Title

Prior chronic alcohol exposure enhances Pavlovian-to-instrumental transfer

Authors

Shields, Chloe N

Gremel, Christina M

Publication Date

2021-11-01

DOI

10.1016/j.alcohol.2021.07.004

Peer reviewed

HHS Public Access

Author manuscript

Alcohol. Author manuscript; available in PMC 2023 May 23.

Published in final edited form as:

Alcohol. 2021 November ; 96: 83–92. doi:10.1016/j.alcohol.2021.07.004.

Prior Chronic Alcohol Exposure Enhances Pavlovian-to-Instrumental Transfer

Chloe N. Shields[1], **Christina M. Gremel, Ph.D.**[1,2]

[1]Department of Psychology, University of California San Diego, La Jolla, CA 92093, USA

[2]The Neurosciences Graduate Program, University of California San Diego, La Jolla, CA 92093, USA

Abstract

Alcohol dependence is associated with aberrant decision-making processes, particularly in the presence of alcohol-related environmental cues. For instance, alcohol cues can trigger alcohol seeking, consumption, and even relapse behavior. Recently, works have suggested that alcohol dependence may induce more general alterations in cued processes that support adaptive behavior, including enhanced cue control of volitional behavior unrelated to alcohol use. Here we examine this hypothesis by combining prior exposure to chronic intermittent ethanol and repeated withdrawal (CIE) procedures with a Pavlovian-to-instrumental transfer (PIT) task in mice. The PIT task entails training a Pavlovian association, separately training an instrumental contingency, and a final test during which the Pavlovian cue and instrumental action are combined for the first time. We first tested two variants of the PIT procedure in ethanol-naïve mice, differing in part in the duration of Pavlovian conditioned cues (short or long). We found in the PIT test that the short cue procedure produced negative transfer, whereas the long cue procedure produced positive transfer. We then used the long cue variant to examine PIT behavior in mice previously exposed to either CIE or air vapor. We found that prior CIE exposure strengthened PIT behavior, with enhanced instrumental responding during presentation of the food-associated cue. We further found that this enhancement in CIE mice persisted even after devaluation of the food outcome. Our findings suggest that ethanol dependence can enhance the influence of reward-predictive cues on ongoing behavior. Greater non-alcohol cue control of behavior may reflect the effect of chronic ethanol exposure on neural circuitry critical for cue-guided behavior in general.

Keywords

alcohol dependence; Pavlovian-to-instrumental transfer; adaptive behavior; devaluation; cues; mice

Correspondence:Christina M. Gremel, Ph.D., University of California San Diego, 9500 Gilman Drive, Mail Code 0109, La Jolla, CA 92093-0109, Phone: 858-594-8933, Fax: 858-534-7190, cgremel@ucsd.edu.

Declaration of Interest

The authors have no conflicts of interest to disclose.

Introduction

Alcohol use and dependence are associated with aberrant decision-making processes, particularly in the presence of alcohol-related cues. For example, viewing an alcoholic drink may trigger craving, alcohol-seeking, or even relapse to drinking behavior in individuals with alcohol use disorder (AUD). Animal models of ethanol use support that cues paired with ethanol can sustain ethanol-seeking and control behavior. In studies where animals are trained to self-administer ethanol, both discrete ethanol-paired cues and the self-administration context can reinstate instrumental ethanol-seeking behavior following extinction (Burattini et al., 2006; Tsiang & Janak, 2006). Ethanol-related cues and contexts also invigorate Pavlovian ethanol-seeking behavior (Cunningham et al., 2006; Remedios et al., 2014; Sciascia et al., 2015) and reinstate Pavlovian ethanol-seeking after extinction (Chaudhri et al., 2010). These altered responses to ethanol cues reflect ethanol's ability to reinforce learning and imbue predictive value to cues and contexts related to ethanol. However, another way that ethanol could alter behavior, particularly with chronic heavy use and dependence, is through ethanol-induced changes to neural circuits that underlie cue-guided behavior. Changes to neural circuitry produced by chronic ethanol exposure could disrupt responses guided by predictive cues in general, including cues associated with non-ethanol rewards. For drug-dependent individuals, such aberrations in cued processes could contribute to deficits in adaptive behavior throughout widespread aspects of life.

Cues in our environment are thought to influence behavior in varied ways. By signaling salient rewarding or aversive outcomes, cues can direct decisions, elicit conditioned behaviors, and incentivize some actions or deter others. One way to examine the influence of environmental cues on ongoing actions is Pavlovian-to-instrumental transfer (PIT), a process through which previously conditioned Pavlovian cues influence instrumental behavior. A basic PIT procedure involves training a discrete Pavlovian conditioned association (e.g., auditory tone functions as a conditioned stimulus to predict an unconditioned stimulus, such as food reward), separately training an instrumental action (e.g., lever press produces same food reward), and then testing performance of the instrumental action in the presence and absence of Pavlovian conditioned cues. Transfer is demonstrated via energized instrumental behavior during presentation of the Pavlovian cue (e.g., rate of lever pressing increases during the auditory tone). Such transfer can be outcome-specific, where cues associated with a specific reward selectively enhance actions performed to earn the same reward. Another form of transfer is referred to as outcome-general, where reward-associated cues enhance instrumental actions in general, independent of which reward the action produces. Importantly, PIT has been demonstrated in rodents and humans (e.g., Garbusow et al., 2016; Lehner et al., 2017; Talmi et al., 2008; van Timmeren et al., 2020), making it a promising translational model.

Indeed, research in alcohol-dependent humans has used PIT models to investigate whether AUD affects cue-guided processes that support adaptive behavior. One prior finding suggested enhanced PIT in human alcohol dependence. In recently detoxified individuals, cues related to monetary wins and losses had a greater influence on volitional behavior compared to that observed in healthy controls (Garbusow et al., 2016). However, a different study examined PIT where cues predicted food rewards. There, they found that cues affected

behavior similarly in abstinent individuals with AUD and controls in both general and specific PIT (van Timmeren et al., 2020). While the reason for this discrepancy is unclear, and could be related to properties of the disparate rewards used, this past work offers some support for the intriguing hypothesis that AUD may enhance the influence of general cues on behavior.

Past examinations of PIT in human AUD have been performed after disorder onset, thus necessarily encompassing both the acquisition and expression of learned associations and their interactions. It is therefore pertinent for animal work to model a similar time course. In the present work, we examined the influence of prior chronic ethanol exposure on food cue-guided PIT behavior, with ethanol exposure prior to behavioral procedures such that all training and testing occurred within the protracted phase of withdrawal (Heilig et al., 2010). Because PIT investigations in humans often rely on short discrete cues (e.g., Lehner et al., 2017; Talmi et al., 2008), but animal PIT models often require the use of longer duration cues (e.g., Crombag et al., 2008; Meltzer & Brahlek, 1970; Van Dyne, 1971), Experiment 1 tested two variants of the PIT procedure in ethanol-naïve mice using either short or long auditory conditioned cues to predict food delivery, with concomitant differences in reinforcement schedule, number of cue-reward pairings, and total number of reinforcers. Replicating previous findings (Crombag et al., 2008), we found that the short cue procedure produced negative transfer whereas the long cue procedure produced positive transfer in the PIT test. Then, in Experiment 2, mice were exposed to chronic intermittent ethanol (CIE) vapor exposure, a well-validated model to examine aspects of alcohol dependence in rodents (Becker, 1994; Becker & Lopez, 2004; Griffin et al., 2009; Lopez & Becker, 2005), and underwent long cue PIT procedures. We found that prior CIE exposure selectively enhanced transfer in the PIT test relative to air controls. Our data suggest that protracted withdrawal from chronic ethanol exposure may enhance the ability of reward cues to guide ongoing actions in mice, perhaps reflecting ethanol-induced changes to neural circuitry critical for cue-guided behavior in general.

Materials and Methods

Animals

Adult female and male C57BL/6J and B6.129S2-*Emx1*$^{tm1(cre)Krj}$/J (Emx1-Cre) mice were acquired from Jackson Laboratory (Bar Harbor, ME) or bred in-house 1 generation from mice acquired from Jackson Laboratory. Strain was kept consistent within each replication, such that Experiment 1A and the first replication of Experiment 2 used all Emx1-Cre mice, and Experiment 1B and the second replication of Experiment 2 used all C57BL/6J mice. There were no strain differences in behaviors examined. Average mouse ages at experiment start were 18.5 ± 0.52 weeks in Experiment 1A, 8.2 ± 0.45 weeks in Experiment 1B, and 17.2 ± 1.03 weeks in Experiment 2. Mice were group housed 2–5 per cage and maintained on a 14-hr light/10-hr dark cycle. Prior to experimental procedures, animals were provided with mouse chow (Lab-diet 5015) and water ad libitum. All experiments were approved by the University of California San Diego Institutional Animal Care and Use Committee, and were conducted in accordance with NIH Guidelines.

Chronic Intermittent Ethanol Exposure

In Experiment 2, mice were exposed to 4 rounds of ethanol vapor or air control with repeated withdrawal (Becker, 1994; Becker & Lopez, 2004; Griffin et al., 2009; Lopez & Becker, 2005). In each round, mice were exposed to 4 consecutive days of 16-hr vapor exposure followed by an 8-hr withdrawal period. After a round concluded, there were an additional 3 days of withdrawal before the next round began. Ethanol was volatilized by bubbling air through a flask containing 95% ethanol at a rate of 2.3 L/min. The resulting vapor was then combined with a separate air stream for a total flow rate of approximately 10 L/min. Ethanol or air vapor was delivered to mice in Plexiglas chambers (Plas-Labs Inc, Michigan, USA). Blood ethanol concentrations (BECs) were collected at the end of each round from sentinel mice (mean BEC = 43.1 ± 6.4 mM; Analox, USA). No injections of Pyrazole or ethanol were administered prior to placement in vapor chambers (Renteria et al., 2018), and animals were monitored for ill effects of vapor procedures.

Pavlovian-to-Instrumental Transfer Task

Overview.—The PIT task, developed from Crombag et al. (2008), was used as a model of cue-guided behavior. The purpose of Experiment 1 was to test PIT transfer using two Pavlovian training procedure variants, differing in part by Pavlovian cue duration (either short, 8-sec, or long, 120-sec), in ethanol-naïve mice. The goal of Experiment 2 was to test the effect of prior chronic ethanol exposure on PIT transfer using the long cue procedure. Both experiments followed similar timelines, beginning with Pavlovian training with auditory tones, then instrumental lever press training, and finally testing for transfer (Figures 1A, 2A).

General Procedures.—In all experiments, mice were food restricted to maintain 85% of baseline bodyweight starting 3 days prior to behavioral procedures. In Experiment 2, to limit the effects of acute withdrawal and examine any lasting effects present in protracted withdrawal, food restriction began 3 days after CIE procedures concluded. Thus, in Experiment 2, behavioral procedures began a total of 6 days after the conclusion of CIE. All behavioral procedures were conducted in standard sound-attenuating operant boxes (Med Associates, Vermont, USA). Each operant box contained two retractable levers situated around a food magazine, and the chamber house light was illuminated during all procedures.

Pavlovian Acquisition.—During Pavlovian training, both a food-predictive conditioned stimulus (CS+) and a conditioned stimulus predictive of an absence of food (CS−) were presented. The inclusion of a CS− allowed for disentanglement of whether any enhancement of instrumental behavior was based on the presence of environmental cues in general, or rather on the presence of cues specifically associated with rewards. During conditioning, two different frequency auditory tones (8 kHz and 15 kHz) were used as conditioned stimuli and were played for a duration of either 8-sec (Experiment 1A) or 120-sec (Experiment 1B, Experiment 2). Food reward (food pellet, Bio-Serve formula F05684) was delivered immediately following tone presentation for Experiment 1A, or on average every 30-sec during tone presentation for Experiment 1B and Experiment 2. During the CS− tone, the stimulus was played but nothing else occurred. Between each tone presentation was

an intertrial interval (ITI) during which no tones were played for 120-sec on average. Mice were exposed to daily sessions of either 16 (Experiment 1A) or 10 (Experiment 1B, Experiment 2) CS+ tones and an equivalent number of CS− tones in each session, presented in pseudorandom order. Entries into the food receptacle were detected via infrared beam breaks and recorded. Pavlovian acquisition sessions were conducted for 8 days in Experiment 1 and 7 days in Experiment 2. One fewer Pavlovian training day was included in Experiment 2 so that the PIT test would occur within the 21-day time period in which disrupted instrumental decision-making has been documented in mice (Renteria et al. 2018).

Instrumental Acquisition.—Following Pavlovian training, mice then underwent instrumental training in the same context used in Pavlovian training. Instrumental training involved daily sessions in which mice learned to press a lever for the same food earned during Pavlovian training. Throughout instrumental training and the transfer test, one lever was always available and the other lever was always retracted. One food pellet was delivered for every lever press under a continuous reinforcement schedule (CRF) for the first 3 days of training. On the following 5 days, 1 food pellet was delivered on average for every 10 lever presses (random ratio 10 schedule, RR). All instrumental training sessions ended when mice earned 30 pellets or 90-min elapsed. The number of lever presses was recorded, as were head entries into the food receptacle.

Pavlovian-to-Instrumental Testing.—Following instrumental training, the influence of conditioned cues on lever press behavior was assessed. The approximately 30-min test was conducted in extinction, in that neither CS+ presentations nor lever presses resulted in food reward. During testing, the trained lever was present at all times. In Experiment 1A, CS+ and CS− tones were presented 8 times each, while in Experiments 1B and 2, CS+ and CS− tones were presented 4 times each. In both experiments, tones were presented in alternating order throughout the test, beginning with the CS+ tone. In addition, between each tone presentation was an ITI period lasting on average 120-sec. Lever presses and head entries into the food receptacle were recorded.

Outcome Devaluation Procedures.—In Experiment 2, the effect of outcome devaluation on PIT responding in Air and CIE mice was assessed. Following the first transfer test, outcome devaluation procedures were conducted. All mice were given free access to the same food pellets earned during training for 60-min. Immediately following this pre-feeding period, all mice went through a second transfer test identical to the first. Lever presses and head entries into the food receptacle were recorded. Of note, outcome devaluation procedures often involve comparing lever pressing or head entries in a devalued state with behavior in a valued state, where satiation has been achieved via pre-feeding of a non-paired reinforcer. In the present work, we excluded a valued comparison as the long nature of the test session can induce greater extinction learning, and the implementation of additional training days could alter either CS or lever press association strength.

Behavioral and Statistical Analysis

Data were analyzed using Prism 6 (GraphPad) and JASP (Version 0.13.1). The alpha level was set at 0.05 for all experiments. All data within groups was found to be normally

distributed with the exception of CS+ food port entries during Pavlovian training in the Air group. Thus, for this group non-parametric analyses of Pavlovian training food port entry data are also presented. In Experiment 2, pre-training bodyweights were compared for Air and CIE mice with an unpaired t-test. For Pavlovian training, food port entry rate was analyzed with 2- or 3-way ANOVA examining the effects of the within-subjects factors Day and Trial Type (ITI/CS+/CS−), with the addition of the between-subjects factor of Group (Air/CIE) in Experiment 2. Rate of lever pressing was analyzed for instrumental training with 1- or 2-way ANOVA examining the effect of Day and, in Experiment 2, Group. For the PIT and devaluation tests, lever press rate was analyzed using 1- or 2-way ANOVA including the factor of Trial Type and, in Experiment 2, the factor of Group. Transfer was evidenced by an increase in lever pressing behavior during presentation of the food-associated CS+ compared to the CS− and the ITI. Planned post-hoc comparisons with Bonferroni correction were conducted to examine performance between trial types in Pavlovian training and in testing. Difference scores for the PIT test and devaluation test were computed as rate of lever pressing during the ITI subtracted from rate of lever pressing during the CS+ tone. Difference scores were compared to 0 in one sample t-tests for Experiment 1A and 1B, and for Air and CIE groups in Experiment 2. In addition, unpaired t-tests were performed to compare difference scores between Experiment 1A and 1B, and between Air and CIE groups in Experiment 2. Group consumption during Experiment 2 devaluation pre-feeding was compared with an unpaired t-test. To reveal any changes in Experiment 2 behavior from the PIT test to the devaluation test, 2- or 3-way ANOVA were conducted with the factors of Group and Test for baseline ITI lever press rates and the additional factor of Trial Type for food port entry rates. Finally, the effects of sex and strain were examined in Experiment 2 using 2-way ANOVA for difference score, with the factors of Group and Sex or Strain.

Results

Attrition

In Experiment 1A, one mouse was excluded from all analyses for failing to learn to lever press, leaving a final n of 7 (2F, 5M). Experiment 1B was performed in two replicate cohorts, with a final n of 16 (16F) and no attrition. In Experiment 2, one mouse was excluded for failing to learn to lever press. Performed in 2 replicates, final group ns in Experiment 2 were $n = 16$ (8F, 8M) in the Air group and $n = 17$ (9F, 8M) in the CIE group. In addition, 2 mice did not consume food pellets during devaluation and so were selectively excluded from devaluation analyses. Final ns for devaluation analyses in Experiment 2 were $n = 16$ Air (8F, 8M) and $n = 15$ CIE (9F, 6M).

Sex and Strain

The effects of sex and strain on PIT were examined in Experiment 2, where strain differed by replication and both sexes were represented in Air and CIE groups. Analyses supported that transfer magnitude (difference between CS+ and baseline ITI lever press rates in the PIT test) and Air/CIE group differences in PIT were not affected by strain. This was supported by a 2-way ANOVA (Strain x Group) for difference score, which found no main effect of Strain ($F(1, 29) = 2.62$, $p = 0.12$), a marginal effect of Group ($F(1, 29) = 3.80$, $p = 0.06$),

and no interaction ($F(1, 29) = 0.13$, $p = 0.72$). Strains were therefore combined for all further Experiment 2 analyses. Sex analyses revealed overall weaker PIT in females, as has been seen previously (Barker & Taylor, 2019). A 2-way ANOVA (Sex x Group) examining difference scores in the PIT test indicated a main effect of Sex ($F(1, 29) = 7.95$, $p < 0.01$) and a main effect of Group ($F(1, 29) = 6.53$, $p < 0.05$), but no interaction ($F(1, 29) = 1.14$, $p = 0.30$). The same analysis for the devaluation test also revealed a main effect of Sex ($F(1, 27) = 7.32$, $p < 0.05$) and a main effect of Group ($F(1, 27) = 12.64$, $p < 0.01$), but no interaction ($F(1, 27) = 0.01$, $p = 0.98$). Together, these results indicate overall weaker PIT in females relative to males, regardless of treatment group. In other words, CIE enhanced PIT strength in both sexes. Males and females were thus combined for all further Experiment 2 analyses.

Experiment 1A: Pavlovian-to-instrumental transfer with short conditioned stimuli

Mice were exposed to a Pavlovian conditioning procedure with 8-sec auditory tones. In this procedure, reward delivery occurred immediately after presentation of the CS+ tone. A benefit of this procedure is that it easily differentiates food port entries that are performed predictively (i.e., in expectation of food delivery) from food port entries that are performed to retrieve food. That is, predictive port entries are those that occur during CS+ presentation, and food retrieval port entries are those that occur soon after CS+ presentation.

Mice acquired Pavlovian conditioned behavior across training and learned that the CS+ tone was predictive of food delivery. This finding was supported by a 2-way ANOVA (Day x Trial Type) for rate of food port entries (port entries per min), which revealed significant main effects of Day ($F(7, 126) = 6.26$, $p < 0.0001$) and Trial Type ($F(2, 18) = 4.26$, $p = 0.03$), and a Day x Trial Type interaction ($F(14, 126) = 4.08$, $p < 0.0001$) (Figure 1B). Post hoc comparisons for the main effect of Trial Type (collapsing across days) supported that the rate of predictive food port entries was significantly higher on average during the CS+ compared to the ITI ($p < 0.05$). There was no significant difference between food port entry rate during the ITI and CS− ($p = 0.09$) or between CS+ and CS− port entry rates ($p = 0.99$). However, post hoc day comparisons for the interaction between Day and Trial Type found that on the final day of training, there were significantly more food port entries performed during the CS+ compared to both the ITI ($p < 0.01$) and the CS− ($p < 0.05$). In subsequent instrumental training, mice learned to press a lever for the same food reward as earned in Pavlovian training and escalated their rate of lever pressing across sessions. This finding was supported by a 1-way ANOVA (Day) for lever press rate, which found a significant main effect of Day ($F(7, 42) = 18.34$, $p < 0.0001$) (Figure 1C).

However, PIT testing with short CS tones suggested negative PIT transfer. This finding was supported by a 1-way ANOVA (Trial Type) for lever press rate during the test, which revealed a significant main effect of Trial Type ($F(2, 12) = 9.50$, $p < 0.01$) (Figure 1D). Post hoc comparisons indicated significantly lower lever pressing rates during the CS+ tone compared to both the ITI ($p < 0.05$) and the CS− tone ($p < 0.01$). There was no significant difference between lever press rates during the CS− and ITI ($p = 0.96$). Further supporting that negative transfer occurred, a one sample t-test found that the difference between lever press rates in the ITI and CS+ was significantly lower than 0 ($t(6) = 4.03$, $p < 0.01$). Thus,

the above data show that the short cue procedure resulted in a decrease in lever pressing during cue presentation.

Experiment 1B: Pavlovian-to-instrumental transfer with long conditioned stimuli

In Experiment 1B, we altered the Pavlovian training procedure described in Experiment 1A to use longer, 120-sec auditory tones. In this modified procedure, food pellets were delivered on average every 30-sec throughout the CS+ tone rather than immediately after tone presentation. This procedure reduces the precise temporal predictability of food delivery and provides larger windows to observe cue-influenced lever pressing behavior.

Animals demonstrated robust Pavlovian learning with long CS tones. This was supported by a 2-way ANOVA (Day x Trial Type) for rate of food port entries, which revealed a main effect of Trial Type ($F(2, 30) = 15.36$, $p < 0.0001$) and Day ($F(7, 105) = 7.31$, $p < 0.0001$), but no significant interaction between the factors ($F(14, 210) = 1.71$, $p = 0.06$) (Figure 1E). Planned post hoc comparisons for the main effect of Trial Type found that food port entry rate was significantly higher during the CS+ tone compared to both the CS− ($p < 0.0001$) and the ITI ($p < 0.001$). There was no significant difference between food port entry rates in the ITI and CS− ($p = 0.99$). Next, animals were exposed to lever press training identical to the procedure described in Experiment 1A. Mice learned to lever press for the same food reward as earned during Pavlovian training and increased their rate of lever pressing across sessions, as demonstrated by a main effect of Day in a 1-way ANOVA ($F(7, 105) = 59.13$, $p < 0.0001$) (Figure 1F).

A PIT test conducted under extinction found that the long cue procedure produced positive PIT transfer (Figure 1G). This finding was supported by a main effect of Trial Type ($F(2, 30) = 8.64$, $p < 0.01$) in a 1-way ANOVA for lever press rate during the test. Post hoc comparisons found that lever press rate was selectively energized during the CS+ tone relative to the ITI ($p < 0.01$) and the CS− tone ($p < 0.01$). There was no significant difference between lever press rates in the ITI and CS− ($p = 0.99$). Further supporting that lever pressing was energized during the food-associated CS+, a one sample t-test found that the difference between ITI and CS+ lever press rates was significantly greater than 0 ($t(15) = 3.64$, $p < 0.01$). In addition, an unpaired t-test found that the PIT difference scores for Experiments 1A and 1B were significantly different, supporting that the short cue procedure produced negative transfer, whereas the long cue procedure produced positive transfer ($t(21) = 4.35$, $p < 0.001$) (Figure 1H). These findings suggest that certain aspects of Pavlovian conditioning can affect the direction of PIT transfer in mice. In particular, short cues with greater reward predictability and fewer cue-reward pairings may produce negative transfer, whereas long cues with less predictable reward delivery and more cue-reward pairings may enable a larger window to observe cue-energized instrumental behavior and positive transfer.

Experiment 2: CIE-induced enhancement of Pavlovian-to-instrumental transfer

We next examined the effect of chronic ethanol exposure on PIT behavior using the long cue protocol. A new cohort of mice was exposed to CIE procedures, followed by training conducted identically to Experiment 1B, except with 1 fewer day of Pavlovian training. Following CIE exposure but prior to food restriction, average weights were 31.24 ± 1.82

grams in the Air group and 28.78 ± 1.63 grams in the CIE group. An unpaired t-test supported that there was no significant difference in weight between groups ($t(31) = 1.01$, $p = 0.32$).

Robust Pavlovian conditioned behavior was observed across groups, with learning supporting similar levels of conditioning between Air and CIE mice (Figures 2B, 2C). A 3-way ANOVA (Day x Trial Type x Group) for rate of food port entries during training did not show a significant 3-way interaction ($F(12, 372) = 2.62$, $p = 0.96$), nor a significant 2-way interaction of Trial Type x Group ($F(2, 62) = 0.68$, $p = 0.51$). However, there was a significant Day x Trial Type interaction ($F(12, 372) = 5.42$, $p < 0.001$), and a significant Day x Group interaction ($F(6, 186) = 2.66$, $p < 0.05$). There were main effects of Trial Type ($F(2, 62) = 39.06$, $p < 0.001$) and Day ($F(6, 186) = 4.78$, $p < 0.001$), but no main effect of Group ($F(1, 31) = 0.001$, $p = 0.98$). Post hoc comparisons for the main effect of Trial Type showed that regardless of Air/CIE group, food port entry rates were significantly higher during the CS+ compared to both the ITI ($p < 0.001$) and the CS− ($p < 0.001$), whereas there was no significant difference between the ITI and CS− ($p = 0.99$). Further supporting similar levels of conditioning across groups, post hoc comparisons for the Day x Group interaction revealed no significant differences in overall food port entry behavior (collapsing across trial type) between Air and CIE on any given training day (ps = 0.99). Non-parametric tests also supported similar levels of training between groups. A Mann-Whitney test found no difference in CS+ food port entry rates between Air ($Mdn = 8.00$) and CIE ($Mdn = 6.30$) on the final day of training ($U = 107$, $z = -1.03$, $p = 0.30$). Confirming learning in the Air group, a Friedman test found a significant effect of Trial Type on food port entry rates during the last day of training in Air mice ($\chi^2(2) = 18.38$, $p < 0.0001$), with Dunn's multiple comparisons indicating significantly higher food port entries in the CS+ relative to the ITI and CS− (ps < 0.001) and no difference between ITI and CS− ($p = 0.99$).

Both Air and CIE groups acquired similar levels of lever press behavior across instrumental training, with no differences between groups (Figure 2D). This was supported by a 2-way ANOVA (Day x Group) for lever press rate, which revealed no main effect of Group ($F(1, 31) = 0.34$, $p = 0.56$) and no Day x Group interaction ($F(7, 217) = 0.76$, $p = 0.62$). A significant main effect of Day ($F(7, 217) = 79.71$, $p < 0.0001$) supported that mice increased lever pressing across training.

A subsequent PIT test revealed strengthened PIT transfer in CIE animals compared to Air animals (Figure 2E). A 2-way ANOVA (Trial Type x Group) for lever press rate found a significant main effect of Trial Type ($F(2, 62) = 21.89$, $p < 0.0001$) and a Trial Type x Group interaction ($F(2, 62) = 3.51$, $p < 0.05$). There was no main effect of Group ($F(1, 31) = 0.90$, $p = 0.35$). Post hoc comparisons for the Trial Type x Group interaction indicated that CS+ lever press rates were selectively enhanced in CIE animals compared to the CS− ($p < 0.0001$) and ITI ($p < 0.0001$), and there was no significant difference between the CS− and ITI ($p = 0.94$). In Air animals, CS+ lever press rates were selectively enhanced compared to the CS− ($p < 0.05$) but less so compared to the ITI ($p = 0.06$). There was no significant difference between the CS− and ITI periods ($p = 0.99$). An analysis of difference scores supported that transfer occurred in both groups. In one sample t-tests, the difference between CS+ and ITI lever press rates was found to be significantly greater than 0 for both the Air

group ($t(15) = 2.28$, $p < 0.05$) and the CIE group ($t(16) = 5.12$, $p < 0.0001$). Supporting that PIT was enhanced in the CIE group, an unpaired t-test found that the difference between CS+ and ITI lever press rates was greater in CIE animals compared to Air animals ($t(31) = 2.27$, $p < 0.05$) (Figure 2F).

Outcome devaluation testing was performed to investigate whether devaluation procedures differentially influence PIT expression in Air versus CIE mice. We found that pre-feeding prior to PIT testing did not disrupt the enhanced PIT observed in CIE mice compared to Air controls (Figure 2G). A 2-way ANOVA (Trial Type x Group) for lever press rate revealed a significant main effect of Trial Type ($F(2, 58) = 18.32$, $p < 0.0001$) and a Trial Type x Group interaction ($F(2, 58) = 5.21$, $p < 0.01$), but no main effect of Group ($F(1, 29) = 0.45$, $p = 0.51$). Post hoc comparisons revealed that in the CIE group, CS+ responding remained significantly higher than during both the ITI ($p < 0.0001$) and the CS− ($p < 0.0001$). Lever press rates did not differ between the CS− and ITI in the CIE group ($p = 0.86$). In the Air group, CS+ responding was not significantly energized relative to the ITI ($p = 0.49$), but was significantly higher than during the CS− ($p < 0.05$). There was no significant difference between lever press rates during the ITI and CS− in the Air group ($p = 0.63$). Analysis of the difference between CS+ and ITI lever press rates for each group supported that PIT was still enhanced in the CIE group following devaluation. One sample t-tests revealed that whereas the CIE group difference score was significantly greater than 0 ($t(14) = 4.90$, $p < 0.001$), the Air group difference score was not ($t(15) = 1.55$, $p = 0.14$). Further, an unpaired t-test comparing difference scores between groups found that the CIE group score was significantly higher than the Air group score ($t(29) = 3.05$, $p < 0.01$) (Figure 2H). Groups did not differ in consumption levels during the pre-feeding period (0.60 ± 0.11 grams in Air and 0.62 ± 0.07 grams in CIE; unpaired t-test, $t(29) = 0.14$, $p = 0.89$). A 2-way ANOVA (Group x Test) also confirmed that across groups, baseline lever press rates in the ITI period decreased from the PIT test to the devaluation test. This was supported by a main effect of Test ($F(1, 29) = 105.70$, $p < 0.0001$) and no main effect of Group ($F(1, 29) = 0.12$, $p = 0.74$) or interaction ($F(1, 29) = 0.30$, $p = 0.59$). Similarly, food port entries decreased from the PIT test to the devaluation test. This was supported by a 3-way ANOVA (Trial Type x Group x Test) for food port entry rate, which found a main effect of Test ($F(1, 29) = 19.51$, $p < 0.001$) and no other significant main effects or interactions (ps > 0.05). Together, these results suggest that CIE-strengthened transfer in the PIT test was resistant to devaluation of the food reward outcome.

Discussion

In the present study, we adapted a model of Pavlovian-to-instrumental transfer in mice to examine the influence of prior exposure to chronic intermittent ethanol on cue control of behavior. In ethanol-naïve mice, we found that a short cue procedure produced negative transfer and a long cue procedure produced positive transfer in the PIT test (Figure 1), supporting that procedural differences in Pavlovian training can influence the expression and direction of transfer in rodent models (Crombag et al., 2008). We then found that chronic ethanol exposure prior to all behavioral procedures resulted in enhanced PIT transfer compared to controls (Figure 2). These results suggest that protracted withdrawal following chronic ethanol exposure strengthened the ability of conditioned cues to guide ongoing

instrumental actions. Importantly, as we used non-drug cues and rewards throughout all experiments, these findings suggest that ethanol dependence may alter behavior guided by reward cues in general, rather than just cues associated with ethanol.

We found that though performance did not differ significantly between groups during training, PIT transfer was selectively enhanced in ethanol animals compared to controls. This finding of CIE-enhanced PIT is consistent with one previous study showing increased influence of non-drug Pavlovian cues on behavior in abstinent humans with alcohol dependence (Garbusow et al., 2016). However, another recent report found no differences in transfer strength between abstinent individuals with AUD and controls (van Timmeren et al., 2020). Two notable differences between these studies are the type of reward and the type of PIT procedure used. Garbusow et al. (2016) used cues associated with monetary wins and losses, and van Timmeren et al. (2020) used cues associated with food rewards. However, as the present work also used food rewards, more investigation is necessary to understand any differences in performance between procedures using primary or secondary reinforcers. A second difference between these studies is the method of PIT used. Whereas van Timmeren et al. (2020) explicitly examined both specific and general PIT, finding no differences between patients and controls with either form of PIT, Garbusow et al. (2016) used a PIT procedure that cannot determine which form of PIT was observed. These experimental differences may underlie the disparate results observed, or other undetermined factors may be involved.

We found that PIT transfer persisted in ethanol animals, but not control animals, following devaluation procedures. Though we did not directly test devaluation to avoid possible training and extinction effects, several factors support that devaluation occurred. Groups showed similar consumption during pre-feeding, and across all groups there was a reduction in baseline rate of lever presses and food port entries made during the devaluation test compared to the first PIT test. As CIE exposure has previously been shown to produce insensitivity to devaluation of ethanol self-administration as well as operant food-seeking (Lopez et al., 2014; Renteria et al., 2018; Renteria et al., 2020), the current findings suggest that cue-induced motivational influences on lever pressing are open to devaluation in control mice but not ethanol mice. Past works have revealed conflicting effects of devaluation on PIT, with procedures similar to those used here demonstrating devaluation susceptibility (Aitken et al., 2016; Corbit et al., 2007; Dailey et al., 2016). In contrast, outcome-specific PIT often shows no effect of devaluation in both rodents and humans (Holland, 2004; Rose et al., 2018; but see Seabrooke et al., 2019). Holland (2004) additionally found that sickness-induced devaluation did not alter presumed outcome-general PIT, perhaps indicating differing effects based on method of devaluation. As the procedure in the present work is thought to produce outcome-general PIT (Cartoni et al., 2016), and devaluation was induced via satiation, our results support that devaluation affected transfer in the control group but not the ethanol group.

It is well established that exposure to ethanol is associated with altered responses to cues for ethanol. In rodents, cues and contexts related to ethanol invigorate ethanol-seeking behavior (Cunningham et al., 2006; Remedios et al., 2014; Sciascia et al., 2015), and ethanol self-administration both augments ethanol-seeking (Remedios et al., 2014; Sciascia

et al., 2015) and reinstates Pavlovian and instrumental ethanol-seeking following extinction (Burattini et al., 2006; Chaudhri et al., 2010; Tsiang & Janak, 2006). Using PIT tasks, a number of rodent studies have found that ethanol-paired conditioned stimuli elicit increases in instrumental responding that was previously maintained by ethanol (Corbit & Janak, 2007; Corbit & Janak, 2016; Glasner et al., 2005; Krank, 2003; Krank et al., 2008; Milton et al., 2012). Though this effect has not been studied extensively in humans, one study of detoxified individuals with AUD interestingly found that alcohol cues had an inhibitory effect on instrumental behavior in PIT (Schad et al., 2019). This inhibitory effect may reflect the negative properties of alcohol and related cues experienced by patients during the process of detoxification (Schad et al., 2019). The present work moves beyond drug-specific cues to investigate the effect of chronic ethanol exposure on PIT behavior in the presence of reward cues in general, not just ethanol cues.

Notably, one prior study in mice using CIE and PIT procedures similar to those in the present work found that transfer was present in Air animals but abolished in CIE animals (DePoy et al., 2015). This study differed from the current work in that ethanol exposure was conducted after training had concluded, when associations were already learned (DePoy et al., 2015). In addition, DePoy et al. (2015) conducted testing immediately after the acute phase of ethanol withdrawal (2–3 days in nonhuman animals; Heilig et al., 2010). This timeline is markedly different from that used in the present work and previous work in humans with AUD, where ethanol exposure occurred prior to all behavioral procedures. Thus, DePoy et al. (2015) may have revealed an effect of more recent withdrawal on PIT performance, and would not have captured any possible effect of chronic ethanol exposure on training. Together, these results suggest that the sequence of drug exposure and conditioning may be an important factor in determining whether prior ethanol dependence enhances or inhibits PIT. Indeed, previous work supports that the timing of exposure to psychostimulants determines whether PIT is enhanced or impaired (Wyvell & Berridge, 2001; Hall & Gulley, 2011).

Though human PIT studies commonly utilize short, discrete Pavlovian cues (e.g., Garbusow et al., 2016; van Timmeren et al., 2020), previous work supports that procedures using short duration cues often do not produce positive PIT transfer in rodent models. Crombag et al. (2008) found that 10-sec Pavlovian tones did not produce transfer, whereas 120-sec Pavlovian tones produced robust transfer. Earlier work found similar results, with long Pavlovian cues producing positive transfer and short Pavlovian cues suppressing instrumental responding (Meltzer & Brahlek, 1970; Van Dyne, 1971). In the present work, mice trained with short cues demonstrated expected performance in both the Pavlovian and instrumental phases of training, but negative transfer in the PIT test. Mice trained with long cues, however, demonstrated expected performance in training and positive transfer in the PIT test. These findings could reflect several differences in the short and long cue procedures, including the temporal pairing of cue-food delivery, difference in the number of cue-reward pairings, and number of rewards delivered. Competition between lever press and food port entry behaviors may also be at play, as mice cannot perform these behaviors simultaneously; a short cue could bias checking of the food port in expectation of imminent food delivery, at the expense of lever pressing. Regardless of differences

between procedures, these findings support using Pavlovian training procedures with long conditioned cues to examine the effect of ethanol dependence on transfer in rodent PIT.

Though several brain areas are thought to contribute to PIT, the two regions most heavily implicated are the amygdala and nucleus accumbens (NAcc; Blundell et al., 2001; Corbit et al., 2001; de Borchgrave et al., 2002; Hall et al., 2001; Holland & Gallagher, 2003), with relatively more recent studies uncovering a dissociation between select subregions. Lesions to the basolateral amygdala (BLA) and NAcc shell have been shown to abolish outcome-specific PIT, whereas lesions to central amygdala (CeA) and NAcc core abolish outcome-general PIT (Corbit et al., 2016; Corbit & Balleine, 2005; Corbit & Balleine, 2011). In amygdala, the dissociation between subregions has been corroborated in human PIT using high-resolution fMRI (Prévost et al., 2012). Further, lesions to CeA and substantia nigra pars compacta (SNpc) or ventral tegmental area (VTA) were shown to reduce transfer in presumed outcome-general PIT (El-Amamy & Holland, 2007), and VTA inactivation itself attenuates or abolishes transfer (Corbit et al., 2007; Murschall & Hauber, 2006).

Chronic alcohol exposure produces functional changes in amygdala and NAcc that could contribute to altered PIT behavior. In rodent CeA, CIE exposure was shown to augment GABAergic signaling and produce a net inhibition (Pleil et al., 2015; Roberto et al., 2004), potentially leading to disinhibition of downstream regions (Gilpin et al., 2015; Roberto et al., 2021). In rodent NAcc, CIE exposure increased glutamatergic activity (Griffin et al., 2014; Griffin et al., 2015), and in alcohol-dependent humans, NAcc activity in PIT was found to correlate with subsequent relapse (Garbusow et al., 2016). The overlap in brain regions implicated in PIT and affected by alcohol dependence is significant, and suggests that chronic exposure to alcohol could alter neural circuitry underlying the motivating influence of Pavlovian cues on instrumental behavior. As similar procedures to that used here are thought to produce outcome-general PIT (Cartoni et al., 2016), changes to subregions involved in that type of PIT are of greatest interest in regard to the present findings. For instance, an intriguing hypothesis is that CIE inhibition of CeA, and possible downstream disinhibition of areas such as SNpc and VTA, could underlie the facilitation of transfer demonstrated here in CIE mice.

One alternative explanation for enhanced transfer is that the CIE group could have undergone changes in motivational processes unrelated to ethanol. Though altered motivation can be difficult to measure directly, some evidence that could support increased motivation for the food reinforcer in CIE animals would be significantly lower weights, generally heightened responding, increased levels of consumption prior to the devaluation test, and possibly enhanced performance in other measures of motivational processes, such as incentive learning tasks. The present work, however, reflects a pattern of results that supports similar levels of food motivation between groups. There were no significant differences between Air and CIE groups in baseline weights, and across Pavlovian and instrumental training, animals exhibited very similar patterns of responding and no significant differences in behavior between groups. The two groups also did not significantly differ in consumption of the food reinforcer prior to the devaluation test. Though incentive learning was not examined here, recent work found that CIE-exposed animals actually exhibited impaired incentive learning although there was no weight loss during or after CIE

(Galaj et al., 2020). Thus, though changes in motivational processes cannot be completely ruled out, findings in the present work do not appear to be consistent with CIE-increased food motivation.

Together, our data suggest that chronic exposure to ethanol and repeated withdrawal can enhance the control of cues on volitional behavior. Importantly, this change was induced by prior chronic ethanol exposure and observed in a non-ethanol related task state. This suggests that ethanol dependence and protracted withdrawal may be associated with changes to neural structures key for guiding cue responses in general, rather than just cues for ethanol. This work sets the stage for future research investigating the neural circuitry underlying altered cue control of behavior in ethanol dependence, and may suggest that ethanol-induced changes in brain areas such as the nucleus accumbens and amygdala are key to enhancements in cue control. Future work dissecting the distribution of lever presses and head entry behavior across a session could also be of interest, in particular to clarify any behavioral differences between varying training procedures. Elucidating the neural and behavioral effects of ethanol dependence on cue control of behavior is crucial for developing improved methods of assessing and treating individuals with AUD.

Funding

This work was supported by the National Institutes of Health (R01 AA026077) awarded to Dr. Gremel.

References

Aitken TJ, Greenfield VY, & Wassum KM (2016). Nucleus accumbens core dopamine signaling tracks the need-based motivational value of food-paired cues. Journal of Neurochemistry, 136(5), 1026–1036. 10.1111/jnc.13494 [PubMed: 26715366]

Barker JM, & Taylor JR (2019). Sex differences in incentive motivation and the relationship to the development and maintenance of alcohol use disorders. Physiology & Behavior, 203, 91–99. 10.1016/j.physbeh.2017.09.027 [PubMed: 28974459]

Becker HC (1994). Positive relationship between the number of prior ethanol withdrawal episodes and the severity of subsequent withdrawal seizures. Psychopharmacology, 116(1), 26–32. 10.1007/BF02244867 [PubMed: 7862927]

Becker HC, & Lopez MF (2004). Increased ethanol drinking after repeated chronic ethanol exposure and withdrawal experience in C57BL/6 mice. Alcoholism: Clinical and Experimental Research, 28(12), 1829–1838. 10.1097/01.ALC.0000149977.95306.3A [PubMed: 15608599]

Blundell P, Hall G, & Killcross S (2001). Lesions of the basolateral amygdala disrupt selective aspects of reinforcer representation in rats. Journal of Neuroscience, 21(22), 9018–9026. 10.1523/JNEUROSCI.21-22-09018.2001 [PubMed: 11698612]

Burattini C, Gill TM, Aicardi G, & Janak PH (2006). The ethanol self-administration context as a reinstatement cue: acute effects of naltrexone. Neuroscience, 139(3), 877–887. 10.1016/j.neuroscience.2006.01.009 [PubMed: 16516392]

Cartoni E, Balleine B, & Baldassarre G (2016). Appetitive Pavlovian-instrumental transfer: a review. Neuroscience & Biobehavioral Reviews, 71, 829–848. 10.1016/j.neubiorev.2016.09.020 [PubMed: 27693227]

Chaudhri N, Sahuque LL, Schairer WW, & Janak PH (2010). Separable roles of the nucleus accumbens core and shell in context-and cue-induced alcohol-seeking. Neuropsychopharmacology, 35(3), 783–791. 10.1038/npp.2009.187 [PubMed: 19924113]

Corbit LH, & Balleine BW (2005). Double dissociation of basolateral and central amygdala lesions on the general and outcome-specific forms of Pavlovian-instrumental transfer. Journal of Neuroscience, 25(4), 962–970. 10.1523/JNEUROSCI.4507-04.2005 [PubMed: 15673677]

Corbit LH, & Balleine BW (2011). The general and outcome-specific forms of Pavlovian-instrumental transfer are differentially mediated by the nucleus accumbens core and shell. Journal of Neuroscience, 31(33), 11786–11794. 10.1523/JNEUROSCI.2711-11.2011 [PubMed: 21849539]

Corbit LH, Fischbach SC, & Janak PH (2016). Nucleus accumbens core and shell are differentially involved in general and outcome-specific forms of Pavlovian-instrumental transfer with alcohol and sucrose rewards. European Journal of Neuroscience, 43(9), 1229–1236. 10.1111/ejn.13235 [PubMed: 26970240]

Corbit LH, & Janak PH (2007). Ethanol-associated cues produce general Pavlovian-instrumental transfer. Alcoholism: Clinical and Experimental Research, 31(5), 766–774. 10.1111/j.1530-0277.2007.00359.x [PubMed: 17378919]

Corbit LH, & Janak PH (2016). Changes in the influence of alcohol-paired stimuli on alcohol seeking across extended training. Frontiers in Psychiatry, 7, 169. 10.3389/fpsyt.2016.00169 [PubMed: 27777560]

Corbit LH, Janak PH, & Balleine BW (2007). General and outcome-specific forms of Pavlovian-instrumental transfer: the effect of shifts in motivational state and inactivation of the ventral tegmental area. European Journal of Neuroscience, 26(11), 3141–3149. 10.1111/j.1460-9568.2007.05934.x [PubMed: 18005062]

Corbit LH, Muir JL, & Balleine BW (2001). The role of the nucleus accumbens in instrumental conditioning: evidence of a functional dissociation between accumbens core and shell. Journal of Neuroscience, 21(9), 3251–3260. 10.1523/JNEUROSCI.21-09-03251.2001 [PubMed: 11312310]

Crombag HS, Galarce EM, & Holland PC (2008). Pavlovian influences on goal-directed behavior in mice: the role of cue-reinforcer relations. Learning & memory, 15(5), 299–303. 10.1101/lm.762508 [PubMed: 18441288]

Cunningham CL, Patel P, & Milner L (2006). Spatial location is critical for conditioning place preference with visual but not tactile stimuli. Behavioral neuroscience, 120(5), 1115. 10.1037/0735-7044.120.5.1115 [PubMed: 17014262]

Dailey MJ, Moran TH, Holland PC, & Johnson AW (2016). The antagonism of ghrelin alters the appetitive response to learned cues associated with food. Behavioural Brain Research, 303, 191–200. 10.1016/j.bbr.2016.01.040 [PubMed: 26802728]

de Borchgrave R, Rawlins JNP, Dickinson A, & Balleine BW (2002). Effects of cytotoxic nucleus accumbens lesions on instrumental conditioning in rats. Experimental Brain Research, 144(1), 50–68. 10.1007/s00221-002-1031-y [PubMed: 11976759]

DePoy L, Daut R, Wright T, Camp M, Crowley N, Noronha B, … & Holmes A. (2015). Chronic alcohol alters rewarded behaviors and striatal plasticity. Addiction biology, 20(2), 345–348. 10.1111/adb.12131 [PubMed: 24666522]

El-Amamy H, & Holland PC (2007). Dissociable effects of disconnecting amygdala central nucleus from the ventral tegmental area or substantia nigra on learned orienting and incentive motivation. European Journal of Neuroscience, 25(5), 1557–1567. 10.1111/j.1460-9568.2007.05402.x [PubMed: 17425582]

Galaj E, Barrera E, Morris D, Ma YY, & Ranaldi R (2020). Aberrations in incentive learning and responding to heroin in male rats after adolescent or adult chronic binge-like alcohol exposure. Alcoholism: Clinical and Experimental Research, 44(6), 1214–1223. 10.1111/acer.14341 [PubMed: 32311102]

Garbusow M, Schad DJ, Sebold M, Friedel E, Bernhardt N, Koch SP, … & Müller DK. (2016). Pavlovian-to-instrumental transfer effects in the nucleus accumbens relate to relapse in alcohol dependence. Addiction biology, 21(3), 719–731. 10.1111/adb.12243 [PubMed: 25828702]

Gilpin NW, Herman MA, & Roberto M (2015). The central amygdala as an integrative hub for anxiety and alcohol use disorders. Biological psychiatry, 77(10), 859–869. 10.1016/j.biopsych.2014.09.008 [PubMed: 25433901]

Glasner SV, Overmier JB, & Balleine BW (2005). The role of Pavlovian cues in alcohol seeking in dependent and nondependent rats. Journal of studies on alcohol, 66(1), 53–61. 10.15288/jsa.2005.66.53 [PubMed: 15830903]

Griffin III WC, Haun HL, Hazelbaker CL, Ramachandra VS, & Becker HC (2014). Increased extracellular glutamate in the nucleus accumbens promotes excessive ethanol drinking in ethanol

dependent mice. Neuropsychopharmacology, 39(3), 707–717. 10.1038/npp.2013.256 [PubMed: 24067300]

Griffin III WC, Lopez MF, & Becker HC (2009). Intensity and duration of chronic ethanol exposure is critical for subsequent escalation of voluntary ethanol drinking in mice. Alcoholism: Clinical and Experimental Research, 33(11), 1893–1900. 10.1111/j.1530-0277.2009.01027.x [PubMed: 19673744]

Griffin III WC, Ramachandra VS, Knackstedt LA, & Becker HC (2015). Repeated cycles of chronic intermittent ethanol exposure increases basal glutamate in the nucleus accumbens of mice without affecting glutamate transport. Frontiers in Pharmacology, 6, 27. 10.3389/fphar.2015.00027 [PubMed: 25755641]

Hall DA, & Gulley JM (2011). Disruptive effect of amphetamines on Pavlovian to instrumental transfer. Behavioural brain research, 216(1), 440–445. 10.1016/j.bbr.2010.08.040 [PubMed: 20817041]

Hall J, Parkinson JA, Connor TM, Dickinson A, & Everitt BJ (2001). Involvement of the central nucleus of the amygdala and nucleus accumbens core in mediating Pavlovian influences on instrumental behaviour. European Journal of Neuroscience, 13(10), 1984–1992. 10.1046/j.0953-816x.2001.01577.x [PubMed: 11403692]

Heilig M, Egli M, Crabbe JC, & Becker HC (2010). Acute withdrawal, protracted abstinence and negative affect in alcoholism: are they linked? Addiction biology, 15(2), 169–184. 10.1111/j.1369-1600.2009.00194.x [PubMed: 20148778]

Holland PC (2004). Relations between Pavlovian-instrumental transfer and reinforcer devaluation. Journal of Experimental Psychology: Animal Behavior Processes, 30(2), 104. 10.1037/0097-7403.30.2.104 [PubMed: 15078120]

Holland PC, & Gallagher M (2003). Double dissociation of the effects of lesions of basolateral and central amygdala on conditioned stimulus-potentiated feeding and Pavlovian-instrumental transfer. European Journal of Neuroscience, 17(8), 1680–1694. 10.1046/j.1460-9568.2003.02585.x [PubMed: 12752386]

Krank MD (2003). Pavlovian conditioning with ethanol: sign-tracking (autoshaping), conditioned incentive, and ethanol self-administration. Alcoholism: Clinical and Experimental Research, 27(10), 1592–1598. 10.1097/01.ALC.0000092060.09228.DE [PubMed: 14574229]

Krank MD, O'Neill S, Squarey K, & Jacob J (2008). Goal-and signal-directed incentive: conditioned approach, seeking, and consumption established with unsweetened alcohol in rats. Psychopharmacology, 196(3), 397–405. 10.1007/s00213-007-0971-0 [PubMed: 17965977]

Lehner R, Balsters JH, Herger A, Hare TA, & Wenderoth N (2017). Monetary, food, and social rewards induce similar Pavlovian-to-instrumental transfer effects. Frontiers in Behavioral Neuroscience, 10, 247. 10.3389/fnbeh.2016.00247 [PubMed: 28101010]

Lingawi NW, & Balleine BW (2012). Amygdala central nucleus interacts with dorsolateral striatum to regulate the acquisition of habits. Journal of Neuroscience, 32(3), 1073–1081. 10.1523/JNEUROSCI.4806-11.2012 [PubMed: 22262905]

Lopez MF, & Becker HC (2005). Effect of pattern and number of chronic ethanol exposures on subsequent voluntary ethanol intake in C57BL/6J mice. Psychopharmacology, 181(4), 688–696. 10.1007/s00213-005-0026-3 [PubMed: 16001125]

Lopez MF, Becker HC, & Chandler LJ (2014). Repeated episodes of chronic intermittent ethanol promote insensitivity to devaluation of the reinforcing effect of ethanol. Alcohol, 48(7), 639–645. 10.1016/j.alcohol.2014.09.002 [PubMed: 25266936]

Meltzer D, & Brahlek JA (1970). Conditioned suppression and conditioned enhancement with the same positive UCS: An effect of CS duration. Journal of the Experimental Analysis of Behavior, 13(1), 67–73. 10.1901/jeab.1970.13-67 [PubMed: 5415041]

Milton AL, Schramm MJ, Wawrzynski JR, Gore F, Oikonomou-Mpegeti F, Wang NQ, … & Everitt BJ. (2012). Antagonism at NMDA receptors, but not β-adrenergic receptors, disrupts the reconsolidation of Pavlovian conditioned approach and instrumental transfer for ethanol-associated conditioned stimuli. Psychopharmacology, 219(3), 751–761. 10.1007/s00213-011-2399-9 [PubMed: 21766171]

Murschall A, & Hauber W (2006). Inactivation of the ventral tegmental area abolished the general excitatory influence of Pavlovian cues on instrumental performance. Learning & Memory, 13(2), 123–126. 10.1101/lm.127106 [PubMed: 16547159]

Pleil KE, Lowery-Gionta EG, Crowley NA, Li C, Marcinkiewcz CA, Rose JH, … & Kash TL. (2015). Effects of chronic ethanol exposure on neuronal function in the prefrontal cortex and extended amygdala. Neuropharmacology, 99, 735–749. 10.1016/j.neuropharm.2015.06.017 [PubMed: 26188147]

Prévost C, Liljeholm M, Tyszka JM, & O'Doherty JP (2012). Neural correlates of specific and general Pavlovian-to-Instrumental Transfer within human amygdalar subregions: a high-resolution fMRI study. Journal of Neuroscience, 32(24), 8383–8390. 10.1523/JNEUROSCI.6237-11.2012 [PubMed: 22699918]

Remedios J, Woods C, Tardif C, Janak PH, & Chaudhri N (2014). Pavlovian-conditioned alcohol-seeking behavior in rats is invigorated by the interaction between discrete and contextual alcohol cues: implications for relapse. Brain and behavior, 4(2), 278–289. 10.1002/brb3.216 [PubMed: 24683519]

Renteria R, Baltz ET, & Gremel CM (2018). Chronic alcohol exposure disrupts top-down control over basal ganglia action selection to produce habits. Nature communications, 9(1), 1–11. 10.1038/s41467-017-02615-9

Renteria R, Cazares C, & Gremel CM (2020). Habitual Ethanol Seeking and Licking Microstructure of Enhanced Ethanol Self-Administration in Ethanol-Dependent Mice. Alcoholism: Clinical and Experimental Research, 44(4), 880–891. 10.1111/acer.14302 [PubMed: 32020644]

Roberto M, Kirson D, & Khom S (2021). The role of the central amygdala in alcohol dependence. Cold Spring Harbor perspectives in medicine, 11(2), a039339. 10.1101/cshperspect.a039339 [PubMed: 31988201]

Roberto M, Madamba SG, Stouffer DG, Parsons LH, & Siggins GR (2004). Increased GABA release in the central amygdala of ethanol-dependent rats. Journal of Neuroscience, 24(45), 10159–10166. 10.1523/JNEUROSCI.3004-04.2004 [PubMed: 15537886]

Rose AK, Brown K, MacKillop J, Field M, & Hogarth L (2018). Alcohol devaluation has dissociable effects on distinct components of alcohol behaviour. Psychopharmacology, 235(4), 1233–1244. 10.1007/s00213-018-4839-2 [PubMed: 29480437]

Schad DJ, Garbusow M, Friedel E, Sommer C, Sebold M, Hägele C, … & Eichmann U. (2019). Neural correlates of instrumental responding in the context of alcohol-related cues index disorder severity and relapse risk. European archives of psychiatry and clinical neuroscience, 269(3), 295–308. 10.1007/s00406-017-0860-4 [PubMed: 29313106]

Sciascia JM, Reese RM, Janak PH, & Chaudhri N (2015). Alcohol-seeking triggered by discrete Pavlovian cues is invigorated by alcohol contexts and mediated by glutamate signaling in the basolateral amygdala. Neuropsychopharmacology, 40(12), 2801–2812. 10.1038/npp.2015.130 [PubMed: 25953360]

Seabrooke T, Hogarth L, Edmunds CE, & Mitchell CJ (2019). Goal-directed control in Pavlovian-instrumental transfer. Journal of Experimental Psychology: Animal Learning and Cognition, 45(1), 95. 10.1037/xan0000191 [PubMed: 30604997]

Talmi D, Seymour B, Dayan P, & Dolan RJ (2008). Human Pavlovian–instrumental transfer. Journal of Neuroscience, 28(2), 360–368. 10.1523/JNEUROSCI.4028-07.2008 [PubMed: 18184778]

Tsiang MT, & Janak PH (2006). Alcohol seeking in C57BL/6 mice induced by conditioned cues and contexts in the extinction-reinstatement model. Alcohol, 38(2), 81–88. 10.1016/j.alcohol.2006.05.004 [PubMed: 16839854]

Van Dyne GC (1971). Conditioned suppression with a positive US in the rat. Journal of comparative and physiological psychology, 77(1), 131. 10.1037/h0031576 [PubMed: 5120676]

van Timmeren T, Quail SL, Balleine BW, Geurts DE, Goudriaan AE, & van Holst RJ (2020). intact corticostriatal control of goal-directed action in Alcohol Use Disorder: a Pavlovian-to-instrumental transfer and outcome-devaluation study. Scientific reports, 10(1), 1–12. 10.1038/s41598-020-61892-5 [PubMed: 31913322]

Wyvell CL, & Berridge KC (2001). Incentive sensitization by previous amphetamine exposure: increased cue-triggered “wanting” for sucrose reward. Journal of Neuroscience, 21(19), 7831–7840. 10.1523/JNEUROSCI.21-19-07831.2001 [PubMed: 11567074]

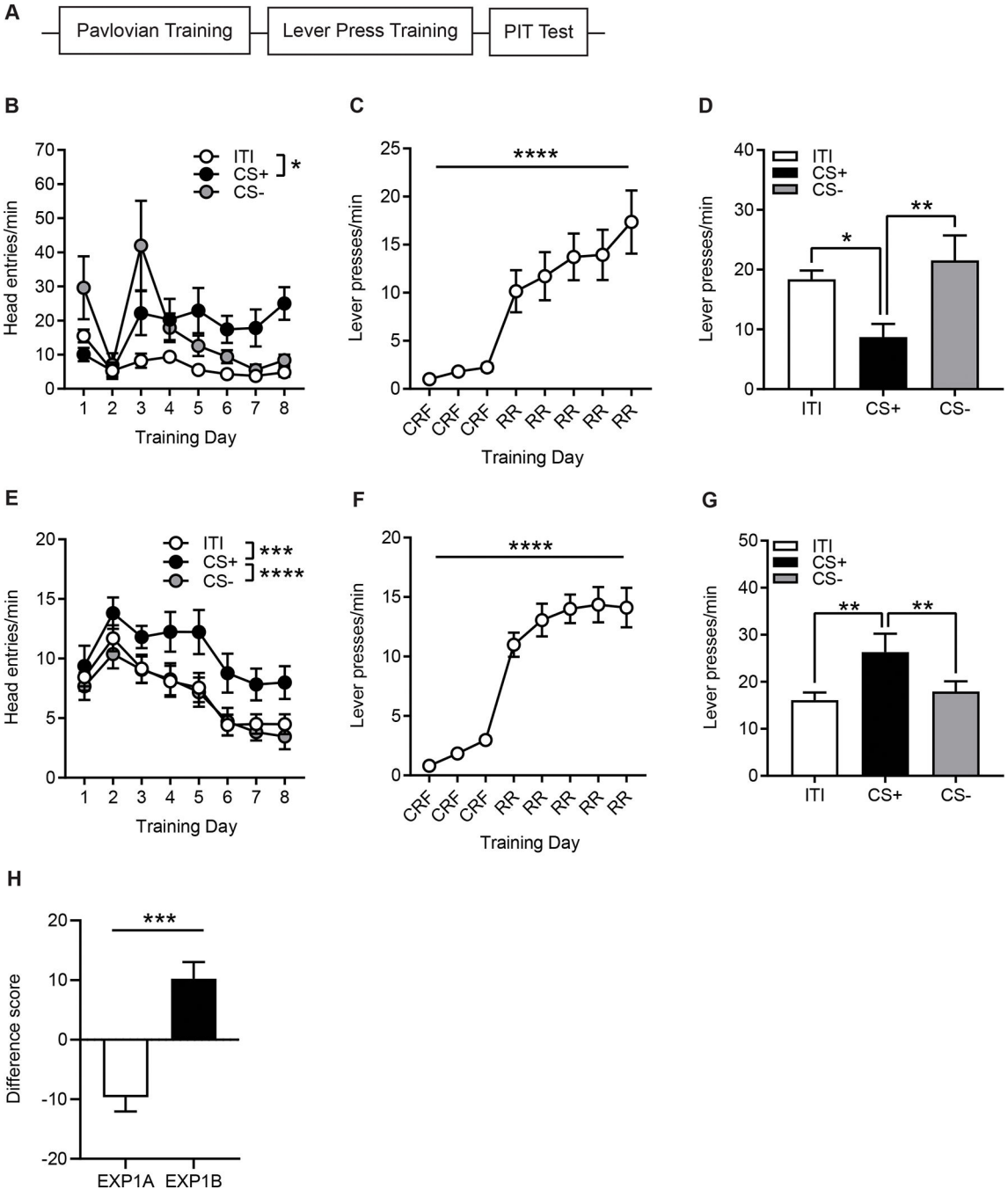

Fig. 1. Long cue procedure produces positive Pavlovian-to-instrumental transfer (PIT) and short cue procedure produces negative PIT.

(**A**) All mice first went through Pavlovian training including a food-associated tone (CS+), a non-food-associated tone (CS−), and intertrial intervals (ITI) where no tone was played; next, mice went through instrumental lever press training on continuous reinforcement (CRF) and random ratio (RR) schedules; finally, transfer was assessed in a PIT test. (**B-D**) Experiment 1A, short cue procedure. (**B**) Rate of food port entries across Pavlovian training days. (**C**) Rate of lever presses across instrumental training days. (**D**). Rate of lever pressing

during each trial type in the PIT test. (**E-G**) Experiment 1B, long cue procedure. (**E**) Rate of food port entries across Pavlovian training days. (**F**) Rate of lever presses across instrumental training days. (**G**) Rate of lever presses during each trial type in the PIT test. (**H**) Average difference between rate of lever pressing during CS+ minus rate of lever pressing during ITI for Experiments 1A and 1B. Data points represent mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ represent significant comparisons.

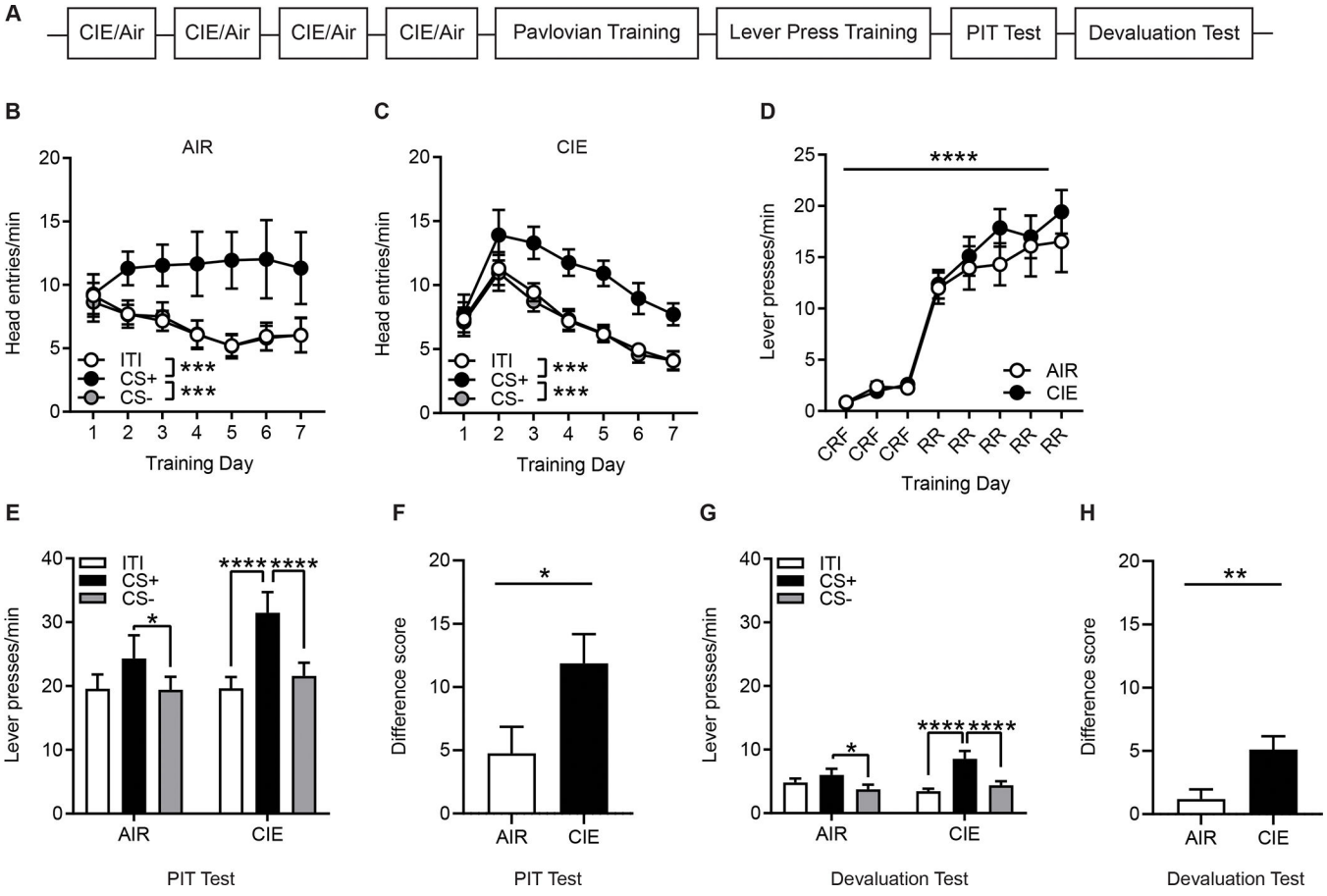

Fig. 2. Chronic intermittent ethanol (CIE) exposure enhances Pavlovian-to-instrumental transfer (PIT).

(**A**) All mice were exposed to either CIE or Air control procedures; next, animals went through long cue procedure Pavlovian training including a food-associated tone (CS+), a non-food-associated tone (CS−), and intertrial intervals (ITI) where no tone was played; next, mice received instrumental lever press training on continuous reinforcement (CRF) and random ratio (RR) schedules; transfer was then assessed in a PIT test; finally, a second PIT test was performed following devaluation of the food outcome. (**B**) Rate of food port entries across Pavlovian training days in Air group. (**C**) Rate of food port entries across Pavlovian training days in CIE group. (**D**) Rate of lever presses across instrumental training days. (**E**) Rate of lever pressing during each trial type in the PIT test. (**F**) Average difference between rate of lever pressing during CS+ minus rate of lever pressing during ITI in the PIT test. (**G**) Rate of lever pressing during each trial type in the devaluation test. (**H**) Average difference between rate of lever pressing during CS+ minus rate of lever pressing during ITI in the devaluation test. Data points represent mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ represent significant comparisons.