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Outcome-specific Pavlovian-to-instrumental transfer (PIT) with alcohol cues and its extinction

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Abstract

The acquired motivational impact of conditioned stimuli has been studied using the Pavlovian-to-instrumental transfer (PIT) task, where a cue paired with a reward is consistently shown to energize responses separately trained with that same reward (“specific” PIT). However, most alcohol studies have shown that alcohol-related cues elevate responses trained with either the same alcohol reward or with other non-alcoholic rewards (“general” PIT). The effects of extinction on this alcohol PIT effect have not been fully explored. We tested the hypothesis that cues signaling different tasting alcohols might acquire specific craving reactions for those alcohols leading to specific PIT, but that these effects might be sensitive to extinction. Three experiments examined the specificity of PIT using alcohol and non-alcohol outcomes. Rats first consumed different-flavored alcohol solutions in their home cages. Then they were trained to perform two responses, each reinforced with distinctly flavored solutions, using a Polycose fading procedure. The outcomes were sweet (4% sucrose) or salty (0.9% NaCl) ethanol (10% v/v) solutions (Experiments 1 and 2) or one plain or salty alcohol vs a nonalcoholic sweet solution (Experiment 3). Then, two cues were each differentially paired with these outcomes. In PIT tests animals performed both responses in the presence and absence of these cues without any rewards. Experiments 1, 2, and 3 showed that a cue paired with a flavored alcohol produced a small but consistent bias towards the response trained with the same alcohol solution (specific PIT). Experiment 2 showed that extinction eliminated this effect. Experiment 3 demonstrated that specific PIT occurred when contrasting salty, or plain, alcohol to a non-alcoholic solution. These results provide evidence that alcohol-related stimuli can elicit craving for specific types of alcohol (as revealed by specific PIT), but that this effect is sensitive to extinction. This paradigm of contrasting two distinctly flavored alcohols may be an especially useful animal model of alcohol addiction.

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Keywords

Pavlovian-to-instrumental transfer; alcohol; addiction; self-administration; extinction

Associative learning has played an important role in theories of addiction (Conklin & Tiffany, 2002; Davis & Gould, 2008; Everitt, Dickinson & Robbins, 2001; Everitt & Robbins, 2005; Hyman, Malenka & Nestler, 2006; Koob et al., 1989; Stewart et al., 1984; Robinson & Berridge, 1993), and there is ample evidence confirming that many of the phenomena observed in the literature, using non-drug rewards, can translate directly into addiction research (e.g., Bevins & Palmatier, 2004; Di Chiara, 1999; Everitt et al., 2001; Kelley & Berridge, 2002). For example, research on alcohol-motivated behavior has demonstrated that cues paired with alcohol can energize independently-trained alcohol-seeking behaviors (Milton et al., 2012) – a phenomenon known as Pavlovian-to-instrumental transfer (PIT) – in a way that parallels the effect seen when non-drug rewards are used (e.g., Holland, 2004). This PIT effect is especially of interest not only because it is related to problems of relapse following extinction of drug taking (Garbusow et al., 2016; O'Brien et al., 1998) and it provides the researcher with alternative measures of addiction-relevant responding (e.g., Kosten & Meisch, 2013; Nieto & Kosten, 2017), but also because it shows how Pavlovian processes may factor into the motivation to initiate drug taking more generally.

Research investigating the PIT phenomenon (e.g., Cartoni, Balleine & Baldassarre, 2016; Holmes, Marchand & Coutureau, 2010) has revealed that Pavlovian cues can stimulate instrumental responding in two ways. The *outcome-specific* PIT effect has been demonstrated when a Pavlovian conditioned stimulus (CS) selectively modulates separately-trained instrumental responses that were previously reinforced with the same reward compared to a different reward from that signaled by the CS (e.g., Colwill & Rescorla, 1988; Delamater, 1995; Kruse, Overmier, Konz & Rokke, 1983). However, *general* PIT has also been shown and this refers to the finding that a CS can also non-selectively elevate instrumental responding even when that response was previously reinforced with a different outcome from that signaled by the CS (e.g., Corbit & Balleine, 2005; 2011).

These two forms of PIT illustrate how Pavlovian stimuli can acquire the capacity to affect instrumental behaviors in different ways. Whereas outcome-specific PIT is usually considered to be mediated by a specific outcome expectancy process, general PIT is thought to reflect activation by some general “drive” or “emotional” process (see Corbit & Balleine, 2005; Delamater, 2012; Balleine & Killcross, 2006). In addition, neurobiological studies have shown that each form of PIT depends on different neural substrates (Corbit & Balleine, 2005; 2011), and, therefore, the study of PIT using alcohol outcomes provides us with a tool to assess the neurobiological underpinnings of how alcohol cues affect alcohol seeking (Corbit, Fischbach & Janak, 2016).

There have been a number of reports examining PIT effects on alcohol seeking, and the data generally show that Pavlovian cues activate alcohol seeking in a non-specific way (reflecting general PIT), and there has been little evidence that outcome-specific PIT operates in these settings (for a review see Lamb, Schindler & Pinkston, 2016). This is somewhat surprising given that human alcohol drinking often occurs in naturalistic situations where there is a

plethora of alcohol choices as well as cues advertising those choices. Under such conditions, one might anticipate that different environmental stimuli could acquire the capacity to elicit specific craving reactions for distinctly flavored alcohols. This, in turn, could strongly affect choice to consume a particular alcohol. PIT studies exploring this possibility have often trained animals to learn two response-outcome (R-O) and two stimulus-outcome (S-O) relationships using alcohol as one of the outcomes and liquid sucrose as the other. They have shown that a CS paired with alcohol not only elevates alcohol-motivated responding, but also responses trained with non-alcoholic rewards (Corbit et al., 2016; Corbit & Janak, 2007; Glasner, Overmier & Balleine, 2005). For instance, Corbit and Janak (2007; Experiment 2) presented rats with two auditory cues, one of them paired with a 10% ethanol solution and the other with a 2% sucrose solution. In the next phase of the experiment, pressing one lever was reinforced with ethanol and the other with sucrose. During an extinction test session, presentations of the CS paired with sucrose selectively elevated sucrose-reinforced lever pressing, but had little effect on alcohol-reinforced lever pressing, i.e., outcome-specific PIT. In contrast, the CS paired with ethanol increased both levers equally, a result consistent with general PIT (Corbit et al., 2016; Corbit & Janak, 2007; Glasner et al., 2005).

However, there is recent evidence supporting the view that specific PIT can be found using alcohol cues. Lamb and colleagues (Lamb, Ginsburg & Schindler, 2016; Experiment 3) trained rats on a concurrent schedule to press one lever reinforced with an ethanol solution (fixed ratio 5 schedule) and another with food pellets (fixed ratio 10 schedule). In the Pavlovian conditioning phase only one CS was trained, and it was paired with ethanol. At test, CS presentations elevated responding to the lever reinforced with ethanol relative to a baseline (pre-CS period), but the CS did not elevate responding to the lever trained with food pellets. Furthermore, after this test, animals received additional presentations of the CS in the absence of ethanol, i.e., extinction, and a new PIT test revealed that the CS lost its ability to elevate responding.

These data are interesting because not only do they support a role for specific PIT in alcohol seeking, but they also suggest that such effects are sensitive to extinction – a result that is not always found in the literature with non-drug rewards (e.g., Delamater, 1996; 2012; but see Delamater, Schneider & Derman, 2017). However, one shortcoming of this study is that the selectivity of the cue effects cannot so easily be observed when only one CS is tested. Responses may differ in the degree to which they can be energized by external stimuli. Perhaps the alcohol response in this study was more sensitive than the pellet reinforced response to any CS, regardless of what that CS predicts. This interpretation would be ruled out if a pellet CS were to selectively energize a pellet response, while an alcohol CS were to selectively energize an alcohol response. It was with this experimental design that other studies have consistently failed to reveal specific PIT (Corbit et al., 2016; Corbit & Janak, 2007; Glasner et al., 2005). Another potential shortcoming of the Lamb et al. (2016) study is that the generality of the alcohol PIT effect has not been well established. In the same report Lamb et al. (2016; see also Lamb, Ginsburg & Schindler, 2017) failed to observe PIT when only a single alcohol-reinforced response was trained on a random interval schedule and several different Pavlovian training procedures were employed. Moreover, the extinction manipulation used in the Lamb et al. (2016) report did not include a non-extinguished control, so it is impossible to determine if the loss of any PIT effect was due to the

manipulation or the simple passage of time. In spite of these shortcomings, we think these results are worth pursuing because identifying the conditions responsible for specific and/or general PIT and its extinction could, ultimately, be helpful in understanding the mechanisms of alcohol abuse.

Given the findings noted above, it is not clear why an alcohol CS might affect instrumental responding differently from a CS that signals a non-drug reward. Understanding how Pavlovian stimuli might instigate alcohol drinking requires a determination of whether or not both of these types of PIT effect contribute to alcohol seeking. For instance, it seems possible that different alcohol cues may elicit “craving” for specific alcoholic drinks, in which case we would expect to find outcome-specific PIT effects. On the other hand, prior research suggests that alcohol cues often operate in a different way, by non-selectively energizing the motivation to work for alcoholic and non-alcoholic rewards alike. This latter possibility suggests that the sensory features of the alcohol used in these studies do not support outcome-specific PIT in the same manner as those of the sucrose reward.

Alternatively, it is possible that discrimination training may be required among differently flavored alcohol solutions before the animal’s behavior is strongly controlled by their distinctive sensory features. The aim of the study reported here is to evaluate the selectivity of PIT produced by CSs that signal alcohol on instrumental responses trained with alcohol (Experiment 1 and 2), and on responses trained with non-drug rewards (Experiment 3), by using a design that allows a distinction between general and outcome-specific PIT. In Experiment 1 two levers were each trained with a distinctively flavored ethanol solution (sweet or salty), and two CSs were then differentially paired with these solutions. The aim of Experiment 2 was to assess the effect of extinction on PIT: one group of subjects received Pavlovian extinction sessions before the PIT tests while a control group only received context exposure. Experiment 3 assessed the generality of our findings of outcome-specific PIT when one of the outcomes was an ethanol solution and the other a non-drug solution (sucrose), as had been done in prior work. One group of rats was trained with sucrose and flavored ethanol solutions while a second group was trained with sucrose and unflavored ethanol solutions (as was done by Corbit & Janak, 2007). In all these experiments, the effect of the CSs on instrumental responding was assessed in a series of PIT tests during which the animals chose between two responses in the presence and absence of the Pavlovian CSs.

Experiment 1: Ethanol Specific PIT

Method

Subjects—Subjects were 8 experimentally naïve Long-Evans rats (4 males, 4 females), bred at Brooklyn College, but derived from Charles River laboratories. At the beginning of the experiment the males’ weights varied between 506 g and 679 g, and the females’ weights between 314 g and 355 g. All the animals were individually housed under a 14 hr light/ 10 hr dark cycle, and they had unrestricted access to food and water throughout the entire experiment.

Apparatus—The experiments were conducted in 8 operant chambers (ENV-008; Med-Associates), each of them with two retractable levers in one of the walls. A 5 cm trough was

located between the two levers (3 cm from each), and it contained two wells into which the outcomes were dispensed. Each chamber was equipped with two syringe pumps, and each of them delivered a 0.1 ml droplet of a solution into the trough. One pump delivered the liquid into the right well from an opening in the wall behind the well, and the other pump dropped the solution on the magazine floor in the left compartment. The chambers were also equipped with speakers that, when activated, produced a white noise 70 dB above background. Also an 1820 mini bulb located on the top of the opposite wall of the trough emitted, when activated, a flashing light. This light remained on for 0.5 s and off for 0.3 s, cycle that was repeated for the duration of the stimulus. The chambers were dark during the experimental sessions, except when the flashing light was presented. A computer equipped with Med-PC software (Med-PC IV; Med-Associates), controlled the stimuli presentation, and recorded the magazine entries and lever presses.

Induction of ethanol drinking—Animals had alternating access to a sweet (4% sucrose) or salty (0.9% NaCl) solution in their home-cages for 30 days (see Table 1). This concentration of NaCl was chosen because intake studies suggest it to be more closely matched to the palatability of our sucrose solution (Tordoff, Alarcon, & Lawler, 2008). On days 1–2, animals received a Polycose solution (16% w/v) without ethanol. Polycose was chosen as a carrier solution because it appears to stimulate a distinct non-sweet carbohydrate receptor, rats readily discriminate it from sucrose and salt, and rats find it highly palatable on its own (Sclafani & Mann, 1987). Then, the sucrose and NaCl were diluted in a high content polysaccharide (Polycose) solution (14% w/v), with a low content of ethanol (2% v/v). The amount of Polycose was gradually reduced across days until it was completely removed from both sweet and salty solutions. In contrast, the ethanol concentration was increased, until it reached a concentration of 10% v/v. In addition, the access to these solutions was gradually reduced from 24 hours to 1 hour per day. Table 2 shows how the relative concentrations used and hourly access periods to these EtOH/Polycose solutions changed across days. The animals were weighed and the amount of solution consumed was recorded daily.

Instrumental training phase—Subjects were then trained to press two levers, each reinforced with a different ethanol solution (sweet or salty). To increase the reinforcing properties of the outcomes, Polycose was added to the solutions during the first part of this phase, but its concentration was progressively reduced until it was completely removed from the solutions. Initially both outcomes consisted of a 10% EtOH v/v/10% Polycose w/v solution (with either 4% sucrose or 0.9% NaCl). Animals were first trained on a continuous reinforcement (CRF) schedule, until they reached 50 responses within a session. Then they received, for each lever, 2 sessions under a random ratio (RR) 2 schedule, followed by 2 sessions under a RR 4, and 2 sessions under a RR 6 schedule. After this, the Polycose concentration of the solutions was reduced to 5%, and animals received 4 additional sessions under the RR 6 schedule. Then the Polycose was completely removed from the solutions and subjects received 4 more sessions with the RR 6 schedule. Two 40minute sessions were conducted daily, one for each lever, with at least one hour between them. For half of the animals, the left lever was reinforced with the sweet alcohol solution and the right lever with the salty alcohol solution. The reverse was true for the remaining animals. In addition,

subjects were first trained on the left lever half of the days, and on the right lever the rest of days.

Pavlovian conditioning phase—Subjects received eight sessions of Pavlovian conditioning, in which the flash and the noise were each paired with one of the outcomes. For half of the animals, the flash was paired with the sweet alcohol solution (4% sucrose w/v/10% EtOH v/v), and the noise was paired with the salty alcohol solution (0.9% NaCl w/v/10% EtOH v/v). Polycose was not used during this phase. The reverse was true for the other half of the animals. Each trial consisted of a CS presented for 90 seconds (to provide enough sampling time to subsequently assess PIT effects), during which the corresponding outcome (0.1 ml) was delivered on a random time (RT) 30 s schedule. On average, there were 3 outcome deliveries on each trial, but because of the random nature of the schedule individual trials could include fewer or more than 3 deliveries. Each session lasted 52 minutes, and contained four trials of each CS type, presented in a random order, with an average intertrial interval (ITI) of 5 minutes (ranging from 3 to 7 minutes). Magazine entries were recorded during the CS presentations and also during the 90 seconds that preceded the CS onset (baseline pre CS period).

PIT tests—Subjects received four PIT tests, in which both levers were concurrently available, and the effect of the CSs on instrumental performance was assessed in the absence of the outcomes. The duration of the tests was 28 minutes, and they comprised four trials of each CS type. In the first four minutes, no stimuli were presented in order to familiarize subjects with the choice procedure and to moderately extinguish their responses. This was followed by eight cycles of a pre-CS period (90 s) immediately followed by a CS presentation (90 s). Magazine entries and lever responses were recorded during the pre-CS and CS periods. Animals received an instrumental retraining session for each lever the day before each test. These sessions were identical to the last four sessions of the instrumental training phase. Multiple PIT tests were conducted in order to ensure a high level of sampling of behavior for each stimulus condition.

Experiment 2 Extinction of Ethanol Specific PIT

Subjects and apparatus

Subjects were 32 experimentally naïve Long-Evans rats (16 males, 16 females) from Charles River laboratories. At the beginning of the Experiment the males' weights varied between 329 g and 385 g, and females' weights between 237 g and 261 g. The animals were maintained under the same conditions as in Experiment 1, and the apparatus was the same.

Induction of ethanol drinking

Animals had alternating 24 hours access to a sweet (4% sucrose) and a salty (0.9% NaCl) solution in their home-cages for 12 days (rather than for 30 days as in Experiment 1 because the procedures described below more efficiently induced alcohol consumption prior to instrumental training). Initially, the ethanol/Polycose concentration was 2%/16%, but the amount of ethanol was gradually increased as the amount of Polycose was reduced, until the solutions were at 10%/10% ethanol (v/v)/Polycose (w/v) concentration from days 7 to 12 of

this induction phase (see Table 3). Animals received intermittent access to the solutions. After two days of ethanol access (one day for each solution type) the ethanol bottles were removed for 24 hours (Pinel & Huang, 1976; Simms et al., 2008; Wise, 1973). After this, ethanol access was resumed. The animals were weighed and the amount of solution consumed was measured daily.

Instrumental training phase

Because it was noted in Experiment 1 that most of the instrumental responses were performed in the first half of the sessions, the length of these sessions was reduced from 40 to 20 minutes. Also, animals received two rather than four sessions with an RR 6 schedule using the 5% Polycose solutions. The rest of this phase was identical to that of Experiment 1.

Pavlovian conditioning phase

Acquisition—Subjects received 12 instead of 8 sessions of Pavlovian conditioning. Based on the PIT results of Experiment 1 we gave additional training to determine if this might amplify the magnitude of the PIT effect. The rest of this phase was identical to that of Experiment 1.

Extinction—Subjects were divided into two groups with equal number of males and females, and with similar rates of responding in the instrumental and Pavlovian conditioning phases. Group Extinction received 12 sessions in which the CSs were presented as in acquisition sessions, but no outcomes were delivered. Animals in Group No Extinction also received 12 sessions of exposure to the experimental contexts, but no CSs or outcomes were presented. Magazine entries were recorded during the pre CS and CS periods in Group Extinction and during equivalent “dummy” periods in Group No Extinction.

PIT tests—As in Experiment 1, animals received one session of instrumental retraining for each lever the day before each of the PIT tests. In addition, and to familiarize the animals with the choice situation prior to the tests, in this experiment all subjects were placed in the operant chambers for five minutes the day between the first instrumental retraining session and the first PIT test. During this time, both levers were available but no outcomes were delivered. The rest of this phase was identical to that of Experiment 1, and included a total of 4 PIT tests.

Experiment 3 Specific PIT: Ethanol vs Sucrose

Subjects and apparatus

Subjects were 32 experimentally naïve Long-Evans rats (16 males, 16 females) bred at Brooklyn College, but derived from Charles River laboratories. At the beginning of the Experiment the males' weights varied between 462 g and 603 g, and females' weights varied between 234 g and 314 g. The animals were maintained under the same conditions as in Experiment 1 and 2, and the apparatus was the same.

Induction of ethanol drinking

There were two differences in this stage compared to Experiment 2. First, subjects were divided into two groups. Group EtOH Alone received access to a 4% sucrose solution in water (w/v) half of the days and to an ethanol solution the other half. Group Ethanol+NaCl received access to the same 4% sucrose solution on some days, but the ethanol solution was flavored with 0.9% NaCl on other days. The ethanol (v/v)/ Polycose (w/v) concentrations of the solutions in both groups changed as in Experiment 2 (see Table 4). In order to match overall intakes of these two solutions, the amount of sucrose was limited to approximate the consumption of the corresponding alcohol solution. The second difference was that animals had access to one of these solutions each day without any off days. Because animals had access to a non-alcoholic sweet solution one of the days, we regarded this ethanol access regime to be intermittent.

Instrumental training phase

For all the animals one of the levers was reinforced with the sucrose solution (4% sucrose), and the other with the ethanol solution (counterbalanced). For group EtOH+NaCl the ethanol solution also included 0.9% NaCl. The ethanol and Polycose concentrations were manipulated as in Experiment 2, and the rest of this phase was also identical to that of Experiment 2.

Pavlovian conditioning phase

Subjects were divided into two groups as in Experiment 2. Both groups received pairings of one CS with the sucrose solution (4% sucrose), and the other CS with the ethanol solution. The only difference between the groups was that for the group EtOH+NaCl the ethanol solution also included 0.9% NaCl. The rest of this phase was identical to acquisition in Experiment 2.

PIT tests

Everything was identical to Experiment 2, except that after two PIT tests all animals received two additional days of instrumental retraining followed by four days of Pavlovian conditioning. These retraining sessions were performed in order to reestablish both instrumental and Pavlovian baselines prior to the additional round of PIT tests. All subjects then received two additional PIT tests, each preceded the day before by a single instrumental retraining session with each lever.

Data treatment

The PIT test data were analyzed by using one-way repeated measures ANOVA tests. Significant differences were further analyzed with post-hoc tests and the tables of critical F values of Rodger (1974; 1975). For all significant ANOVAs, this method examines the source of differences between means with a set of ν_1 linearly independent and mutually orthogonal posthoc contrasts. Type I error, by this method, has a decision-based error definition whereby the proportion of true null contrasts rejected in error was set at 5%. The analysis provides two measures of effect size. The first is an estimate of the noncentrality parameter (), based on Perlman and Rasmussen's uniformly minimum variance unbiased

estimator (Perlman & Rasmussen, 1975), that reflects the overall amount of variation among the population means justified by the empirical data. The second is a calculation of the implied population means, which are derived from the set of statistical decisions for the post-hoc contrasts. These implied means are expressed as a difference between an individual mean, in question, and the overall average of all the population means included in the analysis, and they are reported in σ units that can be interpreted similar to Cohen's d (Cohen, 1988). Thus, a difference of 1 σ unit represents a large difference between two conditions. These methods of analysis were chosen over other ANOVA techniques because they are relatively powerful at detecting true effects (Rodger & Roberts, 2013) and because they offer quantitatively precise conclusions regarding effect sizes. All statistical procedures were conducted using software that is freely available for download from the following site: <https://sites.google.com/site/spsprogram/home>. Additional analyses using more conventional mixed-design ANOVA methods largely agreed with the analyses reported in the text below, and these are provided in the supplemental material. The data from the induction of ethanol drinking period, instrumental training phase, and Pavlovian conditioning phase were analyzed by using *a priori* t-tests.

Before the analysis of the PIT tests the data were grouped as 'same' and 'different', depending on whether the CS presented and the response performed in a particular trial were both paired in training with the same or different outcome. For instance, if a CS signaled the delivery of the sweet solution, the responses performed during the pre-CS and CS periods that were previously reinforced with the sweet solution were grouped as 'same'. The responses previously reinforced with the alternative outcome, e.g., salty solution, were grouped as 'different'. Both types of responses (same and different) were monitored both during the CS and during a pre CS period. If the CS had non-selectively elevated both types of response equally during the CS period, this was taken as evidence of general PIT. In contrast, if the CS biased responding in favor of the 'same' over the 'different' response, the PIT effect was considered *outcome-specific*. To further illustrate this effect, difference scores were calculated by subtracting pre CS responding from CS responding, and by further subtracting 'different' responses from 'same.' This gave an overall specific PIT score that was then compared between groups.

Results

Experiment 1 Ethanol Specific PIT

Induction of ethanol drinking—The animals' daily mean solution intakes (sweet and salty), and the ethanol consumed (in grams per kilogram) are presented in Table 2. Animals consumed more quantities of the sweet than of the salty solution. In the last day of one-hour access to the solution, the ethanol intakes from the sweet and salty solutions, respectively, were 2.4 g/kg (SEM = 0.2) and 1.6 g/kg (SEM = 0.2), a difference that was statistically significant, $t(7) = 6.86$, $p < .001$.

Instrumental training—Lever responding (rpm) in the instrumental phase was grouped in 2-session blocks (see Figure 1, top panel). Subjects performed both instrumental responses, but they responded more for the sweet than for the salty reinforcer. In the last two days of

training subjects performed a mean of 6.3 rpm (SEM = 0.6) for the sweet solution, and 3.7 rpm (SEM = 0.6) for the salty alcohol solution. This difference was statistically significant, $t(7) = 3.3$, $p < .05$. The average amount of the sweet solution consumed in the last two days of this phase was 3.94 ml (SEM = 0.26), corresponding to 0.87 g/kg (SEM = 0.13) of ethanol, and of salty solution was 2.68 ml (SEM = 0.31), corresponding to 0.61 g/kg (SEM = 0.12) of ethanol. Responding to both levers numerically increased from the beginning of training (overall mean = 4.1 rpm (SEM = 0.2)) to the end of training (mean = 5.0 rpm (SEM = 0.5)), but this difference was not statistically significant, $p = .07$.

Pavlovian conditioning phase—Mean magazine responses within the CS periods are shown across training in the form of different scores (CS - pre CS) (Figure 1, bottom panel). The mean magazine responses during the pre CS periods were stable across training and averaged 5.0 rpm (SEM = 0.6). The rats increased the rate of responding across training to both CSs. The mean rate of magazine responding (to both CSs combined) in the first day of training was 2.3 rpm (SEM = 1.3), and in the last three days was 17.3 rpm (SEM = 3.1). This difference was statistically significant, $t(7) = 4.1$, $p < .01$. In each session animals received, on average, 1.2 ml of each solution, and the average ethanol consumption in the last session of this phase was 0.52 g/kg (SEM = 0.06). Although the Figure suggests that animals responded more during the CS paired with the sweet solution than during that paired with the salty solution, this apparent difference was not reliable over the last three days of training. It is worth pointing out that this data is difficult to interpret as a measure of Pavlovian learning since it does not distinguish between magazine responses that may have occurred before versus after the alcohol solution was delivered. Thus, it can be seen as a mixture of unconditioned and conditioned magazine responses alike.

Pavlovian-to-instrumental transfer tests—The data of primary interest is presented in Figure 2. We collapsed over the sex variable (in all of the experiments reported here) because no differences were observed between males and females. Lever responding across the four PIT tests were collapsed and presented in the form of ‘same’ and ‘different’ responses for the pre CS and CS periods of the PIT tests (see top panel of Figure 2). The CSs elevated lever responding when the CS and the lever were both paired with the same outcome in training, but they had no effect on responding when they were paired with a different outcome. An ANOVA on these data showed a significant main effect, $F(3, 21) = 2.11$, $MSE = 0.3$, $\eta^2 = 2.86$, $p < 0.05$. Post-hoc contrasts confirmed that ‘same’ and ‘different’ responses did not differ in the pre CS period and that these did not differ from ‘different’ responses during the CS, $F_s < 1$. Most critically, ‘same’ responses during the CS were significantly higher than responding in the other three conditions combined, $F(3, 21) = 2.14$, $p < 0.05$. The implied population means estimate the size of the same vs different effect to be approximately 0.57 σ units, which we interpret to be a small-to-moderate sized effect.

To further explore the differential effect produced by the cues’ presentations on responding, difference scores (CS – pre CS) were calculated and plotted in the bottom panel of Figure 2, which shows elevation on the ‘same’ but not on the ‘different’ response. A one-tailed t-test confirmed more ‘same’ than ‘different’ responses, $t(7) = 2.71$, $p = .03$. In addition, and to

illustrate the specificity of the effect, the overall specific PIT score (same – different) revealed a difference of approximately 0.6 rpm (see ‘S-D’ in bottom panel of Figure 2).

Experiment 2 Extinction of Ethanol Specific PIT

Induction of ethanol drinking

The mean daily alcohol intake in the induction period of Experiment 2 is presented in Table 3, which shows that both groups consumed similar quantities of both types of solutions. The mean ethanol consumption in the last day of access to the sweet solution was 13.9 g/kg (SEM = 1.1) in Group Extinction, and 14.0 g/kg (SEM = 0.7) in Group No Extinction, a difference that was not statistically significant, $p = .88$. The mean ethanol consumption of the salty solution was 12.3 g/kg (SEM = 1.2), in Group Extinction, and 13.7 g/kg (SEM = 0.8), in Group No Extinction, $p = .17$. Overall, no differences were found in the consumption of ethanol between the sweet and salty solutions, $p = .08$.

Instrumental training

Mean responding (rpm) to the levers reinforced with either the sweet or salty solutions is presented in Figure 3 in 2-session blocks. Subjects increased their rate of responding across training; however, by the end of training responding was higher for the lever reinforced with the sweet than the salty alcohol solution, $t(31) = 4.3$, $p < .001$. The average sweet solution consumed in the last two days of training was 3.69 ml (SEM = 0.24), corresponding to a 0.96 g/kg (SEM = 0.08) of ethanol, and the average consumption of the salty solution was 2.79 ml (SEM = 0.15), which corresponds to 0.71 g/kg (SEM = 0.04) of ethanol. In addition, the overall rate of responding in the first block of training was lower (5.4 rpm (SEM = 0.3)) than in the last block (10.1 rpm (SEM = 0.6)), $t(31) = 8.4$, $p < .001$.

Pavlovian conditioning phase

Mean magazine entries (CS - pre CS) to the two CSs across 3-session blocks shows that subjects increased their rate of responding across training, and also that responding during both CSs was comparable (Figure 4). No significant differences were found between the groups in responding during the pre CS periods (5.6 rpm (SEM = 0.3) for Group Extinction and 5.9 rpm (SEM = 0.35) for Group No Extinction). Responding in the last block of training during the CSs paired with the sweet and salty alcohol solutions did not differ, $p = .36$, but responding to both CSs, combined, increased from the first to the last block of training (means = 8.4 rpm (SEM = 0.8) vs 28.2 rpm (SEM = 1.6)), $t(31) = 11.12$, $p < .001$. The average ethanol consumption in the last session of this phase was 0.58 g/kg (SEM = 0.02) for Group Extinction, and 0.59 g/kg (SEM = 0.02) for Group No Extinction. Magazine entries (CS - pre CS) performed during extinction in each of the groups are also presented on Figure 4. Responding to the CSs during extinction showed a rapid decline in the first block of extinction with responding decreasing further by the end of extinction, $t(31) = 3.61$, $p = .001$. Responding during “dummy CS” and “dummy Pre CS” periods in Group No Extinction did not change across the extinction phase.

Pavlovian-to-instrumental transfer tests

The data from the PIT tests are presented in Figure 5 (as in Figure 2 above). Group No Extinction displayed a pattern similar to that seen in Experiment 1 with greater “same” than “different” responding occurring during CS presentation, but not during pre CS periods (top panel). However, this pattern was completely absent in Group Extinction (middle panel), as the stimuli lost their effect following the Pavlovian extinction phase. Separate repeated measures ANOVAs performed on each group using a pooled error term ($MSE = 0.231$) revealed significant differences in Group No Extinction, $F(3, 90) = 5.58$, $\eta^2 = 13.4$, $p < 0.01$ but no significant differences in Group Extinction, $F < 1$. Post-hoc contrasts for Group No Extinction showed that ‘different’ responses during the CS were significantly lower than pre-CS same and different responses combined, $F(3, 90) = 2.82$, $p < 0.05$, that themselves did not differ. Furthermore, the highest level of responding was shown to the ‘same’ response during the CS, $F(3,90) = 2.51$, $p < 0.05$, compared the other three periods combined. Overall response levels did not differ between the groups, $F < 1$. The implied population means revealed that, once again, the size of the same-different selective PIT effect in Group No Extinction was approximately 0.62σ units (similar to Experiment 1). This was manifest as a slight increase of same responses over baseline responding and a more substantial decrease of different responses.

Difference scores (CS – pre CS) are presented in the bottom panel of Figure 5, which shows positive ‘same’ scores and negative ‘different’ scores in Group No Extinction, but no evident differences in Group Extinction. Statistical analysis of the difference scores revealed a significant difference between same and different scores in Group No Extinction, $F(1, 30) = 11.39$, $MSE = 0.47$, $\eta^2 = 9.63$, $p < 0.005$, but not in Group Extinction, $F < 1$. Overall responding did not differ between the groups. The specific PIT scores (i.e., taking a difference between ‘same’ and ‘different’ scores) for both groups are also plotted in the bottom panel of Figure 5 (striped bars). This shows that a higher specific PIT score was obtained in Group No Extinction than in Group Extinction. This was confirmed by statistical analysis, 2-tailed $t(30) = 2.13$, $p = .042$.

Experiment 3 Specific PIT: Ethanol vs Sucrose

Induction of ethanol drinking

Animals’ daily intakes in the induction period of Experiment 3, separated by group, are presented in Table 4. Subjects in the group ‘EtOH alone’ consumed slightly less ethanol than those in group ‘EtOH+NaCl’, but this difference was not statistically significant. The mean ethanol intake (g/kg) in the last 24-hours access to the ethanol solution was 12.7 g/kg (SEM = 1.0) and 14.5 g/kg (SEM = 1.0), in the group ‘EtOH alone’ and ‘EtOH+NaCl’ respectively, but the difference was not significant.

Instrumental training

Mean lever press responding generally increased over training and was somewhat higher by the end of training for the response reinforced by sucrose compared to alcohol, $t(15) = 2.19$, $p < .05$ and $t(15) = 3.59$, $p < .01$ for Group EtOH+NaCl and EtOH Alone (Figure 6). The only exception to this was that the response reinforced by unflavored alcohol was decreased

somewhat at the end of training when Polycose was entirely removed (Group EtOH Alone). In addition, overall responding in the final block of training in Group EtOH+NaCl was higher than those in Group EtOH Alone, $t(30) = 2.84$, $p < .01$. The average consumption of the ethanol solution in the last two days of this phase for Gp EtOH+NaCl was 3.18 ml (SEM = 0.41), corresponding to 0.65 g/kg (SEM = 0.06) of ethanol, and for Gp EtOH Alone was 1.19 ml (SEM = 0.13), which corresponds to 0.28 g/kg (SEM = 0.04) of ethanol.

Pavlovian conditioning phase

Mean magazine entries (CS - pre CS) during the Pavlovian phase are presented in Figure 7. No significant differences were found between the groups in responding during the pre CS periods (5.7 rpm (SEM = 1.1) for Group EtOH Alone and 7.4 rpm (SEM = 0.9) for Group EtOH+NaCl). Once again, both groups increased their rates of magazine responding to the CSs across Pavlovian conditioning, ($t(15) = 7.29$, $p < .001$, and $t(15) = 5.8$, $p < .001$, respectively, for Groups EtOH+NaCl and EtOH Alone), and the average ethanol consumption in the last session was 0.54 g/kg (SEM = 0.05) for Group EtOH Alone, and 0.56 g/kg (SEM = 0.05) for Group EtOH + NaCl. Group EtOH Alone (top panel) responded numerically more during the CS paired with sucrose than during the CS paired with ethanol, but this difference was not significant in Group EtOH Alone, $p = .07$ or Group EtOH+NaCl, $p = .61$ (in the final block). However, Group EtOH Alone responded less in the final block of training, overall, than Group EtOH+NaCl, $t(30) = 3.49$, $p < .01$.

Pavlovian-to-instrumental transfer tests

A similar pattern of results was found in the four tests so the data have been collapsed across them. In addition, we observed the same pattern of responding during CS and pre CS periods for the instrumental responses reinforced by sucrose and alcohol solutions. For example, based on the CS – pre CS difference score data there was more responding when the CS and response were both reinforced with the same than different outcomes, and this was true of the ethanol and sucrose levers alike (see Table 5). The overall specific PIT scores (same – different) for the ethanol and sucrose levers, respectively, were 0.65 (SEM = 0.23) and 0.37 (SEM = 0.32) rpm for Group EtOH Alone and 0.79 (SEM = 0.48) and 0.94 (SEM = 0.63) for Group EtOH+NaCl. There were no reliable differences within or across groups on this measure. Furthermore, baseline (i.e., pre CS) responding on the ethanol lever was lower than on the sucrose lever in both groups (means, respectively, were 0.9 (SEM = 0.14) and 2.0 (SEM = 0.31) rpm in Group EtOH Alone, $F(1,30) = 8.80$, $MSE = 0.994$, $\eta^2 = 0.23$, $p < 0.01$, and 1.8 (SEM = 0.30) and 2.6 (SEM = 0.34) in Group EtOH+NaCl, $F(1,30) = 5.18$, $MSE = 0.994$, $\eta^2 = 0.15$, $p < 0.05$).

Because of the similarity in the PIT effect across levers, we collapsed over the response factor to statistically examine the overall selective PIT effect. Figure 8 displays the data in this way (comparable to Experiments 1 and 2). Figure 8 shows more ‘same’ than ‘different’ responding in the CS but not pre CS periods, although this difference appeared somewhat reduced in Group EtOH Alone (top panel) who also responded less overall compared to Group EtOH+NaCl (middle panel). Separate repeated measures ANOVAs performed on each group (pooled $MSE = 0.409$) revealed significant differences in Group EtOH + NaCl, $F(3, 90) = 4.34$, $\eta^2 = 0.13$, $p < 0.01$, but not in Group EtOH Alone ($F(3, 90) = 1.22$, $p > 0.05$).

In addition, the analysis confirmed more responding, overall, in Group EtOH + NaCl than in Group EtOH Alone, $F(1, 30) = 11.45$, $MSE = 2.01$, $\eta^2 = 9.7$, $p < 0.005$. Post-hoc contrasts for Group EtOH + NaCl confirmed that subjects performed more ‘same’ than ‘different’ responses in the presence of the CSs, $F(3, 90) = 3.94$, $p < 0.01$, but not in the pre CS period, $F < 1$. In addition, overall responding during CS and pre CS periods did not differ, $F < 1$. The implied population means from this analysis revealed that the size of the same-different selective PIT effect in Group EtOH + NaCl was approximately 0.78σ units (slightly larger than in Experiments 1 and 2), and that this effect was manifested as a slight above-baseline increase of the same response and below-baseline decrease of the different response.

We also analyzed these data by taking CS – pre CS difference scores, as in Experiments 1 and 2 (Figure 8, bottom panel). The Figure shows positive ‘same’ scores and negative ‘different’ scores in both groups, although this difference seems smaller in Group EtOH Alone. Separate repeated measures ANOVAs performed on each group (pooled $MSE = 0.366$) confirmed greater “same” than “different” responding in Group EtOH + NaCl, $F(1, 30) = 16.4$, $\eta^2 = 14.3$, $p < 0.0005$, but also in Group EtOH Alone, $F(1, 30) = 5.73$, $\eta^2 = 4.3$, $p < 0.05$. Further, there was no overall between group difference, $F < 1$. These data imply a slightly larger selective PIT effect in Group EtOH + NaCl by this measure, 0.86σ than in Group EtOH Alone, 0.48σ . However, when we examined the selective PIT effect in each group (i.e., ‘same’ minus ‘different’ scores – striped bars in Figure 8) the two groups did not differ by a 2-tailed t-test, $t(30) = 1.17$, $p = .25$. Thus, overall, these data support reliable selective PIT effects in both groups. Evidently, taking CS – pre CS difference scores reduced the overall error variability enabling a significant selective effect to be revealed in Group EtOH Alone. It is worth pointing out that although the selective PIT effects appeared relatively small in both groups, 14 of the 16 rats in Group EtOH Alone displayed more ‘same’ than ‘different’ responses whereas 13 of the 16 rats in Group EtOH + NaCl displayed this as well.

General Discussion

Pavlovian stimuli that signal the availability of alcohol can exert a motivational effect on behavior by impacting instrumental responding in PIT tasks. The main aim of the present study was to assess the specificity of this effect and to determine its sensitivity to extinction. Our findings revealed that cues that predicted a distinctively flavored alcohol solution selectively influenced instrumental responses that were trained with the same or different flavored alcohol solution. In particular, the selective effect was manifested as greater responding when the cue and the response were previously reinforced with the same than different alcohol solutions. Although the effect was often numerically small, it was highly consistent across experiments, and it was sometimes noticeable as an above-baseline increase of “same” responses, a below-baseline decrease of “different” responses, or sometimes as both of these effects. That pattern of results is also true of PIT studies that have used non-alcoholic rewards (e.g., Delamater, 1995; 1996; Delamater & Holland, 2008), and likely reflects the separate contribution of competing Pavlovian conditioned magazine directed responses (e.g., see Delamater, 1995; Holmes, et al., 2010). In addition, we found that this specific PIT effect was abolished when subjects received a Pavlovian extinction procedure prior to the test, but it appeared unaffected by the simple passage of time.

Furthermore, specific PIT was also found when one of the outcomes was an alcohol solution (flavored or unflavored) and the other a non-alcoholic sweet solution.

This set of results is not entirely consistent with previous evidence found in alcohol studies that used similar PIT designs (Corbit et al., 2016; Corbit & Janak, 2007; Glasner et al., 2005). Corbit and Janak (2007), for instance, also trained two CSs and two responses in a manner similar to our experiments, but they found that a CS paired with alcohol elevated both alcohol and sucrose instrumental responses, reflecting general PIT, whereas a CS paired with sucrose only elevated the sucrose-reinforced instrumental response, reflecting specific PIT. These seemingly contradictory results, however, might be explained by the many procedural differences between these studies. One salient difference is that in our experiments the rats were given a choice between both instrumental responses during the PIT tests, whereas Corbit and Janak (2007) and Corbit et al. (2016) tested each instrumental response in separate sessions. This difference may have contributed to our divergent findings. In addition, we used two distinct flavors, sweet and salty, to differentiate between two alcohol solutions, and this might have especially promoted learning about the distinct sensory characteristics of alcohol that would be necessary for obtaining outcome-specific PIT (e.g., Trapold & Overmier, 1972). Our final experiment, however, showed that we could also obtain selective, but not general, PIT even when unflavored alcohol was contrasted against sucrose as the two outcomes (though the effect appeared more impressive when alcohol was flavored).

A third procedural difference was that we used a carbohydrate (Polycose) fading procedure to induce alcohol drinking (see also Peris et al., 2006), whereas other studies have not used such a technique. Although we generally faded out Polycose concentration across our induction phase in our three experiments, some level of Polycose was included in our alcohol solutions during the initial instrumental training phase. This was done in order to maintain a higher level of instrumental responding prior to Pavlovian training and subsequent PIT tests. We do not know whether this was critical for obtaining our results, but we point out that during the Pavlovian phase the two CSs were trained with alcohol solutions without Polycose and the animals consumed all of their solutions. The only way for selective PIT to have occurred was if the CSs had entered into separate associations with those distinctively flavored alcohol solutions during this phase of the study. Had the animals not learned about these distinct flavors during their instrumental phase, then selective PIT also would not have been possible. It may be argued, however, that because Polycose was present during instrumental training, responding was motivated by the non-alcoholic properties of the solutions more than it was by alcohol itself. But we note that in Experiments 1 and 2 instrumental responding was little affected by the concentration of Polycose as it was faded out (see Figures 1 and 3). Moreover, prior to conducting these studies we ran a pilot experiment similar to the present studies but without using a fading procedure. The results from that study (as well as Lamb, 2016, Experiment 3) were similar to those reported in the present studies. Thus, we think that the Polycose fading procedure is not critical for our findings.

Another, perhaps important, difference between our task and those noted above is that we used CSs from different sensory modalities. In our experiments we used an auditory cue

(white noise) and a visual cue (flashing light) as CSs, while in the studies mentioned above (Corbit & Janak, 2007; Corbit et al., 2016; Glasner et al., 2005) two auditory cues (white noise and tone) were used. The enhanced discriminability of stimuli from separate modalities may be essential to obtain specific PIT because that effect depends upon animals successfully learning fairly subtle sensory-based discriminations. When using two CSs from the same sensory modality, these more subtle discriminations may be more difficult to make.

It is not clear why we obtained different results from Corbit and Janak (2007) and Corbit et al. (2016), but, nonetheless, our findings are not merely a failure to replicate – they are a positive demonstration of selective PIT with alcohol cues that is also sensitive to extinction. We observed the selective PIT effect in three experiments reported here. For whatever reasons, perhaps in the Corbit and Janak (2007) and Corbit et al. (2016) studies the CS was more likely to have associated only with the motivational value of the alcohol outcome rather than its sensory characteristics. This would lead to general PIT (e.g., Rescorla & Solomon, 1967). In our study, the associations were likely stronger with the sensory characteristics of alcohol.

It is worth pointing out that Glasner et al. (2005) also observed general PIT with both alcohol and non-alcohol CSs when those solutions were distinctively flavored. However, in this study, Glasner et al. (2015) contrasted a saccharin-sweetened alcohol solution to a quinine-adulterated non-alcoholic Polycose solution. The failure to see outcome-specific PIT in this case is, perhaps, due to the fact that saccharin and quinine both taste bitter to the rat (Dess, 1993). This might have promoted associations being formed between the CSs and some common reinforcing aspect shared by the two outcomes and this would be expected to bias the results towards general, rather than specific, PIT.

One of the advantages of using the procedures employed here is that it may more realistically model alcohol drinking in humans. In particular, we used flavors to differentiate between different forms of alcohol. Our PIT task, therefore, more closely resembles the types of choices that people face when they develop and maintain drinking behavior. Outside the laboratory, people rarely will consume unflavored alcohol. Rather, they choose among and consume different flavored alcoholic beverages. We suggest that in these naturalistic settings humans are confronted with a myriad of cues in the environment that may stimulate appetite or craving specifically for one type of drink over another. In this way, specific PIT is, perhaps, more the norm, in these settings, and this aspect of real world situations is what can be modeled in the animal lab with the procedures we have adopted.

Of course, there are many aspects to PIT that have yet to be fully explored. For instance, we found that extinction eliminated alcohol-specific PIT (Experiment 2). Lamb et al. (2016) also reported evidence suggesting that alcohol-specific PIT is sensitive to extinction. Our procedure contrasted a non-extinguished to an extinguished group of animals, and this enables us to show that alcohol-specific PIT survives over time in the control group and that the loss of PIT in rats given extinction depended upon extinction training. Lamb et al. (2016) tested their animals for PIT, and then extinguished all of the rats before retesting for PIT. They observed a loss of the effect following extinction. Although this result is suggestive of an extinction of PIT effect, the results could be explained as an effect of the passage of time

or of multiple tests. Our findings support and strengthen this earlier finding but without these confounds. However, the conditions under which we might expect to observe extinction or not are unknown. Using nondrug rewards Delamater (1996) showed that specific PIT is highly resistant to extinction, but more recent work shows that extinction can undermine or reduce selective PIT when Pavlovian learning is relatively weak (Delamater et al., 2017). In the present studies, our rats were neither food nor water restricted, and the motivational value of the alcohol solutions may be presumed to be relatively low. Under these conditions, it is possible that the specific CS-US associations established to support alcohol-specific PIT were, themselves, relatively weak. More generally, the similarities between extinction of PIT effects when using alcohol and natural rewards is largely unexplored.

Another area of investigation concerns examining extinction effects on alcohol-specific and general PIT, as well as their possible spontaneous recovery, renewal, and reinstatement following extinction – effects that are critical for the study of relapse (Conklin & Tiffany, 2002; Kosten & Meisch, 2013). Whether or not general PIT is equally susceptible to extinction is not known, either with natural or alcohol rewards. Developing a paradigm where both general and specific alcohol-PIT effects can be studied would be important for future work. Corbit and Janak (2007) provided convincing evidence for alcohol-general PIT effects and Corbit et al., (2016) showed that this is mediated by similar neural circuits responsible for general PIT with natural rewards. In particular, on the basis of Corbit et al. (2016) and Corbit and Balleine (2005; 2011), we would expect to find our alcohol-specific PIT effects to depend upon basolateral amygdala, nucleus accumbens shell, and orbitofrontal regions, while alcohol-general PIT effects depend upon central nucleus and nucleus accumbens core regions. These distinct circuits may be affected in different ways throughout the development of alcohol addiction.

In our studies we do not know how the learning and/or expression of PIT is affected by the development of alcohol dependence. We have no data on this, but our findings establish a paradigm where specific alcohol PIT effects can reliably be established and this could lead to additional research questions. For instance, if the development of addiction involves a transition from goal-directed to habitual behavior (e.g., Barker, et al., 2015; Corbit, Nie, & Janak, 2012; Everitt, Dickinson, & Robbins, 2001), how might this influence the expression of PIT? One hypothesis is that alcohol-specific PIT may be especially likely to occur in a goal-directed phase whereas general PIT accompanies the transition to habitual responding. This intriguing suggestion has also been largely unexplored even with non-alcoholic rewards (but see Holland, 2004), but some neurobiological models of addiction point to this as a real possibility (Corbit & Janak, 2016; Everitt et al., 2001; Everitt & Robins, 2005; Robins & Everitt, 1999).

One final issue concerns the overall level of alcohol intakes we observed in our studies. This is an important issue because one could argue that the specific PIT effects we observed could be explained in terms of the sensory differences between our two solutions and that their pharmacological effects were minimal or inconsequential. We think it is likely that our specific PIT effects were controlled by learning about the sensory features of alcohol, and that our levels of alcohol intake were not without meaningful pharmacological effects. The alcohol intake levels that we observed during instrumental and Pavlovian training were

similar, or even larger, than those reported in previous alcohol PIT studies. Although we did not monitor blood alcohol levels, we did monitor intakes and, thus, we could calculate the dosage received. By the end of the instrumental phase of our first experiment, for instance, animals consumed 0.87 g/kg (sweet solution) and 0.61 g/kg (salty solution) of ethanol, both on the same day, and this dosage is comparable or greater than those found by Lamb et al. (2016, Experiment 1; 0.67 g/kg and 0.7 g/kg in the experimental and control groups, respectively), by Corbit and Janak (2007, Experiment 2; 0.54 g/kg), and by Lamb et al. (2016, Experiment 3; 0.35 g/kg and 0.36 g/kg in their experimental and control groups, respectively). Thus, we think that the pharmacological effects of alcohol were not inconsequential in our study (at least no more so than in other studies), and that our observations of outcome-specific PIT reflect the fact that sensory properties of pharmacologically active alcohol solutions were learned about and controlled behavior.

In summary, we provide evidence, using a PIT task, that alcohol-paired stimuli can selectively modulate instrumental responses previously rewarded by alcohol. Also, our results showed that this effect can be abolished by extinction, and that the specificity of this effect can be found when animals chose between pressing a lever trained with alcohol and a lever trained with a non-alcoholic solution. Although the literature on alcohol PIT is somewhat mixed, we believe that our procedures are especially useful in producing outcome-specific, as opposed to general, PIT effects. We also believe that the use of distinctly flavored alcohol solutions both during instrumental and Pavlovian training phases might serve as a more naturalistic model in which to study the acquired incentive motivational and possibly specific craving effects of cues on alcohol-motivated behavior, and that this approach may open the way to further discoveries in alcohol addiction research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Animals were trained to press two levers, each of them reinforced with a distinctly flavored alcohol solution.
- Cues trained to signal the delivery of the flavored alcohol solutions selectively elevated the responses trained with the same solutions, i.e. outcome-specific Pavlovian-to-instrumental transfer (PIT) effect.
- This outcome-specific PIT effect was eliminated when an extinction procedure was conducted before the test.
- The outcome-specific PIT effect was also found when only one of the rewards was an alcohol solution and the other was a non-alcoholic solution.
- These results suggest that the PIT task can be used to model more naturalistically the process whereby arbitrary stimuli can acquire the ability to evoke specific craving responses for particular alcohol solutions.

Figure 1.

Top panel: mean responses per minute for the lever reinforced with the sweet (4% sucrose) and salty (0.9% NaCl) ethanol solutions, grouped in two sessions blocks, in the instrumental phase of Experiment 1. Bottom panel: Mean magazine entries (CS - Pre CS) during the CS paired with the sweet (4% sucrose) and salty (0.9% NaCl) ethanol solutions in the Pavlovian phase of Experiment 1.

Figure 2.

Responses averaged over the four PIT tests of Experiment 1. Top panel: Mean rates of 'same' and 'different' responses in the pre CS and CS periods. The population means implied by our statistical analysis are also reported in σ units. Bottom panel: Difference scores (CS – pre CS responding) for 'same' and 'different', and difference between 'same' and 'different' scores (S-D).

Figure 3. Mean responses per minute for the lever reinforced with the sweet (4% sucrose) and salty (0.9% NaCl) solutions, grouped in two sessions blocks, in the instrumental phase of Experiment 2.

Figure 4.

Mean magazine entries (CS - Pre CS) during the CS paired with the sweet (4% sucrose) and salty (0.9% NaCl) ethanol solutions, for Group Extinction (filled symbols) and Group No Extinction (open symbols), in acquisition and extinction of Experiment 2.

Figure 5.

Responses averaged over the four PIT tests of Experiment 2. Top and middle panels: Mean rates of 'same' and 'different' responses in the pre CS and CS periods for Group No Extinction (top panel) and Group Extinction (middle panel). Bottom panel: Difference scores (CS – pre CS responding) and difference between 'same' and 'different' scores (S-D) for Group No Extinction and Group Extinction. The population means implied by our statistical analysis is also reported in σ units.

Figure 6. Mean responses per minute for the lever reinforced with the sucrose solution (4% sucrose) and the ethanol solution, grouped in two sessions blocks, in the instrumental phase of Experiment 2. Top panel: Group EtOH Alone. Bottom panel: Group EtOH+NaCl.

Figure 7. Mean magazine entries (CS - Pre CS) during the CS paired with the sucrose and ethanol solutions in the Pavlovian phase of Experiment 3. Top panel: Group EtOH alone. Bottom panel: Group EtOH+NaCl.

Figure 8.

Responses averaged over the four PIT tests of Experiment 3. Top and middle panels: Mean rates of 'same' and 'different' responses in the preCS and CS periods for Group EtOH Alone (top panel) and EtOH + NaCl (middle panel). Bottom panel: Difference scores (CS - preCS) and difference between 'same' and 'different' scores for Group EtOH Alone and Group EtOH + NaCl. The population means implied by our statistical analysis are also reported in σ units.

Table 1.

Design of Experiment 1.

Induction of EtOH	Instrumental training	Pavlovian conditioning	PIT tests
Access to sweet and salty EtOH solutions in the home-cages	R1-> Sweet EtOH R2-> Salty EtOH	CS1-> Sweet EtOH CS2-> Salty EtOH	CS1: R1 vs R2 CS2: R1 vs R2

Note: EtOH solutions: sweet (4% sucrose) and salty (0.9% NaCl). R1 and R2: either left or right lever responses. CS1 and CS2: either a 90 s white noise or a flashing light stimulus.

Table 2.

Induction of ethanol drinking in Experiment 1.

Days	EtOH (%)	Polycose (%)	Hours	Sweet solution intake (g/kg)	Salty solution intake (g/kg)
1–2	0	16	24		
3–4	2	14	24	6.1	5.1
5–6	4	14	24	9.9	8.2
7–8	6	12	24	13.7	13.9
9–10	8	12	24	15.9	13.8
11–12	10	12	8	7.0	6.3
13–14	10	10	8	8.5	6.2
15–16	10	10	4	5.2	4.9
17–18	10	8	4	3.3	2.3
19–20	10	8	2	2.9	2.3
21–22	10	6	2 *	6.0	5.1
23–24	10	4	2 *	4.8	3.8
25–26	10	2	2 *	4.2	3.2
27–28	10	0	2 *	3.2	3.2
29–30	10	0	1	2.4	1.6

Note: i) Ethanol and Polycose concentrations in the sweet (4% sucrose) and salty (0.9% NaCl) solutions, and number of hours in each day of this period, ii) Ethanol intake is presented in grams per kilogram for each of the solution types.

* = subjects received onehour access period in the morning and another one-hour access period in the afternoon.

Table 3.

Induction to ethanol drinking period of Experiment 2.

Days	EtOH (%)	Polycose (%)	Hours	Gp Extinction: Sweet solution intake (g/kg)	Salty solution intake (g/kg)	Gp No Extinction: Sweet solution intake (g/kg)	Salty solution intake (g/kg)
1–2	2	16	24	4.5	4.2	4.2	4.3
3–4	4	14	24	9.4	10.0	8.5	9.8
5–6	6	12	24	12.3	11.9	12.0	13.2
7–8	10	10	24	12.5	13.6	12.0	13.0
9–10	10	10	24	14.8	12.8	14.7	14.6
11–12	10	10	24	13.9	12.3	14.0	13.7

Note: Ethanol and Polycose concentrations in the sweet (4% sucrose) and salty (0.9% NaCl) solutions, and ethanol intake in grams per kilogram of 'Group Extinction' and 'Group No Extinction', in the sweet and salty solutions.

Table 4.

Induction to ethanol drinking period of Experiment 3.

Days	EtOH (%)	Polycose (%)	Hours	EtOH solution intake (g/kg):	
				Gp EtOH alone	Gp EtOH+NaCl
1–2	2	16	24	4.6	4.6
3–4	4	14	24	10.0	11.4
5–6	6	12	24	13.1	13.9
7–8	10	10	24	11.6	15.1
9–10	10	10	24	11.7	14.8
11–12	10	10	24	12.7	14.5

Note: Ethanol and Polycose concentrations in the sweet (4% sucrose) and EtOH solutions, and ethanol intake in grams per kilogram of group 'EtOH alone' and group 'EtOH + NaCl', in the EtOH solution.

Table 5.

Difference scores (CS – pre CS) and ‘Same – Different’ scores for Group EtOH Alone and EtOH + NaCl in Experiment 3

	Sucrose Lever			Ethanol Lever		
	Same	Different	S - D	Same	Different	S - D
EtOH Alone	0.13	-0.24	0.37	0.49	-0.16	0.65
EtOH + NaCl	0.47	-0.47	0.94	0.72	-0.07	0.79