CS-.

Extinction: Port entry responses to the CS+ gradually decline across extinction sessions. Port entry responses to the CS- remain low and stable across extinction sessions. Ideally, discriminated responding is abolished by the end of extinction training, as evidenced by the lack of a statistically significant difference between port entries to the CS+ and CS-. However, it is not uncommon for some degree of discriminated responding to remain, even at the end of extinction training. In this case, it is important to verify that port entry responses to the CS+ at the end of extinction training are statistically lower than port entry responses to the CS+ at the start of extinction, as well as at the end of PDT (**Figure 3**). This pattern of results indicates that Pavlovian-conditioned alcohol-seeking behavior has been significantly extinguished.

Context-induced renewal test: Port entry responses to the CS+ increase in the context-induced renewal test, relative to CS+ port entries observed at the end of extinction training. This increase is selective to the CS+, as port entries to the CS- remain unaltered. In addition, port entries to the CS+ are statistically elevated compared to port entries to the CS- in the context-induced renewal test (**Figure 4**). Statistical confirmation of comparisons of CS+ port entries between extinction and test, as well as comparisons of CS+ and CS- port entries within test, indicates a selective renewal of Pavlovian-conditioned alcohol-seeking behavior induced by the alcohol associated context at test.

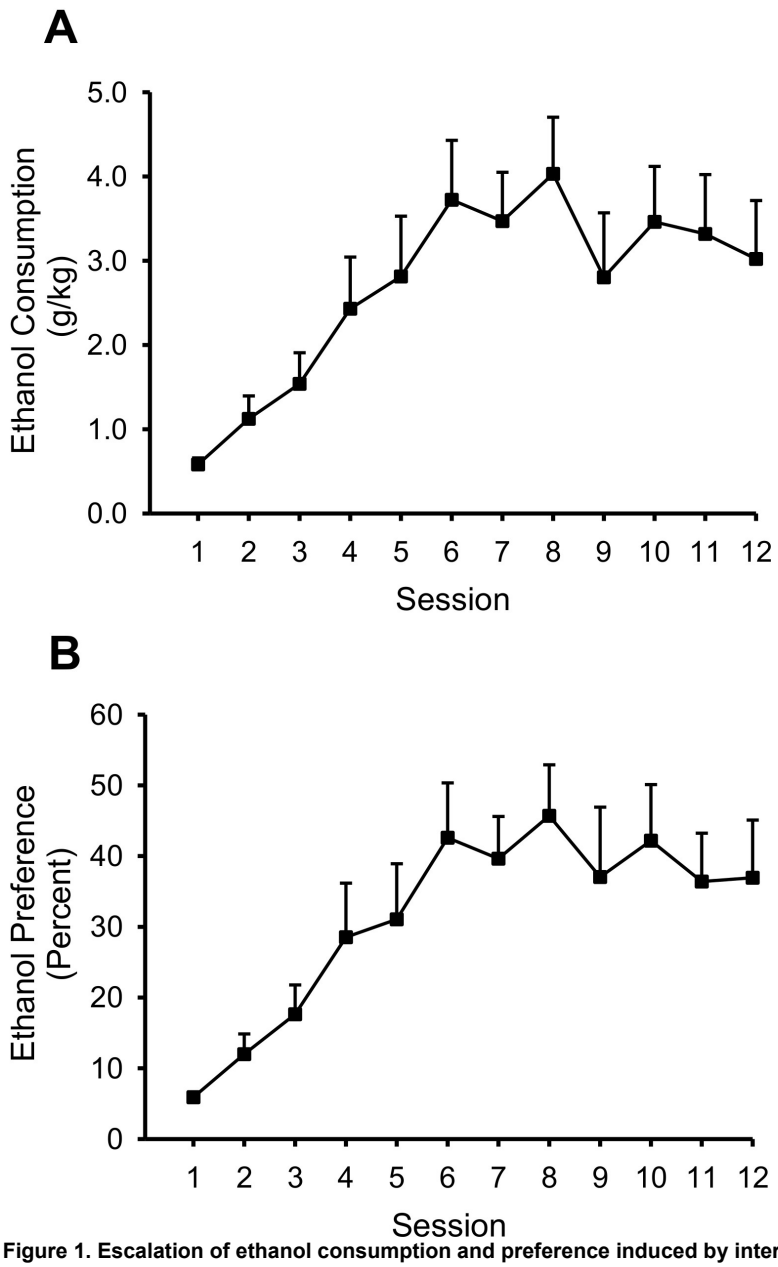

Figure 1. Escalation of ethanol consumption and preference induced by intermittent ethanol access in the home cage. Mean ± SEM **(A)** ethanol consumption (g/kg) and **(B)** ethanol preference across 12 sessions of intermittent ethanol access in the home cage. Both measures typically increase across sessions and then stabilize. Stable ethanol consumption averaged across the last two sessions of home cage ethanol access ranged from 1.33 – 6.44 g/kg for individual rats. These data were obtained using male Long-Evans rats drinking 15% (v/v) ethanol.

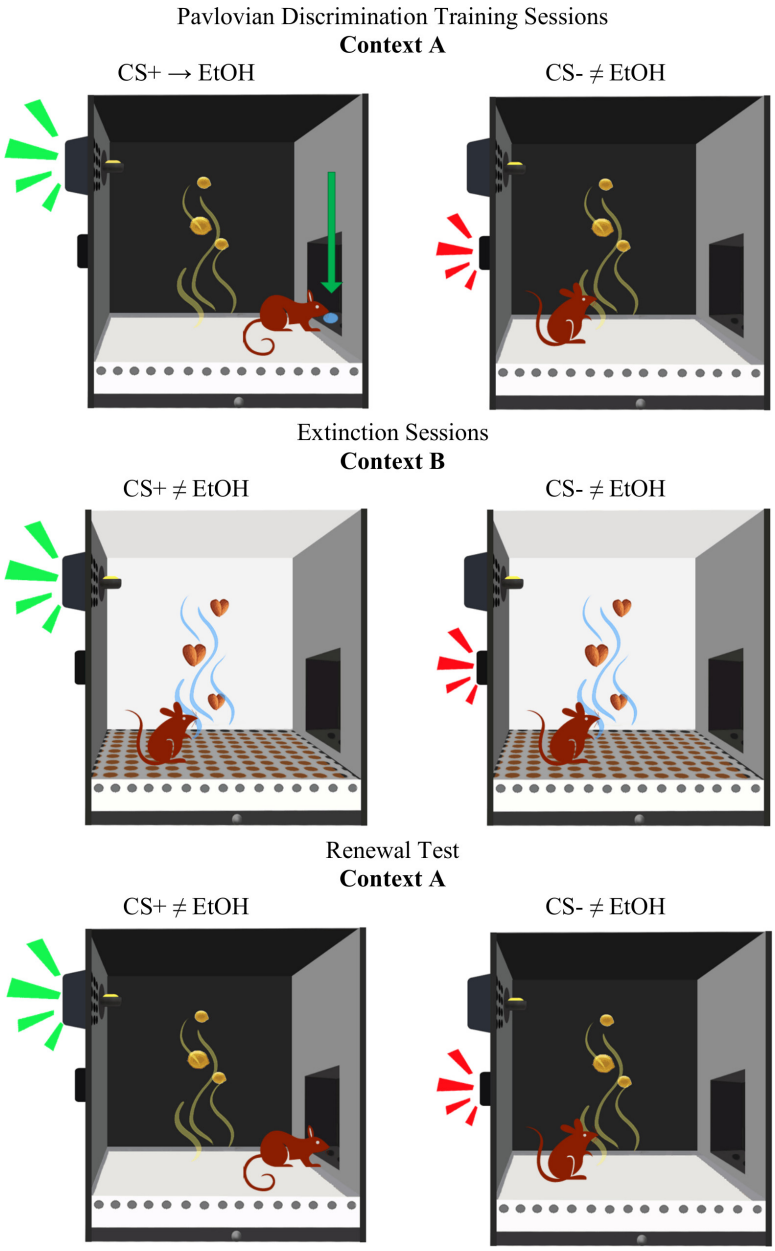

Figure 2. A schematic of the experimental procedure. The procedure consists of three phases: Pavlovian discrimination training (PDT), extinction, and context-induced renewal test. During each PDT session, rats receive 16 trials of one auditory conditioned stimulus (CS+) that signals the delivery of ethanol and 16 trials of a second auditory stimulus (CS-) that is not associated with ethanol delivery. PDT sessions take place in Context A, composed of a specific configuration of visual, olfactory, and tactile stimuli. During extinction sessions, which are conducted in Context B, composed of a different set of visual, olfactory, and tactile stimuli, the CS+ and CS- are presented but ethanol is withheld. For the context-induced renewal test, rats are returned to Context A, and the CS+ and CS- are presented without ethanol. Please click here to view a larger version of this figure.

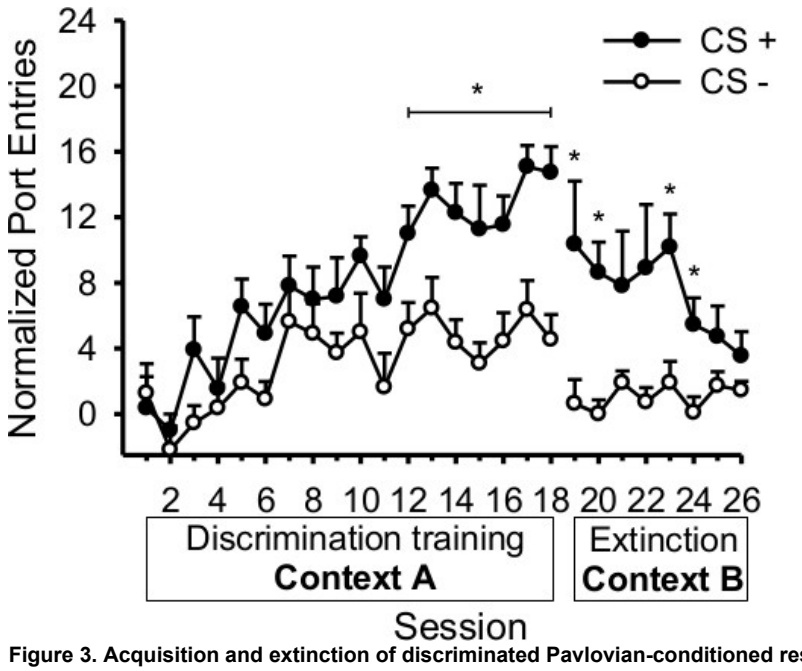

Figure 3. Acquisition and extinction of discriminated Pavlovian-conditioned responding for ethanol. Mean ± SEM normalized port entries made during the CS+ and CS- across PDT (sessions 1 – 18) in Context A and extinction (sessions 19 – 26) in Context B. These data were obtained using male Long-Evans rats, trained with 10% (v/v) ethanol as the unconditioned stimulus (US) that was presented in association with the CS+ during PDT. *p <0.05, CS+ versus CS-. Figure originally published in Chaudhri, Sahuque, & Janak (2008). Used with permission from Elsevier.

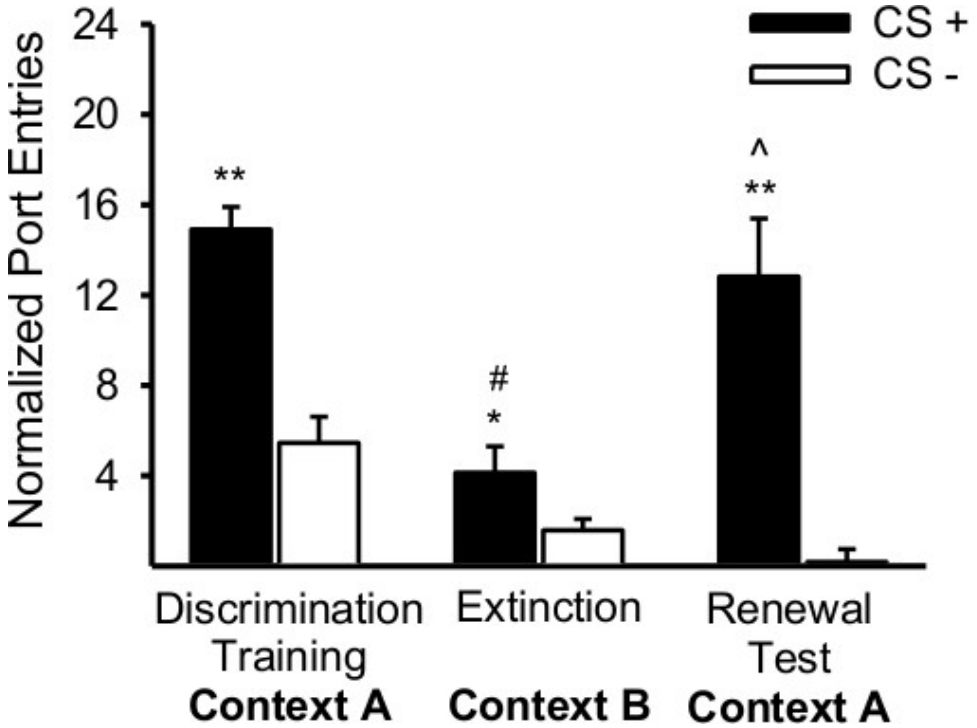

Figure 4. Context-induced renewal of Pavlovian-conditioned responding for ethanol. Mean ± SEM normalized port entries made during the CS+ and CS- in PDT sessions in Context A, extinction sessions in Context B, and renewal test in Context A. Data are averaged over the last two sessions for PDT and extinction; the renewal test is a single session. These data were obtained using male Long-Evans rats, trained with 10% (v/v) ethanol as the unconditioned stimulus (US) that was presented in association with the CS+ during PDT. *p <0.05; **p <0.001, CS+ versus CS-. #p <0.05, CS+ PDT versus CS+ extinction. ^p <0.05, CS+ extinction versus CS+ test. Figure originally published in Chaudhri, Sahuque, & Janak (2008). Used with permission from Elsevier.

Discussion

Results from this procedure reveal that discrete environmental stimuli that routinely accompany alcohol delivery can acquire the capacity to drive alcohol-seeking behavior. They also demonstrate that contextual stimuli associated with the prior availability or absence of alcohol can guide conditioned behavioral responses to discrete alcohol-predictive cues.

Critical steps within the protocol

Contexts in the present task incorporate stimuli that are external to the rat. However, the interoceptive state of the rat at test can also constitute a 'context' that could affect renewal[20]. It is therefore important to habituate rats to any procedures that may occur before the renewal test and change their interoceptive state, like intracranial microinfusions or systemic injections. Moreover, habituation to microinfusions or systemic injections should be conducted prior to both extinction and PDT sessions, to prevent such procedures from becoming associated with one specific phase of the experiment.

Temporal parameters, such as CS duration, CS-US interval, and intertrial interval (ITI), have long been known to influence conditioned responding in a variety of conditioning paradigms[21-26]. Similarly, temporal parameters may influence the observation of the renewal effect in our procedure. Any set of parameters other than those specified in the protocol should be experimentally validated in pilot studies before proceeding to employ them in larger investigations.

Modifications and troubleshooting

Previous research has found that the amount of ethanol that rats consume in the home cage can vary as a function of the supplier from which rats are purchased[17,27]. Supplier is therefore an important consideration for this phase of the study. Similarly, the choice of rat strain should be considered carefully, as there are numerous reports of strain differences in ethanol consumption[28-31].

Prior work in an aversive conditioning paradigm has shown that the developmental age of rats influences the observation of renewal of a Pavlovian conditioned fear response[32]. The lack of a renewal effect in very young rats (younger than postnatal day 20) has been suggested to be due to impaired encoding of contextual information[33]. The developmental age of rats to be used in the present procedure should therefore be taken into consideration.

Renewal of Pavlovian conditioned alcohol-seeking behavior has been observed when 10%[9,16], 15%[14], or 20%[15] ethanol solutions were used during the home cage ethanol access and PDT phases of the experiment. Researchers could use any of these concentrations of ethanol for the purposes of establishing this procedure in their laboratories.

During the acquisition of Pavlovian discrimination training it is important to check fluid ports after each session to ensure that all the ethanol that was delivered has been consumed. Leaving rats in the conditioning chambers for an additional 10 - 15 min after each session may be done early in PDT to encourage consumption of any ethanol that was not consumed during the session. The number of rats leaving unconsumed ethanol in the ports should decrease as PDT progresses. If by the end of PDT rats are either (a) not consuming all the ethanol and/or (b) not responding to the CS+ then these subjects should be dropped from the study. Most rats reliably respond more to the CS+ than the CS-. However, some rats may respond at high levels to both cues, thereby not showing evidence of discrimination. Rats that show high responding to both the CS+ and CS- during PDT are not dropped from the study. In our experience, these subjects show remarkable discrimination during the first extinction session in the absence of ethanol, suggesting that their high levels of responding to the CS- during PDT are driven by the presence of ethanol in the fluid port. These subjects also tend to show robust renewal effects.

Limitations of the procedure

Because the renewal test is conducted in the absence of ethanol, conditioned port entries elicited by the CS+ gradually diminish across the test session. Therefore, the overall amount of behavior generated at test, against which experimentally induced changes can be assessed, is fairly low. Because in this task responding tends to be higher at the start versus the end of the test session, it is important to examine the effects of experimental manipulations on conditioned responding on a trial-by-trial basis.

Entries into the fluid receptacle provide the main dependent measure in this task. However, an erroneous port entry might be registered if an animal is close enough to the fluid receptacle to break the infrared beam across the opening of the fluid port with its whiskers. While infrequent, erroneous responses are obvious as a dramatic increase in total port entries relative to the subject's behavior on prior sessions. Videotaping the animals would facilitate the detection of possible erroneous responses, and allow for a more refined analysis of the behaviors exhibited by the rats throughout training and testing.

Significance of technique with respect to existing/alternative methods

Context-induced renewal of drug-seeking behavior is largely studied using an instrumental conditioning task in which the primary dependent variable is an operant response, such as a lever press[8,34] or nose poke[12]. In the present procedure, the drug-seeking response is 'conditioned approach' to the fluid port that is elicited by presentations of the CS+. This response is likely acquired through Pavlovian learning, which is the learning process that mediates the formation of associations between environmental stimuli and the pharmacological effects of drugs of abuse in humans.

Future applications

This task can be used in combination with neuropharmacology, optogenetics and neurochemistry to study the neural mechanisms that are involved in context-induced renewal of Pavlovian-conditioned alcohol-seeking behavior[16]. In addition, behavioral and neural mechanisms that regulate the acquisition and extinction of Pavlovian-conditioned alcohol-seeking behavior can be investigated. Lastly, this task can be used to

explore manipulations of extinction that might prevent renewal, an important direction for translational research aimed at reducing the impact of drug associated environmental contexts on reactivity to drug predictive cues after cue exposure therapy in human addicts.

Disclosures

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