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Acute Drug Effects on Habitual and Non-Habitual Responding in crossed High Alcohol Preferring Mice

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Abstract

Rationale—Drug reward plays a central role in acquiring drug-seeking behavior. However, subjects may continue using drugs despite negative consequences because self-administration becomes habitual, and divorced from outcome values. Although a history of drug and alcohol use expedite habit acquisition, and in spite of the fact that self-administration leads to intoxication, the acute effects of drugs on habitual responding are not well understood.

Objectives—We sought to observe how acute ethanol and amphetamine affect the balance between habitual and goal-directed behavior, as measured by a fluid-reinforced operant conditioning task.

Methods—Selectively bred crossed High Alcohol Preferring (cHAP) mice were trained on an operant conditioning task reinforced on a variable interval schedule with 1% banana solution, which was subsequently devalued via LiCl pairing in half the animals. Ethanol (1.0 g/kg), amphetamine (2.0 mg/kg), or saline was administered prior to a post-devaluation test.

Results—Overall, mice showed habitual behavior, but when divided into high or low responding groups based on training response rates, saline-treated, low responding animals devalued, while saline-treated high-responding animals didn't. Furthermore, amphetamine elicited devaluation even in high-responding animals, while ethanol prevented devaluation even in low-responding animals.

Conclusions—These data show that ethanol shifts animals toward behaving habitually. This may illuminate why alcohol-intoxicated individuals display impaired judgment about the relative merits of drinking, and potentially serve as a mechanism by which intoxicated subjects resume previously devalued behaviors, such as comorbid drug use. These findings also show that high variable interval response rates facilitate a shift from goal-directed to habitual behavior.

Keywords

alcohol; amphetamine; operant; habit expression; selectively bred; high alcohol preferring

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Introduction

Habit formation is a process by which a subject repeatedly performs a behavior to obtain a desirable outcome and, over time, continues to respond relatively automatically, without considering the outcome (Balleine and O'Doherty 2010). Behavior begins as goal-directed or response-outcome (R-O): a purposeful action that depends upon presentation of a positive outcome (Dickinson 1985). A behavior can also be habitual (or based on a stimulus-response [S-R] association), where the behavior is unaffected by changes in the current value of the outcome (Dickinson 1985). To distinguish between these alternatives requires an extinction test following devaluation of the reinforcer. In the absence of the reinforcer, if a subject is behaving in a goal-directed manner and recalls the value of the outcome, devaluation will decrease responding. However, if that behavior is habitual, devaluing or making the outcome aversive will have no effect on responding because the outcome is not recalled; responding is instead based upon an S-R association.

Habit formation can be used to explain some aspects of problematic drug and alcohol use (Everitt and Robbins 2016). For example, tolerance could decrease the value of a drug reward, or drug use could be accompanied by concomitant health or societal problems, either of which could be viewed as forms of reinforcer devaluation. Failure to consider the rebalanced value of drug use could be explained by habitual responding. This lack of regard for change in outcome value may precede compulsive drug seeking behavior, where the subject continues to self-administer in spite of negative consequences (Everitt and Robbins 2016).

Previous research using rats has shown that an extended history of alcohol consumption accelerates the formation of a habit for alcohol (Corbit et al. 2012) and amphetamine exposure facilitates habitual responding for a conventional reinforcer (Nelson and Killcross 2006). This has been replicated in human studies as well, showing potentiation of habit learning in drug and alcohol users (Panlilio et al. 2004; Sjoerds et al. 2013). Interestingly, acute alcohol intoxication also potentiates habitual responding when patients are matched for alcohol history (Hogarth et al. 2012), but there are no other studies that address acute intoxication and its effects on expression of habitual response patterns. Such data are important because if acute intoxication affects the balance of retrieval of S-R vs. R-O associations, then this could in principle explain puzzling intoxicated behavior, such as continued self-administration of a drug despite tolerance to its rewarding actions (Pudiak et al. 2014) or even during the presence of concurrent aversive effects of drug consumption (Lesscher et al. 2010; Vanderschuren and Everitt 2004). We used drug-naïve animals because we were explicitly interested in how acute drug intoxication could alter instrumental behavior in animals without a drug history, given that the work cited above has already addressed changes resulting from repeated drug exposure.

The goal of the current studies is to examine the acute effect of two drugs of abuse, ethanol (EtOH) and amphetamine (AMP), on the expression of a habit. While effects of an AMP and alcohol history have been studied (Corbit et al. 2012; Mangieri et al. 2012; Nelson and Killcross 2006), their acute effect on retrieval of non-drug related instrumental associations is still unknown.

In two experiments, we administered these drugs acutely prior to a post-devaluation extinction test session to determine whether acute intoxication could shift behavior that would normally be goal-directed towards habitual responding. We hypothesized that both AMP and EtOH would potentiate habitual behavior if drugs of abuse generally facilitate retrieval of S-R associations (i.e., habitual responding) at the cost of R-O.

Methods

Subjects

120 cHAP mice (60 male and 60 female) were divided equally by sex between two experiments and ranged from 60 – 73 days old on the first day of training. All animals were single housed in standard Plexiglas cages with pine bedding and moved to the housing room at least 7 days prior to the first day of magazine training, under a 12-hour reverse light cycle (lights off at 0700). Mice were water restricted and received two hours of water access each day in order to increase motivation to respond for the liquid reinforcer during operant conditioning training, but food was available *ad libitum*. All studies were approved by the Institutional Animal Care and Use Committee (IACUC) of IUPUI and conducted according to the NIH Guide for the Care and Use of Laboratory Animals.

Apparatus

Twelve operant conditioning chambers (Med Associates, St. Albans, VT) were used for the operant conditioning testing in this experiment. Each chamber measured 21.6 × 19.7 × 12.7 cm and was placed inside a light- and sound-attenuating box. The operant conditioning boxes were equipped with yellow lights positioned above the left and right levers, centering the sipper tube opening. A 10-mL sipper tube containing 1% banana solution descended into the chamber's opening upon a correct lever press. Session duration, reinforcers obtained and correct and incorrect lever presses were recorded using MED-PC IV software (Med Associates, St. Albans, VT).

Solutions

For operant conditioning reinforcement, all mice had access to 1% v/v banana flavoring in water. This solution was also devalued for all mice during the devaluation stage using lithium chloride (LiCl). The LiCl solution concentration was 6.36 g/1 L (0.15 M) with an injection volume of 40 mL/kg, resulting in a dose of 0.254 g/kg (6.0 mEq/kg). If animals did not show sufficient aversion after four days, the injection volume was increased to 60 mL/kg, resulting in a dose of 0.382 g/kg (9.0 mEq/kg).

For drug pretreatment, 100% EtOH was diluted to a 10% concentration with 0.9% SAL and injected at a volume of 12.6 mL/kg to achieve a dose of 1.0 g/kg. A 0.2-mg/mL solution of *d*-amphetamine sulfate (Sigma-Aldrich, St. Louis, MO) was administered at a volume of 10 mL/kg in sterile SAL, achieving the dose of 2 mg/kg. Injections were administered ten minutes prior to the extinction test.

Doses of each pretreatment drug were derived from previous research, aiming for a dose that was not so high as to greatly affect operant conditioning responding, but sufficient to induce

a pharmacological effect in conditioned place preference, with additional consideration taken for any motor impairing effects (Grahame et al. 2001; Jerlhag et al. 2010; McKim 1980; Vanhanen et al. 2015).

Procedure

Both experiments followed nearly identical training procedures that are graphically displayed in Fig. 1. Day 1 began with magazine training on an FT-120s protocol where the reinforcer was presented for thirty seconds every two minutes, regardless of lever pressing, to shape the mouse to drink from the sipper tube. Criterion for advancement to the next phase of training was consumption of at least 0.2 mL of the reinforcer. Days 2 – 4 of training consisted of an FR-1 schedule where mice were rewarded for a correct lever press with a 5-s presentation of the reinforcer. Incorrect (opposite lever) presses had no effect, but were recorded. After meeting criterion of twenty lever presses with 0.2 mL of fluid consumed on Day 4, animals moved on to the VI stage of the experiment. On Day 5, mice underwent a 45-minute VI-20s session. On Days 6 – 8, animals proceeded to 45-minute VI-60s sessions.

Conditioned taste aversion (CTA) training began on Day 9. During this phase, mice had 30-minute access to a tube with the reinforcer in their home cage, immediately followed by either a LiCl (devalued) or SAL (non-devalued) injection. Following the first day of CTA, devalued mice were yoked with a non-devalued mouse based on drug group, sex, and amount of banana solution consumed to control for number of injections. This procedure spanned from Days 9 – 11, but continued for devalued mice that did not meet the criterion of consuming no more than 0.5 mL of the banana solution CS and their yoked non-devalued counterparts. If after four days mice failed to meet this criterion, the injection volume increased to a dose of 0.382 g/kg (9.0 mEq/kg) for both devalued and yoked non-devalued animals. All mice met criterion by the 6^{th} day of CTA.

Following the CTA training, mice had a 10-minute reminder session (REM) where they had free access to the banana reinforcer in the operant conditioning chamber. The levers were removed as to not disrupt the S-R association potentially formed during training. Pilot studies indicated that this reminder session facilitated reinforcer devaluation. Criterion for advancement was set at 0.2 mL of banana solution consumed, to ensure that the mouse experienced the banana solution previously paired with aversive LiCl in the operant conditioning chamber. However, no LiCl injection was administered following the CS exposure. Mice were removed from the study if they did not meet this criterion. On the following day, mice had a post-devaluation extinction test. Following a 10-minute EtOH, AMP, or SAL pretreatment, animals had a 15-minute session in the operant conditioning boxes, with an empty sipper tube serving as the reinforcer on a VI-60 schedule. Within each drug pretreatment group, response rates of devalued and non-devalued mice were compared to determine the effect of CTA on lever pressing, thus indicating if the behavior was goal-directed or habitual.

Statistical Analysis

Data were analyzed using SPSS software (SPSS, Version 22, Chicago, IL) and graphed using Prism software (Graphpad Prism, v. 6.0, La Jolla, CA). Significance was set at $p <$

0.05. Repeated measures ANOVA was used to look at changes in responding during the seven days of training. When the assumption of sphericity was violated, Greenhouse-Geisser correction was applied. In order to determine consistency across similar experiments, repeated-measures ANOVAs were used to compare devalued to non-devalued mice within the saline group that was replicated across more than one experiment. To determine the effect of EtOH and AMP pretreatment on post-devaluation responding, we used independent t-tests within each drug condition to compare devalued and non-devalued animals. No significant difference between the devalued and non-devalued animals indicated that LiCl-treated mice were insensitive to devaluation and, therefore, behaving habitually. To examine differences in response rate during training, a median split of total reinforced lever responses during training was performed and animals were labeled as either high or low responders. The EXT test ANOVA was later reanalyzed with high/low responders also introduced as a variable, with Drug condition and Devaluation condition. In the presence of an interaction, we analyzed SAL mice only to determine if devaluation was equally efficacious in both the high and low responders via Responder X Devaluation condition ANOVA. We then separately analyzed EXT behavior within the high- and low-responders by Drug and Devaluation. Following a Drug X Devaluation interaction, we performed t-tests for each Drug condition, comparing devalued and non-devalued subjects.

Results

Collapsing Across Experiments

Five animals were removed from the studies. Two mice did not meet criterion to advance past FR1 training and one died during training due to illness. One DEV mouse died during LiCl treatment. One DEV mouse failed to meet criterion on the REM day by not consuming any banana solution.

The SAL animals were run between two experiments, but there was no significant effect of replication in either the SAL DEV [$t(23) = -1.38$, $p > 0.05$] or SAL NoDEV [$t(24) = 1.06$, $p > 0.05$] groups. We used the absence of differences between experiments to collapse between these two groups of SAL mice. Final group numbers are displayed in Table 1. Similarly, we found no Sex X Drug or Sex X Devaluation interaction, Fs > 1 permitting us to collapse across Sex.

Effects of Acute Drug Administration on Expression of Habitual Behavior

Overall, the control SAL animals had no difference between devalued and non-devalued conditions, $t(43.41) = -0.36$, $p > 0.05$, indicating habitual behavior. EtOH pretreatment did not affect devaluation, with devalued and non-devalued mice showing no difference in response rates ($t(32) = 0.43$, $p > 0.05$). AMP Dev mice showed a strong trend toward reduced responding, as compared to the non-devalued animals [$t(17.25) = -2.11$, $p = 0.050$; Fig. 2].

Low Response Rates During Training Preserve Outcome-Based Responding

Because the range of response rates during acquisition varied greatly among animals in all groups, and increased experience with VI schedules tends to facilitate habitual responding

(Dickinson, et al., 1983), we divided mice into High and Low responding groups based upon their response rates on the reinforced lever during operant conditioning training. We summed lever presses from all four VI days (1 VI20 and 3 VI60) to obtain a "Total VI" (TotVI) response rate, which resulted in a range of 29 – 1,011 presses on the correct lever and a median of 345. Animals responding above and below the median rate were designated High and Low Responders, respectively. We then plotted the total number of correct lever presses against the number of reinforcers earned (Fig. 3) to examine the relationship between lever press behavior and reinforcer delivery. To further explore this relationship, we calculated the ratio of correct responses to reinforcers delivered for each animal. Repeated measures ANOVA of this ratio showed a Day X Responder Type interaction ($F(2.70, 310.74) = 18.55$, $p < 0.001$), indicating the relationship between the active lever press and reinforcer delivery differed over the course of VI training by responder type (Fig. 3A). This suggests that, due to differences in training behavior, high responders might be more likely to form a habit, due to the weaker contingency between response and outcome. Animals within each drug and devaluation subgroup were evenly split between high and low responders (Table 1).

Reanalyzing EXT responding including pretreatment drug, devaluation state, and responder type revealed a significant Drug X Deval X Responder interaction [$F(2, 117) = 4.57$, $p = 0.012$]. To assess how high and low responders differ in the efficacy of devaluation in the absence of drug treatment, we first ran a Responder (High vs Low) X Devaluation ANOVA on saline subjects only. This showed an interaction, $F(1,52) = 7.16$, $p = 0.010$, which we followed with a t-test assessing devaluation efficacy in each Responder group. This indicated that devaluation was effective in low responders, $t(22) = -2.42$, $p = 0.024$, but not high responders, $t < 1.0$. We then stratified by responder group, conducting separate Drug X Devaluation ANOVAs within both the high and low responders. We found a significant Drug × Devaluation interaction in the high responding group [$F(2, 58) = 4.02$, $p = 0.024$] and a trend toward significance in the Low Responding group [$F(2, 57) = 2.71$, $p = 0.076$], supporting the idea that drug treatment altered habitual behavior. Comparing devalued and non-devalued conditions within each drug pretreatment and responder type, we observed that the low responding SAL devalued mice showed decreased responding as compared to non-devalued, indicating goal-directed behavior, $t(22) = -2.42$, $p = 0.024$, but the high responding mice displayed habitual behavior, $t(25) = 1.03$, $p > 0.05$. When pretreated with EtOH, devalued and non-devalued animals responded similarly in both High [$t(14) = -0.026$, $p > 0.05$, Fig. 4A] and Low [$t(16) = 0.82$, $p > 0.05$, Fig. 4B] responding groups, indicating that regardless of response rate, ethanol caused mice to behave habitually. While low-responding mice pretreated with AMP appeared to have a floor effect, perhaps rendering devaluation undetectable [$t(13) = -0.688$, $p > 0.05$] (Fig 4B), there was a strong trend in amphetamine-treated mice towards devaluation [$t(9.590) = -2.202$, $p = 0.053$], suggesting amphetamine restored goal-directed behavior (Fig 4A).

Training, Reminder, CTA Data

To ensure that the differences between devalued and non-devalued mice within each drug group could be attributed to drug effects on devaluation and not preexisting differences, training, REM and CTA behavior were analyzed both as a whole and within each responder

group. Drug pretreatment and devaluation group assignments were counterbalanced based on training data, ensuring there were no baseline differences for either assignment prior to devaluation, $F's < 1.2$, $p's > 0.25$ (Figs. 5A-B). Throughout training, mice showed a significant increase in lever pressing behavior, as indicated by a main effect of day [$F(3.25, 334.71) = 261.81$, $p < 0.001$], as well as a main effect of type of responder [$F(1, 103) = 156.48$, $p < 0.001$] (Fig. 5A-B).

Further analyses were run on the CTA data to determine if there were group differences independent of future drug assignment. All animals hit criterion after six days and none were dropped from the study due to failure to acquire the aversion. Fewer than half of the devalued animals (27/58 mice) required the increased LiCl dose. Non-devalued controls were matched within each drug pretreatment group, so animals within each group had equal number of injections during CTA. Fig. 6 demonstrates the change in banana consumption between the first day of CTA and final day (ranged from Day 3 – Day 6). There was a strong main effect of devaluation condition [$F(1, 113) = 566.12$, $p < 0.001$] and no effect of drug or responder type ($F's < 1.5$, $p's > 0.20$), indicating that only the devalued mice reduced banana consumption.

Looking at the effects of future drug pretreatment and devaluation on REM intake yielded a main effect of devaluation group [$F(1, 114) = 102.93$, $p < 0.001$], but no effect of drug group or training responder, $F's < 0.5$, indicating that only devaluation group, and not future drug treatment, reduced intake at this time (Fig. 7). This also provides evidence that the memory of the CTA transferred to the operant conditioning box.

Discussion

These experiments are the first to systematically examine whether acute drug intoxication can influence the balance between retrieval of goal-directed vs. habitual instrumental behavior. We expected both AMP and EtOH, two drugs of abuse, to potentiate expression of habitual responding. However, only EtOH led to habitual responding in animals that otherwise would have shown devaluation, supporting the idea that acute EtOH intoxication may cause poor decision-making and failure to balance appetitive and aversive outcome values (Hogarth et al. 2012; McCloskey et al. 2009; Miller and Fillmore 2014).

These findings fit within a framework that emphasizes the importance of retrieval in addition to learning factors during instrumental behavior. Previous studies in rodents have shown that a history of alcohol consumption potentiates habitual behavior, and this mechanism might be implicated in addiction (Corbit et al. 2012; Hogarth et al. 2012; Sjoerds et al. 2013). Because these studies used exposure to alcohol either prior to, or concurrently with the instrumental training, they leave open whether the effects of drug exposure are on acquisition of, or retrieval of, instrumental associations. More recently, Gremel and Costa (2013) elegantly described the shift from R-O to S-R behavior as less than an all-or-nothing change and more of a continuum. They were able to show that at the time of extinction testing, a context could favor either S-R or R-O associations in the same mice, depending upon training procedures in those environments. Consistent with this idea and with the present results, Hogarth et al. (2012) showed that, similar to the present study, acute EtOH (0.4 g/kg) in humans facilitated

habitual responding during extinction testing. Together, these findings implicate the importance of retrieval mechanisms as an important influence on habitual behavior, while the current results implicate alcohol intoxication in particular as tipping the balance during testing towards S-R associations. The current results may mean that during times of alcohol intoxication, there is impairment in the ability of intoxicated individuals to respond appropriately to the current balance between appetitive and aversive aspects of reinforcers, including drugs of abuse.

Acute administration of AMP preserved outcome-based behavior even when saline-treated mice showed habitual responding. Although a history of AMP (Nelson and Killcross 2006; Nelson and Killcross 2013) facilitates habitual responding, this effect is likely a result of chronic administration, as compared to acute administration. It is evident that acute AMP, as seen in this study, is not sufficient to facilitate habit expression, and may even facilitate retrieval of information about devaluation.

Analysis of both CTA and REM intake indicated that devaluation was equally effective in the three drug treatment groups. Moreover, on the test day, we compared responding of non-devalued mice between drug pretreatments and there was no effect of drug on response rates. This suggests that the results seen in the devalued animals were not due to motor effects, but instead some other mechanism. Notably, because animals with the lower response rates were more likely to show devaluation than mice with higher response rates, behavioral floor effects cannot explain the lack of devaluation observed in EtOH-treated mice, which also showed no effect of EtOH on response rates in either high- or low-responding animals.

Based on the current findings, future researchers may consider avoiding alcohol intoxication during habit testing if they want to differentiate between alcohol effects on acquisition vs. expression of instrumental associations. For example, Corbit et al. (2012) found that extended training for alcohol promotes habitual behavior more rapidly than in animals with a shorter training period. To devalue EtOH, the authors used satiation devaluation prior to the habit expression test. While the authors saw habitual behavior in the long-training group, they were intoxicated during the test due to their consumption of alcohol immediately prior to extinction testing. Results from the present studies indicate that, following habit training, administration of acute alcohol prior to these tests may promote the expression of a habit where one may not otherwise exist. If sucrose-reinforced mice are sober during testing, while ethanol-reinforced mice are intoxicated, this can make it appear as if reinforcer type influences learning, when in fact it alters retrieval. Thus, satiation devaluation may be problematic when alcohol serves as the reinforcer, a point we also argued in a review (O'Tousa and Grahame, 2015), but now show empirical evidence to support.

Acute EtOH alone could not have been responsible for the shift observed in Corbit et al., as the short training animals still preserved outcome-based behavior. There may have been a weak S-R relationship formed in the short training animals (especially given the use of a variable ratio, rather than variable interval schedule), but they still remained on the "goal-directed" side of the continuum and even acute EtOH was not sufficient to push them far enough to the "habitual" side. However, if acute administration of EtOH increases the probability of the subject behaving in a habitual manner, use of satiation devaluation of

EtOH prior to habit testing should be used with caution. Importantly, a different study by Mangieri et al. (2012) also observed that ethanol reinforcement was more likely to engender habitual responding after long training than sucrose reinforcement, but they (like the present study) used LiCl devaluation rather than satiation devaluation, obviating the need for acute intoxication during the extinction testing.

The overall findings also hold real world implications for understanding addiction. Patients with alcohol use disorder continue to drink in spite of negative consequences, which could arise from habitually responding in the presence of a stimulus without regard for the outcome. This set of experiments investigated how acute intoxication with drugs of abuse might affect this behavior. In principle, drug-induced habitual responding could explain relapse behavior. Although a person may have quit using alcohol and be abstinent for a period of time, one drink may be sufficient for them to “fall off the wagon” and resume problematic drinking behavior, despite the previous devaluation of the intoxication outcome (Keller 1972). In fact, simple placement back into an alcohol-paired context may be sufficient to facilitate a shift back to habitual behavior (Hogarth et al. 2012). Acute intoxication causing a temporary shift toward responding for formerly devalued outcomes may be a driving force behind impaired judgment while drinking, as well as impaired judgment about the value of drinking itself.

Based on these findings, one area to investigate is the underlying neural substrates of habit expression. Based on previous research, the DMS and nucleus accumbens (NAc) are vital for the acquisition of instrumental behavior and the dorsolateral striatum (DLS) is necessary for habitual behavior (Corbit et al. 2001; Yin et al. 2004; Yin et al. 2005). Robbins and Everitt (2002) hypothesized that because drugs of abuse agonize release of dopamine in the striatum, they accelerate the shift toward habitual behavior. Through a series of microdialysis experiments, it was shown that AMP increases DA release to a greater extent in the NAc than the dorsal striatum (Di Chiara and Imperato 1988) and higher doses of EtOH showed a increase of DA release in the striatum and decreased levels in the NAc (Imperato and Di Chiara 1986). More recently, *ex vivo* voltage clamp recording of the DLS by Patton et al. (2016) demonstrated that acute application of alcohol decreased the firing of inhibitory fast spiking interneurons acting upon the inhibitory medium spiny neurons within this region via delta opioid receptors (DORs), thereby disinhibiting the DLS and potentiating action in this “habitual” area. These properties of alcohol may be the cause of its acute effects on habitual behavior observed in these experiments.

One potential limitation of this study is the absence of a dose-response curve. Doses were chosen to ensure that each drug was pharmacologically relevant without greatly affecting response rates, leaving a narrow window of dosages that can be used. We were successful in finding a dose of each drug that did not affect extinction response rates. This means that EtOH impairment of devaluation is not simply due to depressed motor behavior, as we saw no motor effects in the non-devalued control group. While it is possible that a higher dose of any of the drugs used could also affect habit, we may not be able to selectively measure such changes using the current procedure.

Another potential limitation here is that cHAP mice are selectively bred for high alcohol preference. Although the animals in this study did not consume alcohol, they can serve as a model for family history positive human patients. This provided unique insight into subjects with a genetic predisposition to alcoholism that has not yet been studied in an animal model. However, it is also possible that the unique effect of alcohol in these experiments might be caused by an interaction of alcohol with a positive family history. Future work might examine whether alcohol facilitation of habit extends to other populations.

A final caveat is that we re-exposed animals to the banana flavor (reminder treatment) following its pairing with LiCl. We did this based upon pilot experiments in which mice not so reminded failed to show devaluation. It is also possible that REM could cause a second order association to form between the flavor aversion and the test context, as has been observed in rats (Archer and Sjoden 1982), which could contribute to the devaluation seen here in low responders. However, the fact that high responders showed no devaluation suggests that such a classically conditioned aversive response, which should not distinguish between high and low responders, is not what is causing the devaluation effects observed in saline-treated animals here. Thus, the change in devaluation magnitude in EtOH mice is unlikely to be mediated by second order aversive conditioning to the context.

Taken together, these findings demonstrate that acute administration of EtOH is sufficient to promote the expression of a habit that would not be expected under control conditions. This is not seen when animals are under the influence of AMP. This pattern could be explained by the specific effect of acute EtOH on dopamine levels in the dorsal striatum or DORs. Future studies should delve deeper into this field to differentiate the neural changes that underlie the shift to habitual behavior when EtOH is administered acutely, as compared to the propensity toward S-R behavior that occurs following a history of alcohol or drug use. Given this knowledge, it is essential to be cautious when administering EtOH when testing for habitual behavior, as this acute intoxication can alter expression of instrumental learning.

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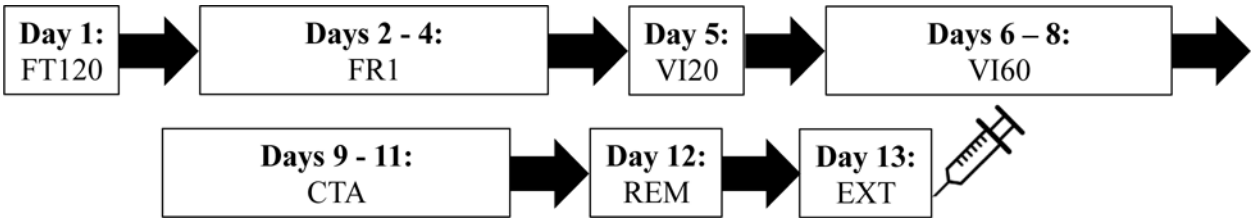

Fig. 1.

Timeline of events for both experiments. Syringe indicates acute drug administration 10 minutes prior to session.

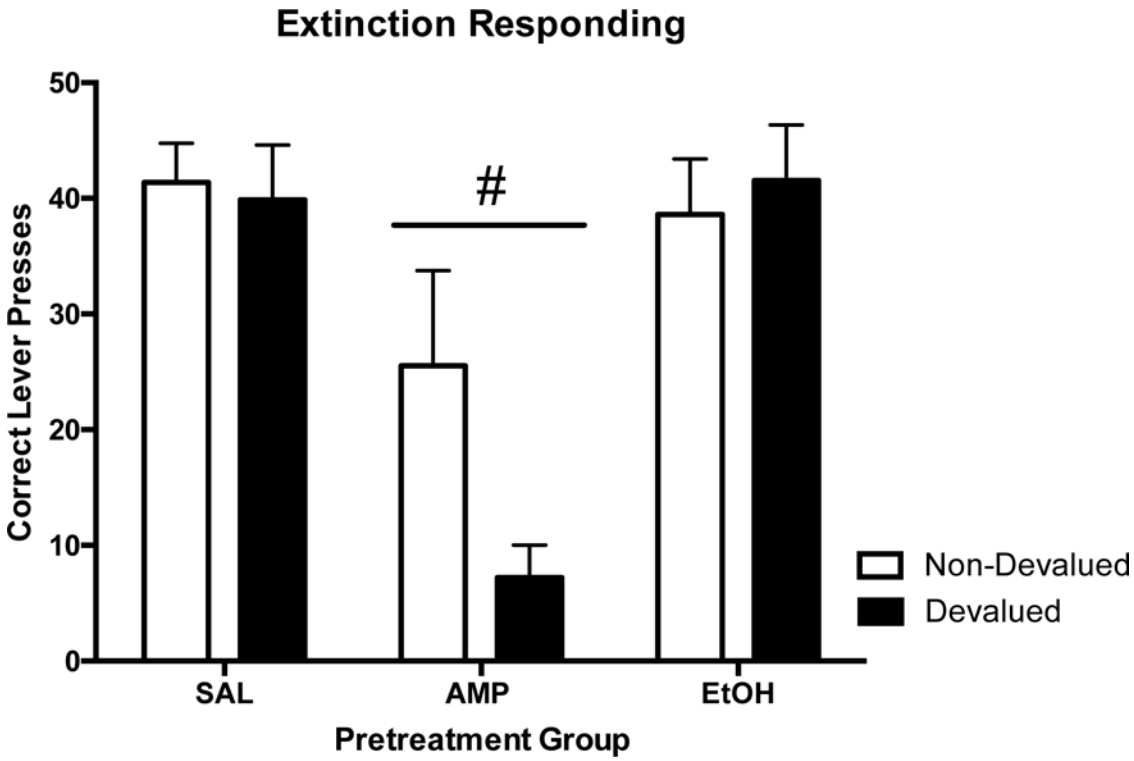

Fig. 2.

Collapsed results from Experiments 1 & 2. Mice were injected with SAL, AMP (2.0 g/kg) or EtOH (1.0 g/kg) 10 min prior to the extinction session. (#$p = 0.059$)

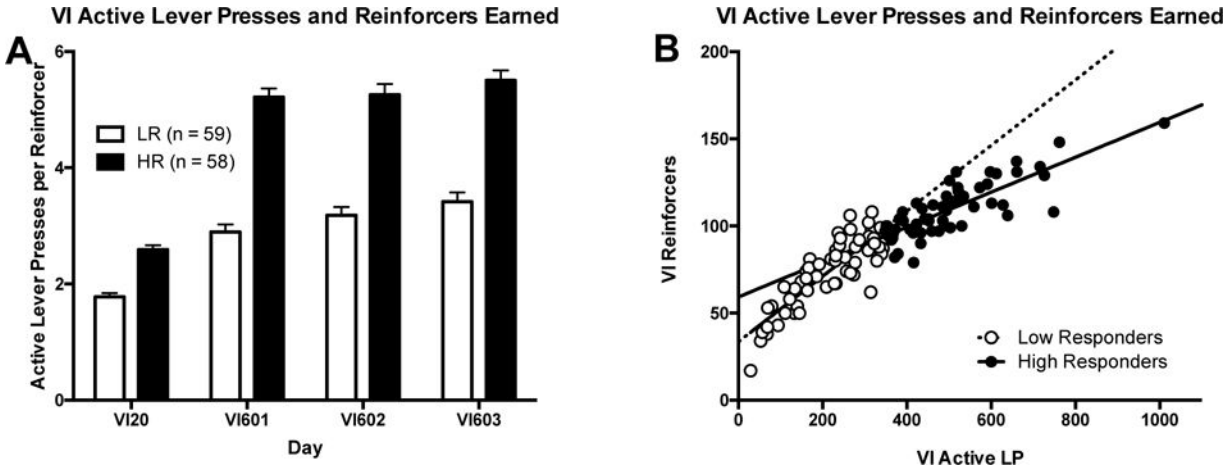

Fig. 3.

a Ratio of correct lever presses to reinforcers earned during the four VI sessions. **b** Scatterplot demonstrating the relationship between total active lever presses during the VI days and reinforcers earned. Regression lines were fitted to each subgroup of responders (LR: dotted; HR: solid).

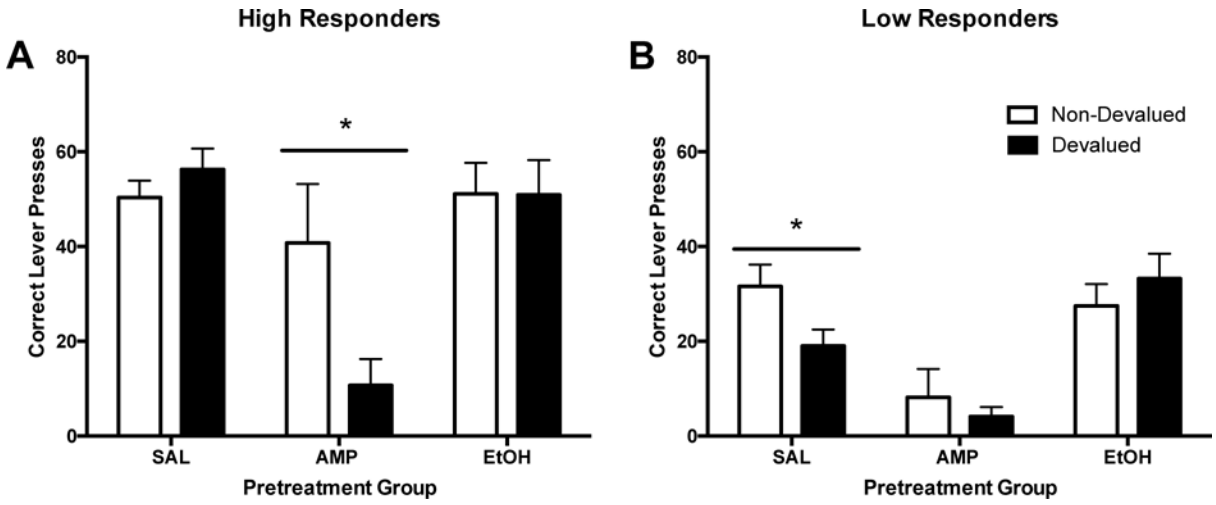

Fig. 4.

Extinction test results. Mice were injected with SAL, AMP (2.0 g/kg) or EtOH (1.0 g/kg) 10 min prior to the extinction session. **a** Lever pressing in high responding mice. **b** Lever press behavior for the low responding mice. (* $p < 0.001$).

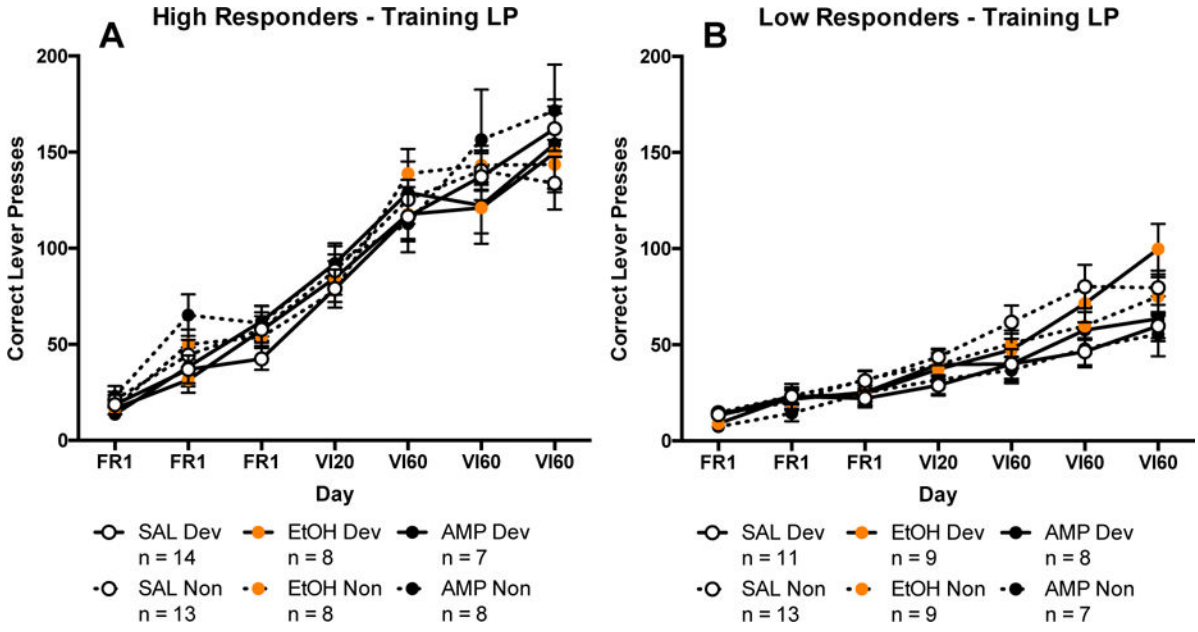

Fig. 5.
Correct lever presses during training.

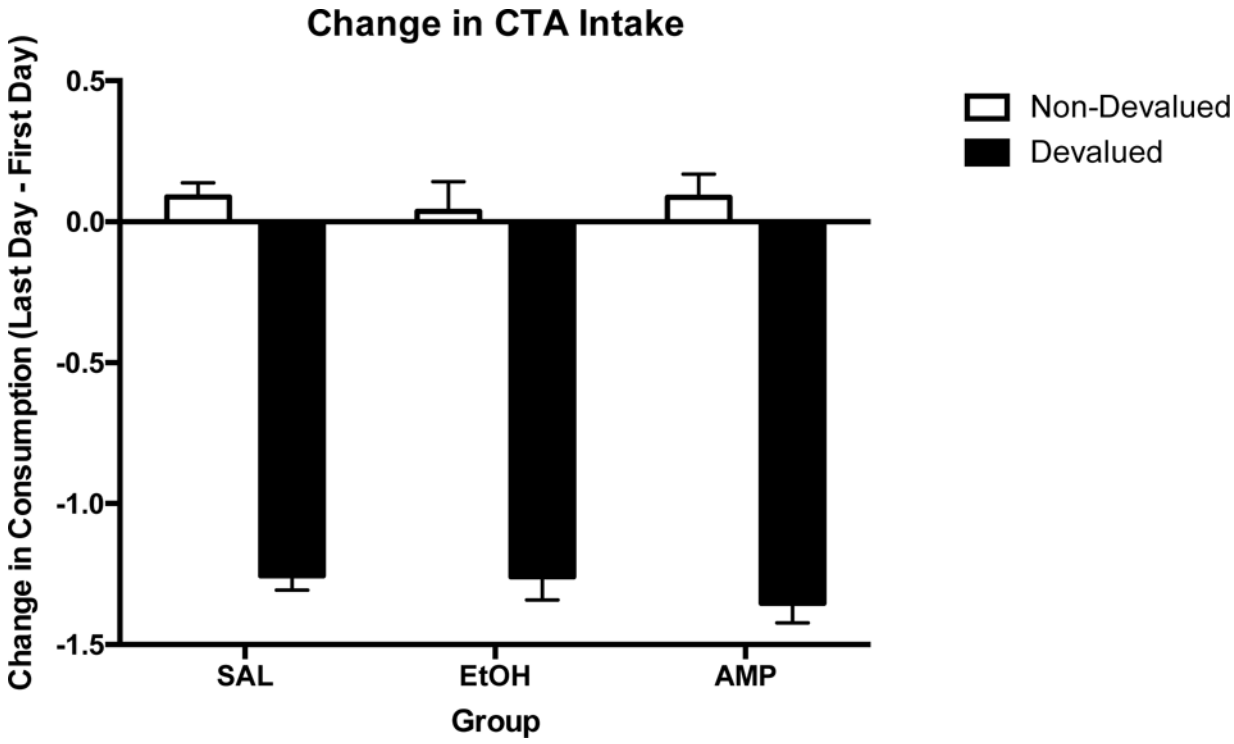

Fig. 6.
Change in CTA intake from first day to last day.

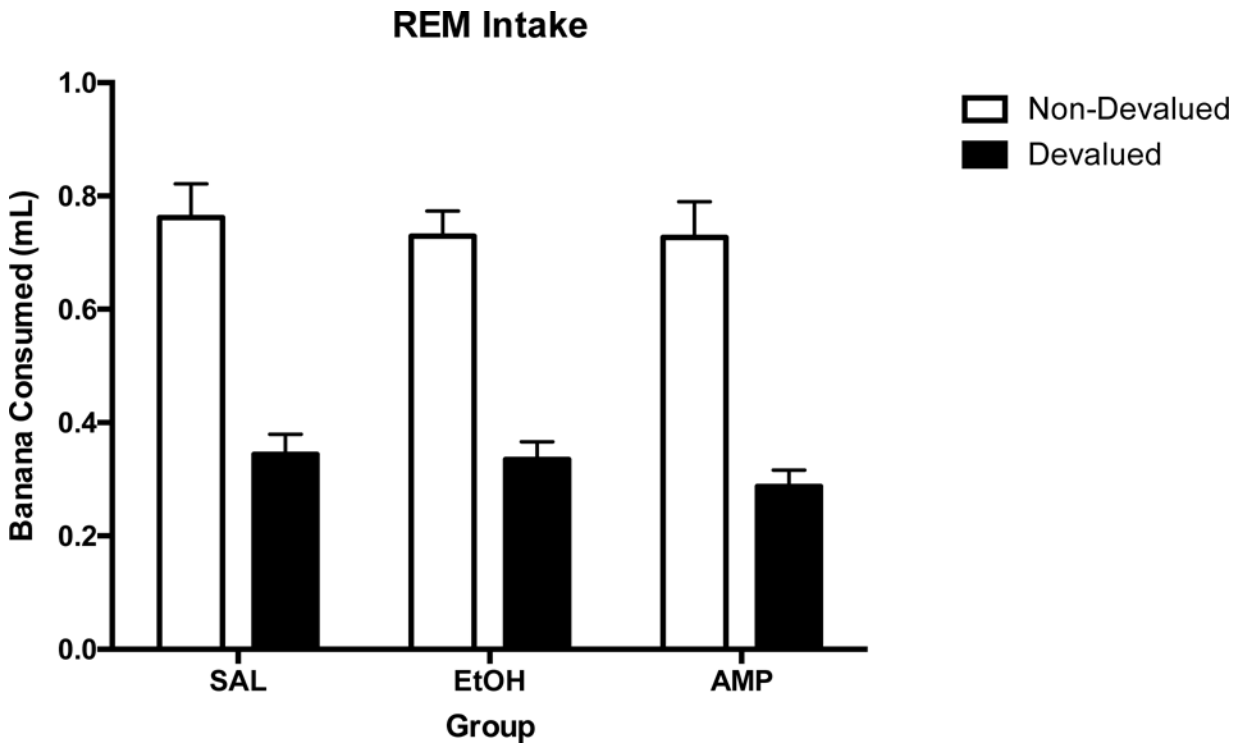

Fig. 7.
Reminder session intake.

Table 1

Final group numbers for both original analyses and when divided into high and low responders.

Original Groups	High/Low Responders
SAL Dev n = 25	High: n = 14
	Low: n = 11
SAL Non n = 26	High: n = 13
	Low: n = 13
EtOH Dev n = 17	High: n = 8
	Low: n = 9
EtOH Non n = 17	High: n = 8
	Low: n = 9
AMP Dev n = 15	High: n = 7
	Low: n = 8
AMP Non n = 15	High: n = 8
	Low: n = 7