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30 Updated 12/26/2015

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By Christa A. Houck

Entitled
PHARMACOLOGICAL MODULATION OF HABIT EXPRESSION

For the degree of Master of Science

Is approved by the final examining committee:

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Approved by: Nicholas Grahame 09/06/2016
Head of the Departmental Graduate Program Date

PHARMACOLOGICAL MODULATION OF HABIT EXPRESSION

A Thesis

Submitted to the Faculty

of

Purdue University

by

Christa A. Houck

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

December 2016

Purdue University

Indianapolis, Indiana

For my incredibly supportive parents who taught me the meaning of hard work.

You two kids from Newark always wonder where I came from, but to me, it has always been so clear.

ACKNOWLEDGEMENTS

I would first like to thank my mentor, Dr. Nick Grahame, for always challenging me to ask harder questions and pursue deeper answers, never blinking an eye whenever I'd show up unannounced at your office multiple times a day, and your unwavering optimism that these data would (eventually) make sense. I would also like to thank Dr. Czachowski and Dr. Goodlett for your help in shaping this project into something I am truly proud of and all of your guidance along the way. I am so grateful for the entire Addiction Neuroscience department, most notably Chelsea Kasten for reading and rereading everything I have ever written and SELB 304 for not kicking me out of the office (even when I deserved it).

I'm so lucky to be surrounded by my family, who provided endless love and support and surprise cookies (thanks, Kelli!). Thank you to my wonderful fiancé, Christopher, for pretending to believe me when I had to write "just one more sentence" and having unshakable confidence in me, even when I had a tough time doing it myself.

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ABSTRACT

Houck, Christa A. M.S., Purdue University, December 2016. Pharmacological Modulation of Habit Expression. Major Professor: Nicholas Grahame.

Habit expression is emerging as a theory of addiction: subjects begin to use drugs to attain positive reinforcing effects but continue to use in spite of negative effects because the behavior becomes habitual, and therefore divorced from its outcome. Many studies have shown that a history of drug and alcohol use lead to expedited acquisition of a habit, but the acute effects of these drugs on behavior is still unknown. Behaviors that result from acute intoxication, such as increased aggression, risky sexual behavior, and impaired judgment, could be interpreted as habitual: actions performed without regard for the outcome. Therefore, we studied the transition from goal-directed to habitual behavior, when a response is made regardless of outcome value, and how acute intoxication of ethanol (EtOH), amphetamine (AMP), nicotine (NIC), and yohimbine (YOH) affect the resulting behavior. Through a series of four experiments, selectively bred crossed High Alcohol Preferring (cHAP) mice were trained on an operant task to self-administer 1% banana solution, which was subsequently devalued via LiCl CTA. EtOH (1 & 1.5 g/kg), AMP (2.0 mg/kg), NIC (0.5 mg/kg), YOH (1.0 mg/kg), or SAL were administered prior to baseline and post-devaluation tests. We found that acute EtOH at 1- and 1.5-g/kg doses facilitated the expression of a habit, whereas all other pretreatments resulted in

devaluation. These data may indicate a unique role for EtOH in facilitating the retrieval of habitual over outcome-based associations. This could shed light on why intoxicated individuals display impaired judgment and a mechanism by which relapse after a period of abstinence can occur.

CHAPTER 1. INTRODUCTION

1.1 Theory of Habit Formation

Habit formation is a process by which a subject repeatedly performs a behavior to obtain a desirable outcome and, over time, continues to perform that action without considering the outcome (Balleine and O'Doherty, 2010). A habit begins with a subject performing a behavior to get something they want. This typical behavior is described as goal-directed: a purposeful action occurs in desire of a positive outcome (Dickinson, 1985). It is also referred to as response-outcome (R-O) behavior, as the response is made while the subject is mindful of the outcome of that action. An action can also be habitual, or stimulus-response (S-R), behavior, where the behavior is an unchanging response to the presence of a particular stimulus, regardless of the current value of the outcome (Dickinson, 1985). If a subject is behaving in a goal-directed manner, a change in the value of the outcome will affect response rate. However, if that behavior is habitual, reducing the value of the outcome will have no effect on responding, as long as the stimulus is still present.

In order to induce this phenomenon where a subject is no longer sensitive to outcome devaluation, it is necessary to cause a stronger S-R association and weaken the R-O relationship. Development of the S-R relationship tends to require the use of extended fixed ratio (FR) training or a variable interval (VI) schedule (Dickinson, 1985).

The latter requires less time; the subject over time begins to dissociate the response (i.e. a lever press) with the outcome (i.e. food pellet delivery), but the relationship between the presence of the stimulus (i.e. a lever) and its elicited response is preserved and strengthened. This was first seen by Dickinson et al. (1983). While observing rats' behavior on a VI schedule, the researchers noted that the rate of lever pressing on an interval schedule did not predict rate of reward delivery, which may decrease the strength of the response-outcome association. Conversely, lever pressing behavior directly predicted the rate of presentation of the reward under a variable ratio schedule, which may tend to preserve the R-O association, and thus requiring longer training to induce a habit.

With extended training or induction of a VI schedule, the subject should begin to strengthen the S-R association enough to tend to behave as such, as opposed to an R-O action. To test which association is strongest, one should decrease the value of the outcome to determine if the subject is mindful of the outcome's value. This can be done in a variety of ways, depending on the type of study used. Typically the reinforcer is either paired with lithium chloride (LiCl) to induce gastric malaise in animal studies (Adams and Dickinson, 1981) or the subject is given free access to the reinforcer prior to testing to induce reinforcer-specific satiety, as is done in human studies and some animal work (Rolls et al., 1983, Colwill and Rescorla, 1985). Satiation devaluation is specific to the reinforcer and typically does not generalize to all appetitive stimuli. Both of these methods seek to reduce the reinforcer's positive effects, either by becoming associated with illness or due to satiation. In theory, subjects who are behaving in a goal-oriented manner will be sensitive to this devaluation procedure and will not continue to respond

for the reinforcer. However, habitual subjects, when placed back into the original training environment, will continue to respond in the presence of the stimulus, as the S-R association is stronger than the weakened R-O one. Typically these test sessions are done in extinction, as to ensure that the presence of the reinforcer does not elicit its own emotional response that could directly influence responding or, in the case of a drug reward, have an effect on motor responding. In addition, these tests tend to be short to prevent complete extinction of the operant response.

1.2 Habitual Behavior & Alcohol and Drug Use

Habit formation can be used to explain problematic drug and alcohol use. Substance use disorders are a particularly debilitating disease marked by excessive use of alcohol or drugs, loss of control in use, and social consequences resulting from using the substance (American Psychiatric Association, 2013). Within these criteria for diagnosis, persons afflicted continue to use drugs or alcohol, in spite of negative consequences. Under normal conditions, a person facing legal or social punishments or physical harm directly resulting from drug or alcohol use should decrease or discontinue use. In other words, the outcome of the drug would be devalued by these negative consequences. Failure to consider these consequences of drug use could be caused by habitual responses.

However, it is important to note that the shift from outcome-mediated instrumental behavior towards habit learning is not absolute or irreversible. Animals and humans may switch between expression of a habit or act in a goal-directed manner and their specific behavior in a given condition can change, dependent on alcohol intoxication or contextual cues (Hogarth et al., 2012, Gremel and Costa, 2013). Furthermore,

lesioning the dorsolateral striatum, a brain region strongly implicated in habitual behavior, causes animals to revert to outcome-based responding after acquisition of a habit (Balleine and O'Doherty, 2010, Corbit et al., 2012), demonstrating that the R-O association persists even after the shift towards habitual responding has occurred and dominated expression.

1.3 Problems in the Habit Formation Literature

Some problems may arise based on the method of devaluation used. Satiation as a devaluation method may have other effects when the reinforcer is a drug that is consumed just before an extinction test. For example, alcohol is known to have depressant effects on motor behavior, such as increased latency to correct loss of righting reflex (LORR) and decreased time spent on a rotarod task (Ornelas et al., 2015). Deficits in motor behavior can impact lever pressing behavior and satiation of alcohol may result in fewer responses for the reinforcer, even in the absence of a devaluation effect. However, rodents do not typically consume pharmacologically relevant doses of alcohol that would significantly interfere with operant behavior, so this is less of a problem with the satiation model (Corbit et al., 2012).

An additional issue with drug reinforcer satiation devaluation is the unknown effect of being under the influence of a drug of abuse on retrieval of instrumental associations. One possibility is that being under the influence of alcohol or drugs of abuse could help shift retrieval towards S-R associations. Even further, just the presence of cues related to alcohol can potentiate the shift to habitual behavior. This effect has been seen in human studies as well as animal studies. Ostlund et al. (2010) trained rats to

respond for a food reinforcer, which was subsequently devalued via satiation. The rats were tested following devaluation in a neutral setting and a different setting in which they had previously received ethanol. Although their training was not sufficiently long to produce a habit, as seen by devaluation effects in the neutral context, rats did not attenuate seeking for the devalued reinforcer in the alcohol-paired context. Under a different paradigm, Porrino et al. (2004) trained rhesus monkeys to respond for either a food or cocaine reinforcer. Via audioradiography, glucose utilization was measured in the monkey striatum throughout training. Researchers found that while the ventral striatum was active in the beginning of cocaine sessions, activity shifted to the dorsal striatum, a region implicated in habitual behavior. This pattern was not seen with the food reinforcer. Both of these studies reveal a unique change that occurs in the presence of drug-related cues or acute intoxication. Perhaps the reminder of the drug cues is sufficient to elicit an anticipatory intoxication response, similar to that of acute intoxication. Because the presence of alcohol and alcohol-related cues may facilitate habit expression, independent of training effects, it can be difficult to truly elucidate the outcomes of previous studies that have used drug reinforcer satiation as a method of devaluation.

In the human literature, Sjoerds et al. (2013) compared adults with a diagnosis of alcohol use disorder with unaffected controls on a habit formation experiment using a monetary reinforcer. While there were no differences in responding during training, meaning that both groups had equal access to the reinforcer, subjects with a history of alcohol use disorder were not sensitive to the devaluation effects as seen in the control subjects. Another study performed by Panlilio et al. (2004) performed a similar

experiment with subjects with a history of cocaine use that did not attenuate responding for cocaine, despite previous extinction trials that devalued that outcome.

These findings seem to fall in line with previous literature describing the effects of long-term drug use, as it is understood that use causes significant structural, physiological, and chemical changes in the brain. However, these findings are not limited to only long-term abuse. Researchers see similar patterns of accelerated habit formation under acute intoxication. Hogarth et al. (2012) used a sample of twenty-four men to determine if acute alcohol administration during training for chocolate and water reinforcers could facilitate habit formation. Subjects receiving alcohol during training showed no change in chocolate responding following satiation of chocolate, whereas control subjects significantly reduced chocolate preference and responded more for water. This can be interpreted in one of two ways: consistent with previous studies of drug history, subjects with an acute history of alcohol use tend to behave more habitually in general. Another theory is that because the task was learned under acute intoxication, this task specifically was shifted to S-R behavior quicker than one learned while sober. However, the second explanation is most likely the cause due to the sample used. Both the alcohol and placebo groups scored an average of 21.3 on the AUDIT questionnaire, indicating that they may be at risk for alcohol dependence due to high drinking behavior. Because alcohol history was matched and the placebo group preserved outcome-based behavior, it is more likely that acute alcohol consumption during the task facilitated the shift to a habit.

Preliminary data from our laboratory supports the hypothesis that drugs of abuse can facilitate retrieval of a habitual basis for instrumental behavior. Specifically, we

observed the effects of acute ethanol administration on the efficacy of reinforcer devaluation using LiCl. Administering a 1.5-g/kg dose of ethanol 10 minutes prior to two extinction tests that occurred pre- and post-devaluation led selectively bred crossed high alcohol preferring (cHAP) mice to act more habitually, as compared to animals receiving a saline pretreatment, as seen in Figure 1. Animals receiving an alcohol pretreatment behaved similarly in both extinction tests, revealing they were not sensitive to the devaluation effects and continued responding in an S-R manner. Saline control animals decreased responding from extinction test 1 to test 2, indicating they devalued the reinforcer, which had been paired with gastric malaise of LiCl. Note that in this experiment, as in the proposed studies, we administered the drug prior to two extinction tests occurring both before and after reinforcer devaluation (CTA). This allowed us to separate the effects of alcohol on the rate of responding in extinction from its effect on the otherwise expected devaluation effect. These data reinforce the idea that part of the reason drugs of abuse promote addictive behaviors is that they shift organisms toward habitual responding, which inherently fails to take into account the current value of the reinforcer.

1.4 Alcohol, Amphetamine, Nicotine, and Yohimbine

Alcohol is one of the most abused substances in the United States. While the effect of an alcohol history has been studied (Corbit et al., 2012), its acute effect on retrieval of instrumental associations is still unknown. In a similar way, amphetamine history, but not single administration, accelerates subsequent habit formation (Nelson & Killcross, 2006) and this accelerated shift is attenuated with the administration of non-

specific and D1 specific antagonists, flupenthixol and SCH23390, respectively (Nelson & Killcross, 2013). However, its effect following acute, not chronic, administration is not yet known. Nicotine is another ubiquitous drug of abuse, but there are very few studies that address its role in habit acquisition or expression, making it of interest to investigate. Any drug that facilitates acquisition or expression of habitual behavior may increase the abuse potential of the drug by interfering with evaluation of positive and negative associations with drug-seeking responses.

Yohimbine is not a known drug of abuse. It serves as a control for amphetamine and nicotine's stimulant actions. These substances, in addition to being drugs of abuse, potentiate the sympathetic nervous system, which may have its own effects on instrumental behavior. Sanger (1988) looked at drug discrimination between amphetamine and yohimbine and determined that rats were able to differentiate *d*-amphetamine from yohimbine at varied dosages with 100% accuracy, showing that the stimulant effects of amphetamine are not generalizable to yohimbine. This demonstrates that the two substances produce different, discernable effects and yohimbine can act as appropriate control in this set of experiments.

Amphetamine and nicotine would be strong additions to the habit expression research to better elucidate how drugs of abuse impact this process and potentially drive the shift from goal-directed behavior to habitual. Previous research has examined the effects of a history of drug use, especially ethanol and amphetamine, on this behavioral shift, but there has been no data to determine if chronic use is necessary to induce these changes. This is pivotal, as a symptom of acute intoxication is impaired judgment and impulsivity, which could be a manifestation of facilitation of habit expression (American

Psychiatric Association, 2013). Looking at these other drugs, with yohimbine as a control, can determine if the effect seen in alcohol, is generalizable to other drugs of abuse, like amphetamine and nicotine, or if the pilot study findings were specific to ethanol. We hypothesize that amphetamine, in addition to increasing response rate in both tests, will lead to a tendency to express a habit, thus preventing us from observing reinforcer devaluation. We also expect nicotine to also potentiate habitual behavior if drugs of abuse potentiate habit expression. Yohimbine, however, has no abuse potential and therefore should not exhibit the same effects as amphetamine and nicotine. Instead, mice pretreated with yohimbine would continue to be sensitive to devaluation effects.

1.5 Specific Hypotheses

1. Known stimulant drugs of abuse, amphetamine and nicotine, will promote habit expression following reinforcer devaluation, but yohimbine, a stimulant with no abuse potential, will have no effect. (Experiments 1, 2 & 3)

2. Acute administration of ethanol will facilitate the expression of a habit in a dose-dependent manner. (Experiment 2)

CHAPTER 2. MATERIALS AND METHODS

2.1 General Design

In all four experiments, male and female cHAP mice were trained on an operant task to respond for 1% banana solution. These animals were given acute injections of ethanol, amphetamine, nicotine, yohimbine, or saline prior to pre- and post-devaluation extinction expression tests. The devaluation procedure consisted of access to the banana solution, followed by an immediate injection of lithium chloride (LiCl). Changes in responding between pre- and post-devaluation testing was measured to determine if animals were behaving habitually or in a goal-directed manner.

2.2 Subjects

190 cHAP mice (95 male) were used throughout these four experiments (34 in the pilot experiment, 48 in Experiment 1, 48 in Experiment 2, and 60 in Experiment 3). All animals were single housed 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 (1430 – 1630) in order to increase motivation to respond for the liquid reinforcer during operant training. All four experiments 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.

2.3 Apparatus

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

2.4 Drugs

For operant reinforcement, all mice had access to 1% v/v banana flavoring in DI H_2O solution. This solution was also devalued for all mice during the devaluation stage (with the exception of non-devalued animals in Experiment 3 that received 0.9% saline), 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 signs of an aversion after seven days, the injection volume was increased to 60 mL/kg, resulting in a dose of 0.382 g/kg (9.0 mEq/kg).

Doses of each pretreatment drug were derived from consideration of previous research. The dose of each drug administered should not be so high as to greatly interfere with operant responding, but also needs to be sufficient to induce a pharmacological effect in some behavioral assay; conditioned place preference was used as a reference behavioral assay. A literature search was conducted to find appropriate doses seen as rewarding (for amphetamine, nicotine, and ethanol) in a conditioned place preference paradigm but with few motor effects. Prior to the pilot experiment, an ethanol probe was conducted to determine a dose of ethanol that does not significantly interfere with lever pressing behavior to ensure that alcohol administration's motor effects are solely responsible for differences seen in the extinction tests. Preliminary data show that the acute administration of 1.5 g/kg ethanol shifts cHAP mice to express habitual instead of goal-directed behavior, as it rendered devaluation ineffective and had few side effects. Although there is not yet published data of cHAP mice in a place preference paradigm, HAP1 animals show a significant place preference at this dose (Grahame et al., 2001), indicating pharmacological reinforcement. Therefore, LoEtOH mice in Experiment 2 received a pretreatment of 1.0 g/kg EtOH (10% v/v) and HiEtOH mice in the pilot experiment and Experiment 2 were injected with 1.5 g/kg EtOH (15% v/v) ten minutes prior to both pre- and post-devaluation testing.

In order to see noted behavioral effects of amphetamine in mice, previous studies have used a range of doses between 1 mg/kg to 10 mg/kg (Jones et al., 1998, Oberlin et al., 2010). 2.0 mg/kg was used because it has been shown to be reinforcing in a CPP paradigm (Jerlhag et al., 2010, Vanhanen et al., 2015) and shows few motor impairing side effects (McKim, 1980). A subcutaneous injection of 0.5 mg/kg nicotine

immediately prior to extinction tests was used due to previous research indicating its reinforcing properties (Al-Hasani et al., 2013) and absence of motor impairing effects (Shoaib et al., 2002, Jackson et al., 2013a, Jackson et al., 2013b). Yohimbine was administered at 1.0 mg/kg, based on previous research using it as a pharmacological stressor at this dose (Mantsch et al., 2010) and shows little motor impairment (Katz, 1984).

2.5 Habit Formation Training – Experiments 1, 2, & 3

All experiments followed nearly identical training procedures that are graphically displayed in Figure 2. Training for the habit formation task started with a fixed ratio (FR) schedule and transitioned to a VI schedule. Day 1 began with magazine training on an FT-120 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 fluid. Days 2-5 of training consisted of an FR-1 schedule where mice were rewarded for a correct lever press with a 5 second appearance of the reinforcer. After meeting criterion of twenty correct lever presses with 0.2 mL of fluid consumed on Day 5, the animals moved on to the VI stage of the experiment. On Day 6, mice underwent a 45-minute VI-20 session. During this session, mice were rewarded for a correct lever press following a varying delay, averaging 20 seconds, after the initial correct press. Incorrect (opposite lever) presses had no effect, but were recorded. On Days 7 – 9, animals proceeded to 45-minute VI-60 sessions, where the random interval was extended to an average of 60 seconds. In Experiment 3 only, all mice received a 10-mL/kg injection of 0.9% saline

approximately 15 minutes following the operant session. These injections were performed in order to habituate the mice to being scruffed and injected prior to their experimental injections on the test days.

2.6 Habit Formation Testing – Experiments 1 & 2

The EtOH pilot experiment and Experiments 1 and 2 all followed a similar within-subjects design. After habit training, baseline extinction responding was measured prior to devaluation. On Day 10, animals received their assigned drug pretreatment prior to operant testing, dependent on the experiment. Pilot mice received either 0 or 1.5 g/kg EtOH 10 minutes prior to the test. Experiment 1 animals were injected with 2 mg/kg AMP, 0.5 mg/kg NIC (s.c.), 1 mg/kg YOH, or SAL immediately before extinction testing. In Experiment 2, the mice received either a 10-minute pretreatment of 0, 1, or 1.5 g/kg EtOH or an immediate pretreatment of 0 or 2 mg/kg AMP. All groups then received a 15-minute extinction pretest in the operant boxes. During this session, mice responded on a VI-60s schedule for an empty sipper tube, maintaining the visual and auditory presentation of the sipper tube as experienced during training, but without the banana reinforcer. If a mouse responded fewer than 10 times on the correct lever, they were removed from the study because it is impossible to detect devaluation from such a low baseline.

After the baseline responding rate was established in the extinction pretest, the conditioned taste aversion (CTA) training began on Day 11. During this phase, all mice had 30-minute access to a tube with the reinforcer in their home cage. Immediately following this session, mice received a devaluation injection of LiCl. This procedure

spanned from Days 11 – 14, but continued for mice that did not meet the criterion of consuming no more than 0.5 mL of banana solution. If after seven days, mice continue to drink, the injection volume increased to a dosage of 0.382 g/kg (9.0 mEq/kg) in order to facilitate taste aversion learning.

Following the CTA training, on Day 15, mice had a 10-minute reminder session where they had free access to the banana reinforcer in the operant chamber without any negative consequence. The levers were removed from the chamber, as to not disrupt the S-R association potentially formed during VI training. Criterion for advancement was set at 0.2 mL of banana solution consumed, to ensure that the mouse was reminded of the availability of the reinforcer in the operant box. Pilot studies indicated that this reminder session facilitated operant devaluation effects. Mice were removed from the study if they did not meet this criterion. On Day 16, mice had a second extinction test, identical to the one administered prior to the CTA training. Following the group-dependent pretreatment, animals had 15-minute session in the operant boxes, with an empty sipper tube serving as the reinforcer on a VI-60 schedule. These extinction post-test results were compared to the pretest results in order to determine the effect of devaluation on lever pressing, thus indicating if the behavior was goal-directed or habitual.

2.7 Habit Formation Testing – Experiment 3

To ensure that repeated administration of AMP had no effect on response rate, a between-subjects design was to assess devaluation effects in Experiment 3. After habit training, baseline responding in extinction was measured. On Day 10, all animals received a SAL pretreatment immediately before to the 15-minute extinction pretest.

This operant session was conducted identically to those in the experiments in Aims 1 & 2. This day was not used in statistical analysis to determine habit expression, but was conducted to keep the procedure of all four experiments relatively similar. Following this pretest, the devaluation phase of the experiment began. Half of the animals in each assigned drug group underwent an identical procedure to that of Aims 1 & 2: 30 minutes of access to banana solution, followed by a LiCl injection for Days 11 – 14. The remaining mice were assigned to the non-devalued group, which received home cage banana access, followed by an equivolumetric injection of saline. Previous findings in our lab have shown that this procedure is not sufficient to induce a taste aversion to banana. In order to match for number of injections, non-devalued mice were yoked to those in the devalued group, based on sex, drug pretreatment, and weight. If, on Day 14, devalued mice drank more than 0.5 mL, they would continue to undergo CTA until they attenuated drinking and their yoked non-devalued animal would receive another day of banana access followed by a SAL injection.

Once the devalued animals met criterion, they would undergo a reminder session identical to that of Aims 1 & 2 on Day 15. As in Aims 1 & 2, all mice needed to consume at least 0.2 mL banana solution to advance to the second extinction test and were removed if they did not. On Day 16, the second extinction test was conducted. This procedure was similar to that of the first test on Day 10, but animals in the AMP group received a 2-mg/kg injection of AMP and SAL mice received a SAL injection. Immediately following the group-dependent pretreatment, animals had a 15-minute session in the operant boxes, responding for an empty sipper tube on a VI-60 schedule. The extinction post-test results of the devalued animals within each treatment group were

compared to the results of the corresponding non-devalued group in order to determine the animal's sensitivity to devaluation.

2.8 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 an α-value of 0.05. To determine whether there were any group differences in training response rates, a repeated measures analysis of variance (ANOVA) was conducted, with training day as the within-subjects measure and group as the between-subjects measure. For Experiments 1 & 2, effect of sex was assessed by a repeated measures ANOVA, comparing extinction tests, group, and sex. Because there was an *a priori* hypothesis that pre- and post-devaluation tests would differ within each drug treatment group, individual paired t-tests were conducted for each group. No significant difference between the pre- and post-devaluation extinction test indicated that they animal was insensitive to devaluation and, therefore, behaving habitually. For each paired t-test, power was calculated in SPSS, as well as effect size, by subtracting the pooled variance of each individual experiment from each group's standard deviation. To determine if mice in Experiment 3 had expressed a habit, two independent t-tests were conducted on the post-devaluation extinction test, comparing the devalued and non-devalued animals within each drug pretreatment group. A one-way ANOVA was used to assess differences between groups in the overall devaluation score (pre-devaluation responding – post devaluation responding). A bivariate correlation was run, comparing

correct lever presses to banana reinforcer intake during training to assess learning of the instrumental behavior and dissociation of the response-outcome relationship.

CHAPTER 3. RESULTS

3.1 Experiment 1 Findings

Three animals were removed from analyses: two NIC mice did not drink on the reminder session and one YOH mouse did not meet criterion on EXT1. To ensure that there were no group differences in exposure to the reinforcer during training, a repeated measures ANOVA was used to examine correct lever presses between groups over the 7 days of training. Responding increased over the training sessions, as indicated by a main effect of day ($F(6, 246) = 93.112$, $p < 0.001$), but there were no group differences, $F(3, 41) = 0.211$, $p = 0.888$) (Figure 3). Following Experiment 1, a repeated measures ANOVA was conducted comparing the devaluation pre-test and post-test to assess the effects of the CTA procedure within each drug group and sex. Overall, there was neither a main effect of test ($F(1, 41) = 0.716$, $p = 0.402$), nor a test x group interaction ($F(3,41) = 0.397$, $p = 0.756$), as seen in Figure 4. Sex did not impact responding within each drug group ($F(3,37) = 0.141$, $p = 0.934$). Due to *a priori* hypotheses predicting differences between tests in each group, planned paired t-tests within each pretreatment were conducted.

Both SAL and YOH pretreatment groups showed a strong trend toward a devaluation effect ($t(11) = 2.122$, $p = 0.057$; $t(11) = 2.113$, $p = 0.058$). AMP pretreated mice showed a weaker effect of devaluation ($t(11) = 1.660$, $p = 0.125$), potentially

showing evidence of habitual behavior. Interestingly, nicotine-treated animals exhibited a very strong devaluation effect, $t(9) = 3.079$, $p = 0.013$. Effect sizes of devaluation for each pretreatment group can be found in Table 1.

3.2 Experiment 2 Findings

Two mice were excluded from analyses due to failure to meet criterion. One SAL mouse was removed following the reminder session and an AMP mouse following EXT1. As demonstrated in Figure 5, a repeated measures ANOVA measured changes operant responding over acquisition training and indicated an overall increase in correct lever presses ($F(6, 252) = 93.771$, $p < 0.001$), but no differences between future drug pretreatment ($F(3, 42) = 0.045$, $p = 0.987$). For Experiment 2, data were analyzed via a repeated measures ANOVA, with test (pre-test vs. post-test) as the within-subjects measure, and group and sex as between-subjects measures. Overall, all animals showed evidence of devaluation, as demonstrated by a significant main effect of test ($F(1,42) = 31.307$, $p < 0.001$). There was also a significant test x group interaction ($F(3,42) = 2.876$, $p = 0.047$), which is demonstrated in Figure 6. There was no test x group x sex interaction ($F(3,38) = 0.271$, $p = 0.846$), indicating that within each drug pretreatment, male and female mice did not perform differently on either test.

Because there were *a priori* hypotheses about the devaluation effects in each treatment group, follow-up paired t-tests were conducted for each drug pretreatment group. Only the low dose EtOH group behaved habitually ($t(11) = 1.613$, $p = 0.135$). SAL, AMP, and high EtOH mice significantly devalued following the CTA procedure

(t(10) = 2.475, p = 0.033; t(10) = 3.690, p = 0.004; t(11) = 2.922, p = 0.014), with effect sizes of each comparison listed in Table 1.

3.3 Experiment 3 Findings

To ensure no differences in training responding between each drug pretreatment and devaluation condition, a repeated measures ANOVA was conducted and confirmed there were no training differences between groups (F(6, 336) = 135.698, p < 0.001) and a main effect of day (F(3, 56) = 0.360, p = 0.782), indicating an overall increase as training progressed (Figure 7). In order to analyze the effects within each drug group, independent t-tests were conducted comparing EXT2 responding to see if there were differences between the devalued and non-devalued animals. As seen in Figure 8, there were no observed differences in post-devaluation responding between these treatment groups in either the AMP or SAL pretreated animals (t(28) = -0.470, p = 0.642; t(28) = 0.050; p = 0.960), indicating that both groups were behaving habitually.

To determine if the non-devalued control condition was effective, two separate paired t-tests were conducted comparing EXT1 and EXT2, as performed in the within-subjects experiments, in the SAL pretreated animals only. This comparison could not be made in the AMP group, due to the differing pretreatments. Interestingly, both groups showed significant devaluation effects (t(14) = 2.584, p = 0.022; t(14) = 2.900, p = 0.012), as demonstrated in Figure 9. This indicates that even the non-devalued animals decreased responding between extinction tests. Interestingly, when analyzing the intake of animals during the CTA procedure, there was a main effect of group (F(3,56) = 11.934, p < 0.001) and a significant day x group interaction (F(9,168) = 8.489, p < 0.001) which

appears to be driven by the reduction of consumption of the devalued, but not non-devalued animals (Figure 10). Follow-up one-way ANOVAs indicated that there were significant group differences on Day 3 and 4 of CTA ($F(3,56) = 5.445$, $p = 0.002$ & $F(3,56) = 23.236$, $p < 0.001$).

3.4 Integrating Across the Project

In order to determine the overall effects across comparable experiments, a repeated-measures ANOVA was used to compare magnitude of devaluation of the pilot ethanol experiment with the SAL and EtOH groups from Experiment 2, using replication as a factor. This failed to obtain a significant pre-test vs. post-test x replication interaction ($F(1,49) = 2.936$, $p = 0.093$), so these groups were collapsed between experiments. When the SAL and AMP groups from Experiment 1 were added, there was no significant effect of replication ($F(1,38) = 1.913$, $p = 0.175$). To ensure that saline groups did not differ among the three experiments (EtOH pilot, Experiment 1, and Experiment 2), these groups were isolated and a repeated measures ANOVA comparing pre- and post-devaluation responding revealed no significant interaction statistic among the three saline replications ($F(2,33) = 0.362$, $p = 0.699$). Therefore, this allowed for analysis of the three within-subjects experiments as a whole.

A repeated-measures ANOVA, with pre-test vs. post-test as the within-subjects measure and group and sex as the between subjects measures, of the entire data set revealed no test x group x sex interaction, $F(5, 101) = 0.508$, $p = 0.770$). Thus, the data were collapsed across sex. There was a significant overall devaluation effect among all of the groups, $F(1, 101) = 44.779$, $p < 0.001$) and a strong trend toward a test x group

interaction $F(5, 101) = 2.161$, $p = 0.064$. Considering the *a priori* hypotheses for group differences, paired t-tests within each drug group were conducted.

Overall, the control SAL animals showed evidence of devaluation ($t(38) = 4.750$, $p < 0.001$), as hypothesized. Similarly, animals pretreated with AMP, NIC, and YOH also all showed evidence of devaluation ($t(22) = 3.588$, $p = 0.002$; $t(9) = 3.079$, $p = 0.013$; $t(10) = 3.187$, $p = 0.010$). While there was no significant difference in devaluation score between the SAL control and NIC mice ($t(47) = -1.357$, $p = 0.181$), AMP pretreated animals showed a strong trend toward a greater devaluation effect than the SAL group ($t(60) = -1.992$, $p = 0.051$). In addition, both the high and low dose ethanol groups behaved habitually ($t(25) = 1.842$, $p = 0.077$; $t(11) = 1.6.13$, $p = 0.135$) (Figure 11). Magnitude of effect sizes are listed in Table 1.

Another way to analyze the effect of drug pretreatment is to calculate the difference scores (post-devaluation responding subtracted from pre-devaluation responding) and compare each drug to saline to determine if devaluation is greater or less than that of the control. As seen in Figure 12, there is no main effect of group using a one-way ANOVA ($F(5, 115) = 2.008$ $p = 0.083$).

To ensure that the differences within each drug group could be attributed to the isolated drug effects and not a result preexisting differences prior to test days, training, REM and CTA behavior were analyzed. In order to determine if the R-O association was learned, correct lever presses were correlated with reinforcer consumption on the final day of FR training. Results showed that there was a significant association between correct responses and intake of banana solution on day 3 of FR1 training ($r = 0.3474$, $p < 0.001$), indicating that there was a relationship between this behavior and intake (Figure

13). When analyzing behavior on the last day of VI training, the association was weaker and only trended toward significance ($r = 0.1393$, $p = 0.0652$), as demonstrated in Figure 14. A t-test confirmed that these two correlations were significantly different ($t(348) = 2.013$, $p = 0.0449$), indicating that the association between responding and a reinforcer was significantly stronger in the FR phase, as compared to the VI phase.

Repeated measures ANOVA looking at amount consumed during the four day CTA training by group indicated there was neither a significant effect of drug pretreatment ($F(5,115) = 0.943$, $p = 0.456$), nor a day x group interaction ($F(51,345) = 1.543$, $p = 0.088$). This indicates that CTA behavior did not differ between groups. This ANOVA also revealed a main effect of day ($F(3,345) = 209.330$, $p < 0.001$), indicating a successful aversion to banana solution over time, as seen in Figure 15. A one-way ANOVA looking at differences in number of days to reach CTA criterion within each drug pretreatment group revealed that there was no significant effect ($F(5,120) = 0.920$, $p = 0.471$), meaning that it took all groups an equal amount of time to devalue (Figure 16). All pretreatment groups consumed the same amount on the REM day, following CTA ($F(5, 120) = 1.108$, $p = 0.360$), indicating that exposure to banana on this session had no effect on subsequent devaluation score. This effect held true even when body weight was taken into account, $F(5, 120) = 0.792$, $p = 0.558$ (Figures 17 A & B).

CHAPTER 4. DISCUSSION

4.1 Overall Discussion

This set of experiments is the first to explicitly examine the effects of acute drug intoxication on the expression of a habit. Based on previous theories, it was thought that, in general, drugs of abuse potentiate the expression of habitual behavior. However, these findings demonstrate that this effect is unique to alcohol and under its influence a subject is more likely to behave habitually. These findings shed light on how acute EtOH intoxication may reflect poor decision-making and loss of control seen in intoxicated persons.

Overall, both low (1-g/kg) and high (1.5-g/kg) doses of ethanol resulted in reduced sensitivity to devaluation, as compared to SAL control mice. While the higher EtOH dose did decrease response rates in the extinction pre-test, we have previously been able to show significant devaluation effects at this baseline rate in our lab, ensuring that this is not a floor effect (O'Tousa & Grahame, personal communication). This effect was also seen in the lower EtOH dose, where pre-devaluation test responding was not different from SAL control mice, indicating that insensitivity to devaluation in intoxicated mice can be interpreted as facilitation of habitual responding, suppression of R-O responding, or both. However, in Experiment 2, EtOH pretreated animals did show evidence of devaluation, contradicting pilot research. Because of the habitual behavior demonstrated in the low dose of EtOH in this experiment and the lack of a group x

experiment interaction when combining the two 1.5 g/kg EtOH groups, it was possible to combine these findings and interpret them as a whole. The exact reason behind this inability to replicate is unknown and could simply be attributed to sampling error. Another cause of this could be that t-tests examining EtOH-treated animals at both doses had very low power, likely due to the fact that the mean difference of response rates was small. Detecting a null result is difficult to prove and could have led to the ambiguity.

Experiments 1 & 2 found differing effects of AMP on the expression of a habit. Experiment 1 found no significant difference between pre- and post-devaluation tests, indicating that administration of AMP promoted habitual behavior. Although the t-test did not detect an effect of devaluation, there was an overall decrease in responding from the pre- to post-test marked by a large effect size ($d = 0.707$, Table 1). This absence of significance, but devaluation-like pattern of responding may be due to the observed high variability of extinction response rates, rather than a small devaluation effect. The t-test was underpowered to detect a true effect. However, Experiment 2 resulted in a strong, significant devaluation effect for animals pretreated with AMP. The overall effect between Experiments 1 and 2 indicated that acute AMP did not facilitate habit expression. While it did not reach significance, the devaluation effect in this group trended toward being greater than that of the control animals, indicating that AMP administration may in fact preserve outcome-based behavior. Although a history of AMP has been shown to produce habitual responding (Nelson and Killcross, 2006, Nelson and Killcross, 2013), this is likely due to procedural differences with acute administration. Chronic administration of AMP may engage different mechanisms than acute AMP.

In both experiments, it was anecdotally observed that AMP animals appeared to be sensitized to its motor stimulating effects by the second extinction test, as these mice showed more locomotor activity upon removal from the operant boxes. Thus, Experiment 3 was designed to address any effects of AMP on motor behavior, and better elucidate the effects of acute AMP on habit expression. However, Experiment 3 yielded uninterpretable findings that made it impossible to elucidate the effect of AMP on habit formation. Initially, it appeared that SAL pretreated animals behaved habitually, therefore making it impossible to determine if AMP exposure accelerated that shift, as this control group displayed S-R behavior. Further examination showed that the non-devalued SAL control animals exhibited decreased responding from the extinction pre-test to post-test. Because these non-devalued animals showed evidence of devaluation, we were unable to interpret how AMP interacted with behavior caused by devaluation.

Contrary to the original hypothesis, acute administration of NIC preserved outcome-based behavior, similarly to SAL pretreated animals. The original hypothesis suggested that nicotine's effects on DA should be sufficient to produce a habit when one is typically not expected. However, throughout this experiment, it was evident that administration of drugs of abuse alone is not sufficient to facilitate habit formation. Deeper research of nicotine's nuanced effects on habit expression, such as a chronic use study, is necessary to truly understand nicotine's effects, if any, on habitual behavior.

Although the devaluation effect in the YOH group was not significant, the pattern of responding was as hypothesized. These animals showed a strong trend toward sensitivity to the devaluation training, indicating that they were still behaving in a goal-directed manner. Previous research using acute administration of yohimbine in a maze

task indicated that it induced reliance on caudate-dependent declarative memory (Packard and Wingard, 2004), which utilizes the DMS (Yin et al., 2004). As previously mentioned, YOH mainly acts on the norepinephrine system, conflicting with the hypothesis that acute increases in DA levels are responsible for accelerated shifts to habitual responding. Therefore, because yohimbine potentiates the use of the DMS and is not known to have any abuse potential, its pattern mirroring the SAL group was predicted. It can be concluded that manipulation of the norepinephrine system should not be a target for habit formation research.

In addition to assessing the effect of drug pretreatment as a within-subjects measure, observing the difference between pre- and post-devaluation responding, a between-subjects analysis was also conducted, quantifying this change in a single "devaluation score" (Figure 13). This method could be considered a stronger type of analysis, as it can measure how different each drug is from the saline control in both a positive and negative direction and it skirts the issue of the within-subjects analysis that seeks to prove the null (if there is no change in responding from pre- to post-devaluation testing). However, there was no main effect of group when the data were analyzed in this fashion and post-hoc paired t-tests to saline could not be performed. This could be due to the between-subjects design that inherently has less power to detect effects.

To ensure these finding are truly a representation of habitual expression, training data were analyzed. There were no group differences in training response rate or intake, so all animals had equal access to the reinforcer during training and could learn the response-outcome association. This is shown by the significant association between lever press responding and banana reinforcer intake in the FR phase, which indicates that this

schedule favors R-O acquisition. Interestingly, although there was a strong trend toward significance in the VI phase, this relationship was found to be significantly weaker. This is likely due to the dissociation of the response behavior with the outcome, as it has been shown that VI strengthens the S-R relationship. These findings provide evidence that the VI phase is likely facilitating the shift toward a habit.

In addition, to ensure that the effects seen were based on drug effects alone analyses of CTA devaluation and REM intake indicated that there were no group differences. Despite differences in post-devaluation responding in the drug pretreatment groups, this appears to be due to acute intoxication effects on the test day and not an inability to learn the CTA. All drug groups showed evidence that this aversion was learned by showing similar time to reach criterion and demonstrating an overall decrease in consumption of the banana solution. In addition, the reminder session successfully helped the mice reacquaint the banana reinforcer to the operant box without acting as a safety signal, as mice that met the 0.2 mL criterion were still able to devalue the following day.

4.2 The Alcohol Effect – Implications on Satiation Devaluation

On the surface, these findings should not be surprising. As mentioned earlier, previous studies in both humans and rodents have shown that a history of alcohol consumption potentiates habitual behavior and this mechanism might be a reason behind addiction (Corbit et al., 2012, Hogarth et al., 2012, Sjoerds et al., 2013). However, upon deeper examination, this study is unique in that the mice did not have a history of ethanol exposure. Instead, these animals received two acute injections and this short exposure

should not be sufficient to induce behavioral changes. In fact, the present findings may create alternative interpretations for researchers using satiation devaluation with ethanol prior to habit testing.

For example, Corbit et al. (2012) found that extended training for alcohol, four and eight weeks, promotes habitual behavior more rapidly than animals with a shorter training period of one to two weeks. Their procedure utilized a random ratio schedule utilized a random ratio schedule, one that has been shown to induce habits at a slower rate than a random interval schedule. In addition, to devalue the reinforcer, ethanol, the authors used satiation devaluation and rats had free access to ethanol prior to the habit expression test. While the authors detected an effect of training in the four and eight week animals, it is in fact possible that the effect does not lie in the training duration, but instead due to the acute alcohol exposure prior to the test. Results from the previous studies indicate that, following habit training, administration of acute alcohol prior to these tests may promote the expression of a habit where one may typically not exist.

Acute ethanol alone cannot be responsible for this shift, as the short training animals still preserved outcome-based behavior. 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, where a subject can behave in either manner, dependent on context and training history. Behavior originates as goal-directed, as only one relationship is learned at this point and that is the relationship between the response and the outcome. Throughout training, the subject is able to learn a new association, S-R, which is strengthened with each training session and at a more rapid pace when a variable interval schedule is used. This begins to decrease the probability of an outcome-based behavior

and make it more likely that the subject will behave habitually. Because of the use of random ratio schedule, it is likely that this slow-forming habit training was not sufficiently long to weaken the R-O relationship. In other words, while there may have been a weak S-R relationship formed in the short training animals, they still remained on the "goal-directed" side of the continuum and even acute ethanol was not sufficient to push them far enough to the "habitual" side. This does not, however, disprove the ability of acute alcohol to promote expression of a habit after relatively brief training.

Because of this, an extended history of ethanol use may not be the only method of facilitating habitual behavior. Instead, perhaps acute intoxication can contribute to an S-R tendency to promote a temporary shift in behavior. Although these data cannot determine if it is possible for acute ethanol alone to induce habit expression, it is likely not the case, as an instrumental response must be learned prior to demonstration of habitual behavior. Due to the devaluation effects observed in the 1 and 2 week training groups from Corbit's study, that there must be another driving force, such as extended variable ratio or variable interval training, to be sufficient to tip the scales toward S-R responding. However, it is evident that acute administration of ethanol increases the probability of the subject behaving in a habitual manner and use of satiation devaluation of ethanol prior to habit testing should be used with extreme caution.

4.3 The Alcohol Effect – Implications on Habit Research

The findings from these studies have a greater impact on habit research than just the issue of satiation devaluation. They further shed light on the actual theory of habit formation and expression and what may contribute to a subject's ultimate behavior.

Initially, the development of a habit was thought to be absolute and marked by a shift of activity from the ventral striatum (nucleus accumbens and VTA) to the dorsal striatum (Belin et al., 2009). These two pathways existed in parallel, with activity being exclusive to one or the other. Behavior originated in the mesolimbic pathway and, over time, shifted to the nigrostriatal pathway, leading to habitual behavior. This shift was absolute following a length of time and dissociation of the response-outcome relationship (either via a VI schedule in a laboratory setting or by no longer reaching the reinforcing drug effects with long-term use). This could also explain relapse behavior, even without craving, because once a subject has made that shift, they continue to favor the nigrostriatal pathway (Robbins and Everitt, 2002).

While parts of this theory may still hold true, that goal-directed behavior is governed by the mesolimbic pathway and habitual by the nigrostriatal, these findings indicate that the switch may be more fluid that originally hypothesized. As previously mentioned, Gremel and Costa (2013) demonstrated that behavior is fluid and dependent on context and other state-dependent effects, one of which could be intoxication. This is further shown by Patton et al. (2016), which indicated that acute alcohol administration can favor the habitual DLS, which under normal conditions has decreased functioning. These experiments support this fluidity, as SAL pretreated animals did not have enough training to become habitual and had not made the absolute switch that Robbins & Everitt had discussed. However, under acute alcohol intoxication, a context that may preferentially increase activity of the nigrostriatal pathway, subjects may be able to behave as if a habit has been acquired.

4.4 The Alcohol Effect – Implications for Addiction

Beyond effects on research, these findings also hold real world implications for understanding addiction. Patients with alcohol use disorders continue to drink in spite of negative consequences, which looks similar to a habitual response: a response in the presence of a stimulus without regard for outcome. This set of experiments serves as a model of detecting the expression of a habit and how acute intoxication of drugs of abuse affect this behavior. The results demonstrate that acute alcohol makes a subject behave habitually, when they otherwise would not. Although these subjects could not be related to patients with substance use disorder, they were acutely intoxicated. DSM-5 describes alcohol intoxication as marked by loss of control, impaired judgment, and increased aggression (American Psychiatric Association, 2013).

This can also be used to 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 devaluing of the intoxication outcome (Keller, 1972). In fact, simple placement back into an alcohol-paired context may be sufficient to facilitate this shift back to habitual behavior (Hogarth et al., 2012). This is also seen in other behaviors, such as smoking (Griffiths et al., 1976), risky sexual behavior (Carroll and Carroll, 1995), and overeating. Lloyd-Richardson et al. (2008) found that college students who consumed moderate levels of alcohol reported increased food consumption while intoxicated, as compared to low- or non-drinking control groups. Acute intoxication causing a temporary shift toward habitual behavior may be a driving force behind impaired judgment while drinking.

4.5 Future Directions & Implications

Based on these findings, one area to investigate is the underlying neural substrates of habit expression. Based on previous research, the dorsomedial striatum (DMS) and nucleus accumbens (NAc) are vital for the acquisition of instrumental behavior and the dorsolateral striatum (DLS) necessary for habitual behavior (Corbit et al., 2001, Yin et al., 2004, Yin et al., 2005a, Yin et al., 2005b). The mesolimbic and mesocortical tracts connect the VTA to the nucleus accumbens and the PFC and OFC, respectively, providing a basis for subjects to make a response to seek out the subsequent positive outcome in a goal-directed manner (Olds and Milner, 1954, Belin-Rauscent et al., 2012). Conversely, habitual responding is rooted in the nigrostriatal dopamine system, which connects the substantia nigra to the dorsal striatum, namely the putamen (DLS) (Knowlton et al., 1996, Faure et al., 2005, Balleine and O'Doherty, 2010). These findings have been replicated in human imaging studies as well (Valentin et al., 2007, Tanaka et al., 2008, Tricomi et al., 2009, Sjoerds et al., 2013), indicating structures implicated in a rodent are translational to humans (McKim et al., 2016).

Within the striatum, approximately 95% of neurons are GABAergic medium spiny neurons (MSNs) that express dopamine D1 and D2 receptors, making dopamine a neurotransmitter of interest, as it affects the functioning of the majority of neurons in this region (Yager et al., 2015). Robbins and Everitt (2002) hypothesized that because drugs of abuse agonize release of dopamine in the striatum, they accelerate the shift toward habitual behavior, via this mechanism. Through a series of microdialysis experiments, it was shown that AMP and NIC increase DA release to a greater extent in the mesolimbic pathway than the nigrostriatal, potentially explaining a preservation of outcome-based

behavior (Di Chiara and Imperato, 1988). Conversely, higher doses of EtOH showed a increase of DA transmission in the nigrostriatal pathway and decreased activity in the nigrostriatal pathway (Imperato and Di Chiara, 1986). These findings, illustrated in Figure 18, could explain the differential drug effects. 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 MSNs acting upon the inhibitory MSNs within this region, thereby disinhibiting the DLS and potentiating action in this "habitual" area. This property of alcohol may be the cause of its acute effects on habitual behavior observed in these experiments. It should be noted that the microdialysis studies were done in Sprague-Dawley rats and the voltage clamp in slices from mice on a C57BL/6J background, so the results may not be generalizable to cHAP mice. Future studies could investigate the potentially unique DA transmission of these selectively bred mice.

If differential dopamine effects are driving the expression of a habit, a deeper investigation of other drugs of abuse may provide more insight into this mechanism. Acute morphine administration elicits equal DA release in the mesolimbic and nigrostriatal pathways and could cause similar results as EtOH. It should be noted that AMP, NIC, and YOH are also all stimulants, which could have an interfering effect on behavior. A future study using morphine could more deeply parse this out, without the added stimulating drug effects.

Beyond a deeper investigation of acute alcohol intoxication on habit expression, future experiments would reexamine the between-subjects design, as it does have promising potential to be useful when sensitization effects may be prominent following repeated AMP administration. However, because the non-devalued SAL animals in

Experiment 3 showed evidence of devaluation when examined as a within-subjects design, this creates problems in interpreting data from this experimental design. A previous experiment utilizing this between-subjects procedure showed no devaluation effects in the non-devalued control animals, as was to be expected. This between-subjects difference could be caused by the injection stress, as the previous experiment used no injections on the test day. However, given these findings, it is important to consider the possibility that differences seen between experimental and control groups are simply the result of drug administration and not an actual behavioral change.

Further, the question of what helps to potentiate a habit can be looked at a different way. This procedure can also be used as a model of inflexible drinking. Lesscher et al. (2010) used the high-drinking C57BL/6J mouse to model a similar behavior. Mice received either zero, two, or eight weeks of home cage alcohol via a two hour limited choice procedure, followed by a quinine test, where the alcohol was adulterated with bitter quinine, which is perceived as aversive. Mice in both alcohol history groups continued to drink the adulterated ethanol, in spite of its aversive taste, whereas alcohol naïve mice avoided the solution in lieu of water consumption. Similar to the procedure in these presented studies, animals had varied "training" durations to determine the length necessary to develop a behavior that is insensitive to outcome value. To test how far these findings extend, pretreating animals with acute injections of a drug of interest prior to a quinine adulteration test could examine the effects of intoxication on outcome-based behavior. Sucrose could be used as an alternative reinforcer, when alcohol is the drug of interest, as to avoid a satiation effect, as quinine adulteration of

sucrose has been shown to reduce preference for this appetitive reinforcer as well (Dess, 2000).

4.6 Limitations & Conclusions

Although these findings are promising and are significant to the habit formation literature, a few issues limit their conclusions. Primarily, there were inconsistencies among the SAL control groups of these experiments. Although there were no statistically significant differences between these groups across experiments, the control group in Experiment 1 did not have a significant devaluation effect and the rest of the groups were not consistent in the magnitude of devaluation, despite identical procedures being utilized. Because these within-subjects experiments require devaluation in the SAL group in order to detect an effect of the drug pretreatment, it is necessary to understand what would cause inconsistencies in these control animals.

One possibility that could explain the variation seen in this series of experiments is the effect of injections on the magnitude of devaluation. As noted in the methods section, pretreatment times for the SAL mice in each experiment were dictated by the pretreatment necessary for the experimental drug groups. This differed by drug and, therefore, experiment, with AMP, NIC, and YOH having no pretreatment delay and the EtOH mice requiring a 10-minute pretreatment time. Within each experiment, SAL animals' pretreatments were matched to the experimental conditions, with no delay in Experiment 1 and 3, and a division of the control group into 0- and 10-minute delay to match the AMP and EtOH groups in Experiment 2. Within Experiment 2, there was no difference in devaluation effect between the differing pretreatments in the SAL group,

but due to the small group sizes, it may have been underpowered. Strikingly, these experiments produced different patterns of devaluation, albeit not significantly different. SAL mice with a 10-minute pretreatment (as seen in the pilot experiment and 2/3 of SAL animals in Experiment 2) showed marked devaluation. However, in Experiment 1 where there was no delay between the pretreatment injection and each test, the devaluation effect was not large enough to be significant. The SAL findings from this experiment did not match those of a previous experiment conducted without test day injections, further implying that this may be cause of different behavior. For the future, a delay following drug pretreatment may be necessary in order to avoid injection effects on instrumental responding.

This immediate injection effect could have contributed to the marked devaluation in the non-devalued SAL animals in Experiment 3. Previously in our lab, a non-devalued control under these parameters successfully showed no change in responding following the non-devalued CTA phase. However, in that experiment, animals did not receive a test day injection, as they did in this experiment. Another explanation for this unexpected behavior could be that EXT1 and EXT2 are not identical, as previously thought. It has been assumed that a change in responding on EXT2 is solely caused by a change in the associative structure of the S-R-O relationship. However, the devaluation-like effect seen in these animals indicates that a change in responding may be attributed to other factors.

Length of training may also play a role in interpreting these findings. Based on previous research in our lab, “medium” training, 1 day of VI20 training and 3 days of VI60, does not produce a habit under normal conditions in cHAP mice, but “long” training (adding two extra VI20 days and 2 VI60 days) is long enough to promote S-R

behavior (O'Tousa & Grahame, personal communication). Therefore, because we wanted to demonstrate accelerated habit formation when it would not typically occur, we adopted this training procedure. However, a recent experiment in our lab using these same mice showed that long training in fact was not long enough to produce a habit and even "x-long" training (1 more VI20 and 5 VI60 days) was still not enough training to elicit S-R behavior in control animals (Millie & Grahame, personal communication). Therefore, while we saw no effect of any drug other than ethanol, it is possible that our training was not long enough, and therefore not sensitive enough, to show effects of the other drugs.

Drug treatment effects on overall operant responding may have also skewed results as well. Pretreatment drugs were administered on both extinction pre- and post-tests to best control for this effect and there were no significant differences among drug treatment groups for pre-test responding in any of the experiments (with the exception of the ethanol pilot experiment). Despite this, there was a pattern of depressed responding in the high-dose ethanol animals. Because the devaluation effect has a relatively small effect size in the SAL control mice, it is possible that even a non-significant decrease in responding could skew the potential for detecting a devaluation effect. However, as previously mentioned, this cannot be attributed to a floor effect. These findings, however, do signify a need to account for this effect when administering these drugs.

In addition, cHAP mice were used in these experiments, which are a line of mice selectively bred for high alcohol preference. The animals in this study did not consume alcohol, but 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, because of this distinctive model, it is possible that the unique effect of alcohol in these experiments might be caused by an interaction of alcohol with a positive family history. In addition, much of the background research these studies were based on was performed in other mouse strains. Future studies should seek to replicate some of these findings, such as the DA microdialysis work, to determine if these mice have any other unique features that may not make them translatable to other rodents or humans.

Taken together, these findings demonstrate that acute administration of ethanol at reinforcing doses 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 amphetamine or nicotine, indicating that this effect is specific to ethanol and not generalizable to all drugs of abuse. This pattern could be explained by the specific effect of acute ethanol on the mesolimbic vs. nigrostriatal dopamine systems, as characterized by Imperato and Di Chiara (1986), which differs from NIC and AMP. Future studies should delve deeper into this field to differentiate the neural changes that underlie the shift to habitual behavior when ethanol 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 ethanol when testing habitual behavior, as this acute intoxication can create a confounding effect.

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TABLES

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Table 1 Effect sizes and power of paired t-tests throughout all studies

Effect size was calculated by subtracting the pooled variance of each experiment from each group's standard deviation. Power was derived from SPSS.

Experiment	Drug Pretreatment	Effect Size	Power
Pilot	Saline	0.643	0.73
	Ethanol (1.5 g/kg)	0.065	0.03
Experiment 1	Saline	0.408	0.24
	Amphetamine (2.0 mg/kg)	0.707	0.22
	Nicotine (0.5 mg/kg)	0.871	0.59
	Yohimbine (1.0 mg/kg)	0.780	0.62
Experiment 2	Saline	0.674	0.42
	Ethanol (1.5 g/kg)	0.668	0.54
	Ethanol (1.0 g/kg)	0.324	0.21
	Amphetamine (2.0 mg/kg)	1.448	0.74
Collapsed Data	Saline	0.545	0.92
	Ethanol (1.5 g/kg)	0.345	0.26
	Ethanol (1.0 g/kg)	0.333	0.21
	Amphetamine (2.0 mg/kg)	1.097	0.72
	Nicotine (0.5 mg/kg)	0.912	0.59
	Yohimbine (1.0 mg/kg)	0.816	0.62

FIGURES

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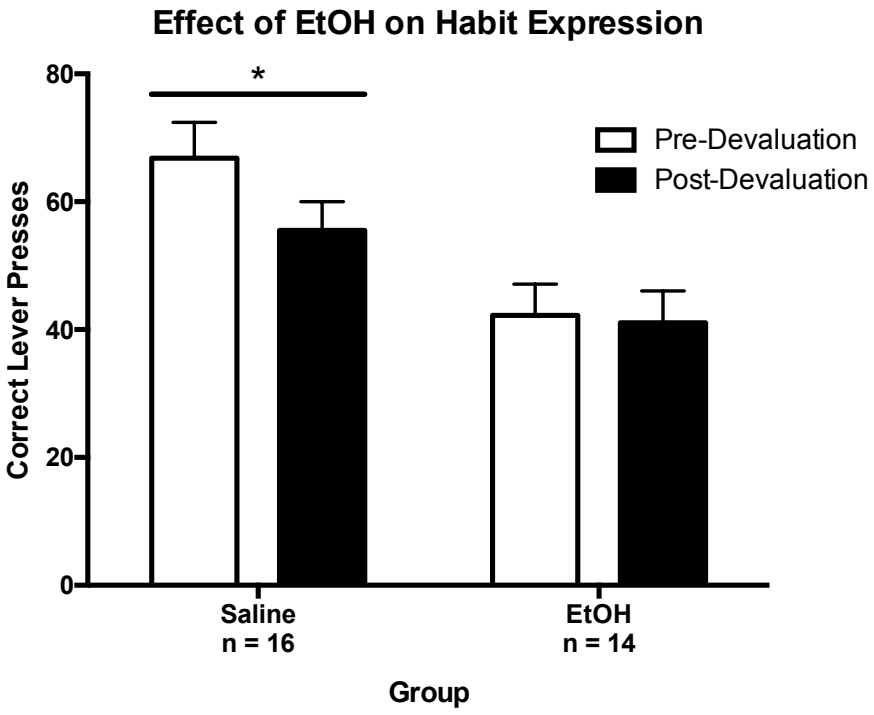


Figure 1: EtOH Pilot Data

Results from the EtOH Pilot Experiment. While SAL pretreated mice showed evidence of significant devaluation, animals that received an EtOH pretreatment showed evidence of habitual behavior. (*p = 0.001)

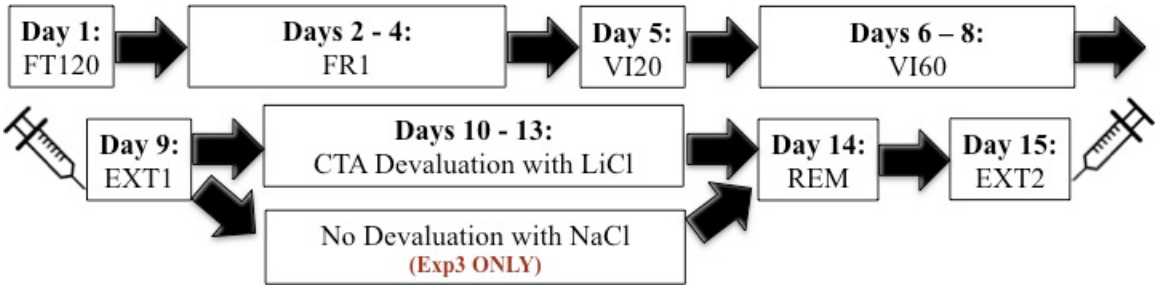


Figure 2: Study Timeline

Timeline of events for Experiments 1 – 3. All experiments began with one day of magazine training, followed by three days on an FR1 schedule. The "habit formation phase" consisted of 1 day of VI20 and 3 days of VI60 training. Baseline responding is assessed during the first extinction test, where animals received their assigned drug pretreatment, except in Experiment 3, where all animals received SAL. Four days of LiCl-induced CTA in the home cage followed until animals stopped consuming banana solution. A one day, ten minute reminder session in the operant box preceded the second extinction test, which was performed to determine the effects of the devaluation training. Animals in all experiments received their assigned drug pretreatment on this day.

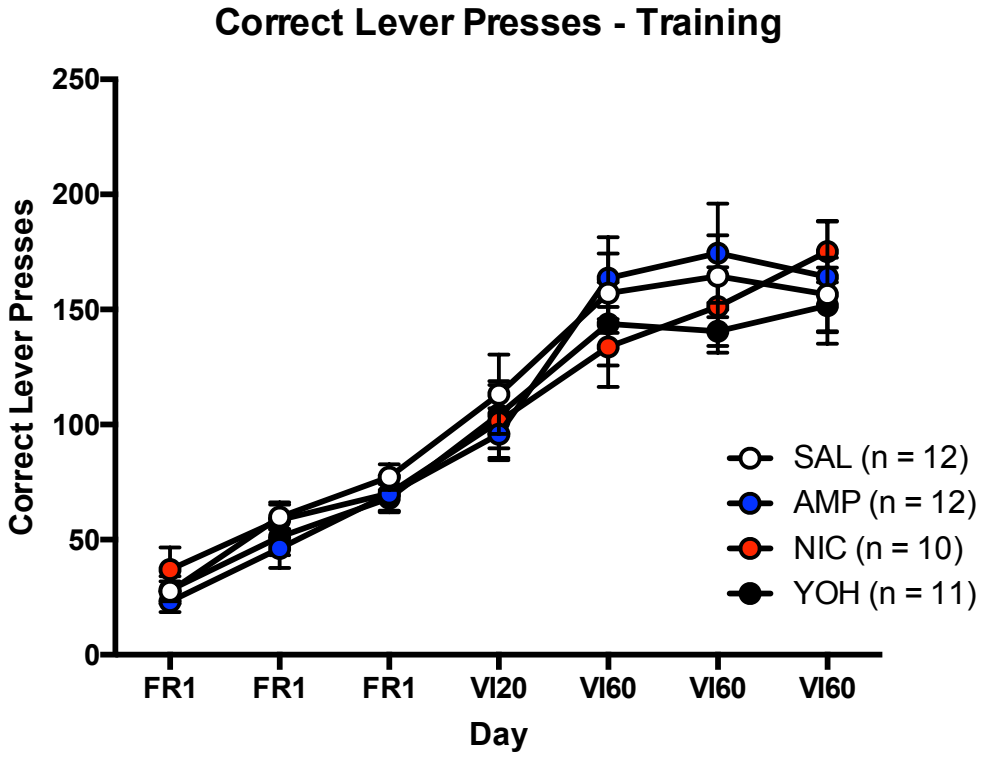


Figure 3: Experiment 1 Training

Correct lever presses during training for Experiment 1. As training progressed, all mice increased responding for the reinforcer. There are no differences in operant responding between drug pretreatment groups. It should be noted that no drugs were administered during these seven sessions.

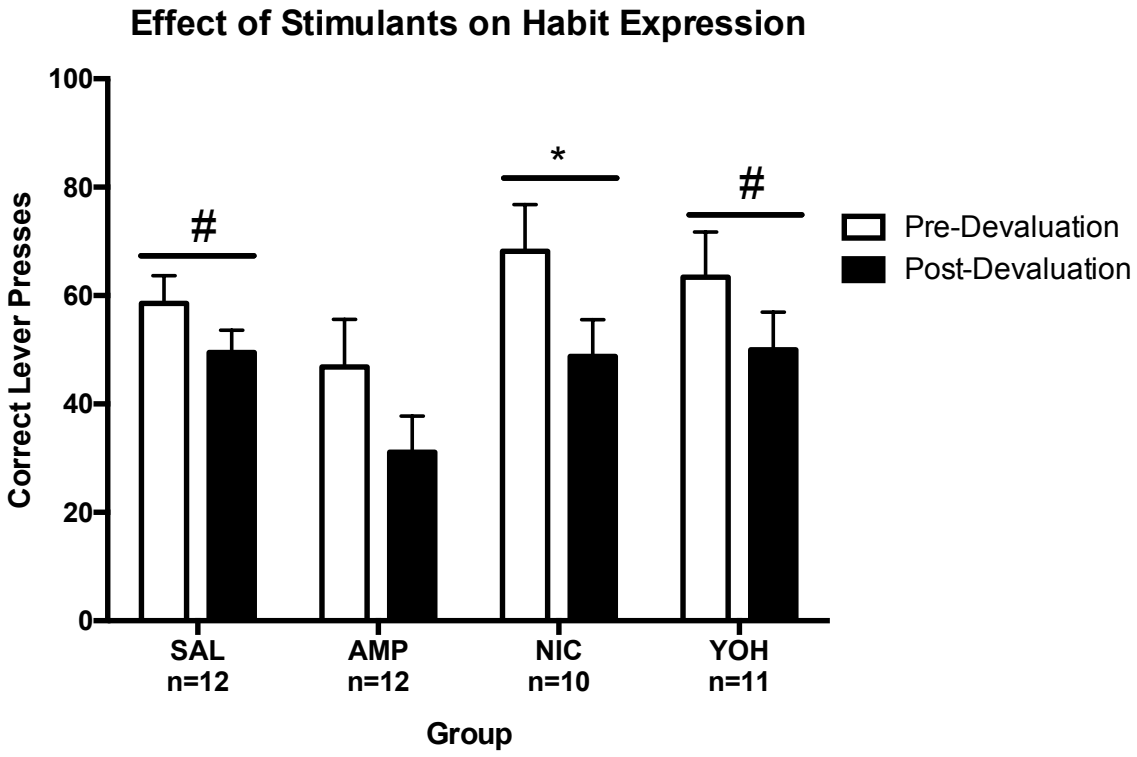


Figure 4: Experiment 1 Results

Results from Experiment 1. NIC pretreated mice were the only group to show significant devaluation. Both SAL and YOH animals showed a strong trend toward devaluation. AMP animals had no significant effect of devaluation on post-devaluation response rates. (#$p < 0.058$; *$p < 0.001$)

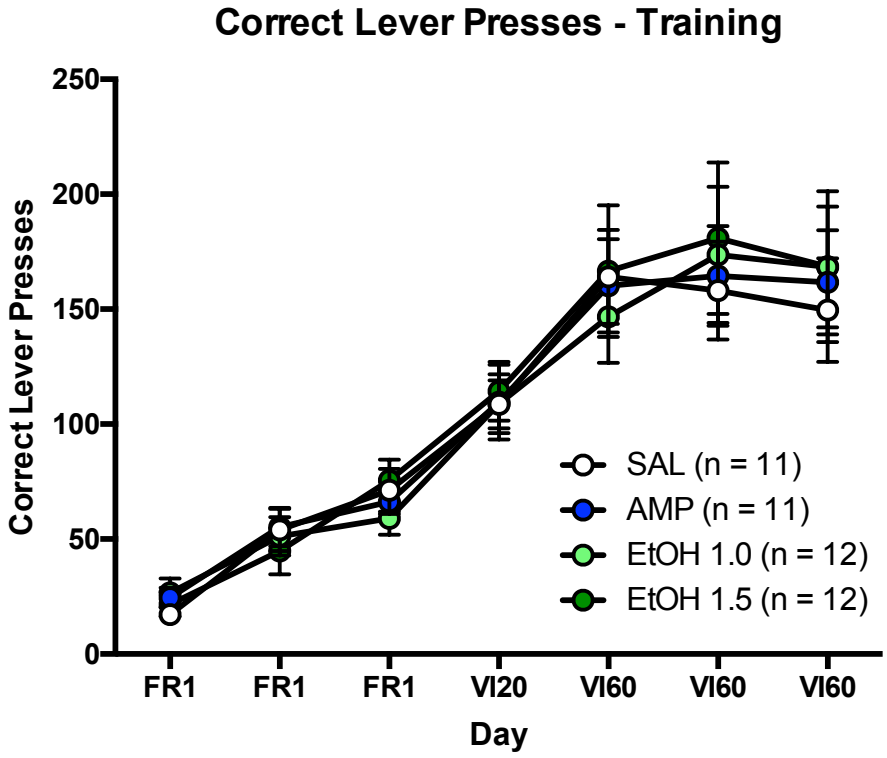


Figure 5: Experiment 2 Training

Correct lever presses during training for Experiment 2. Throughout the seven training sessions, all animals increased response rates and there were no differences between drug treatment groups. Mice had not yet received drug treatments at this point of the experiment.

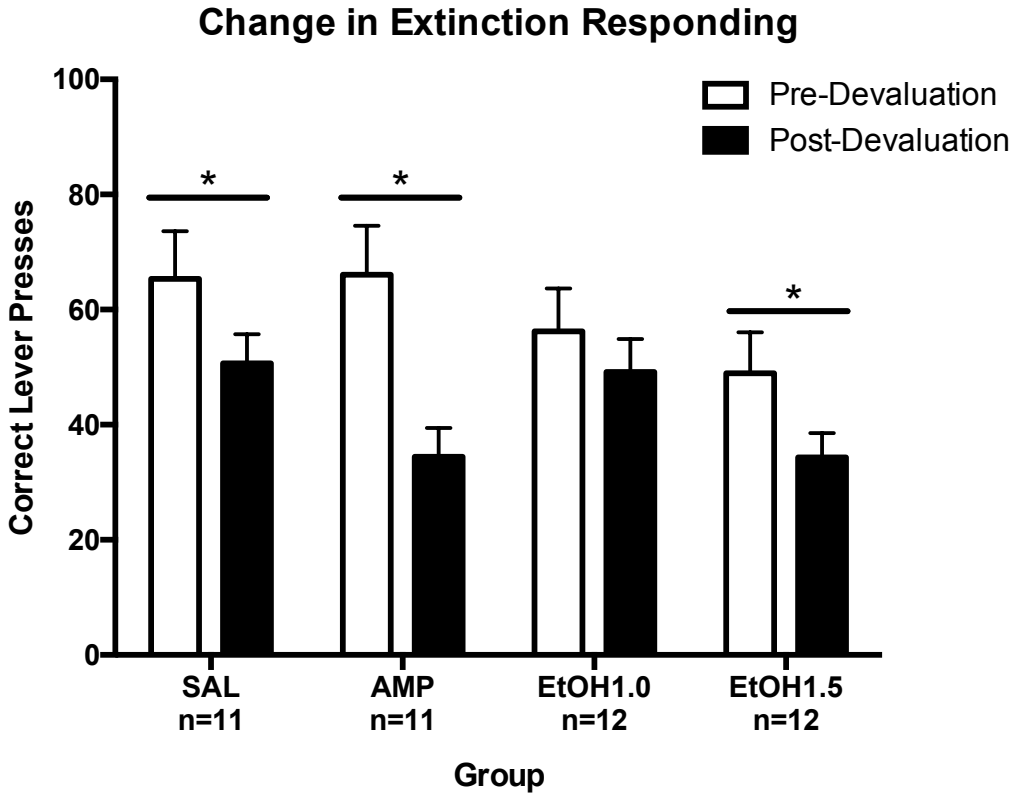


Figure 6: Experiment 2 Results

Results from Experiment 2. Significant devaluation was observed in SAL, AMP, and HiEtOH (1.5 g/kg) mice and LoEtOH (1.0 g/kg) pretreatment showed evidence of habitual behavior. (*$p < 0.05$)

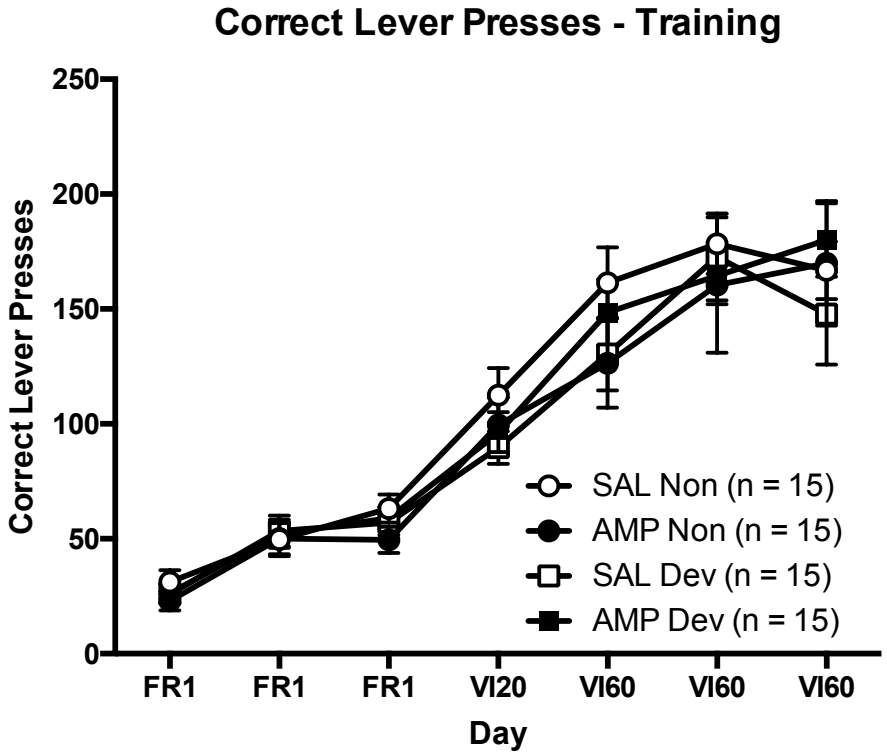


Figure 7: Experiment 3 Training

Correct lever presses during training for Experiment 3. During training, animals increased responding on the correct lever and there were no differences across group assignments. Mice did not receive any injections until the following session, EXT1.

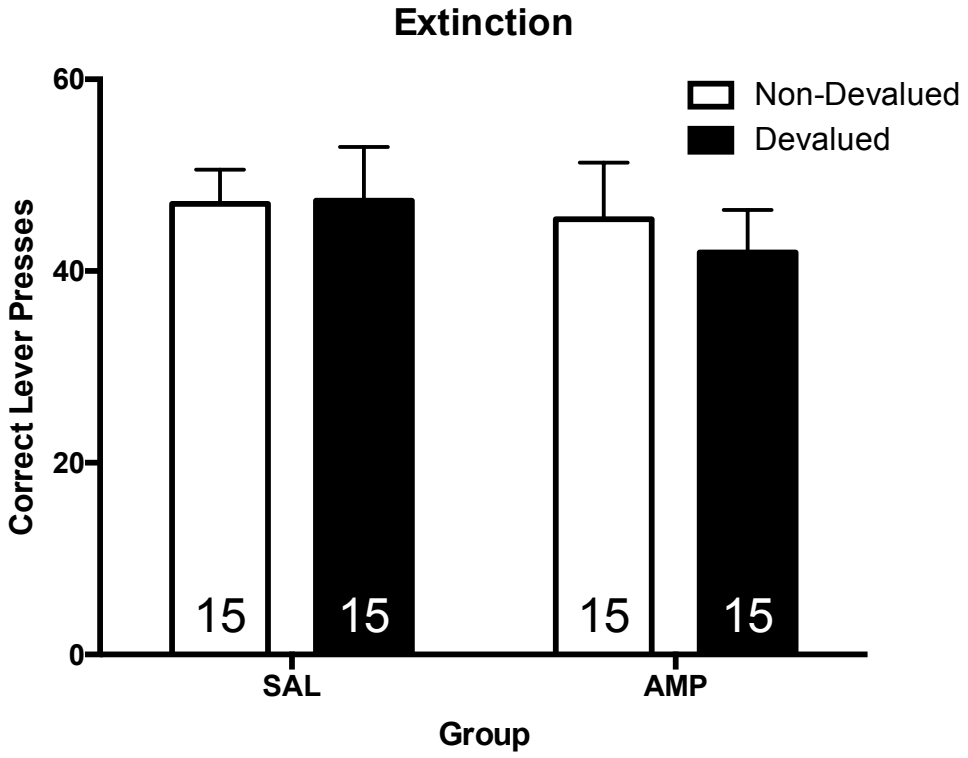


Figure 8: Experiment 3 Results

Results from Experiment 3. Animals that received CTA showed no difference from those in the non-devalued group, indicating a habit in both pretreatment groups.

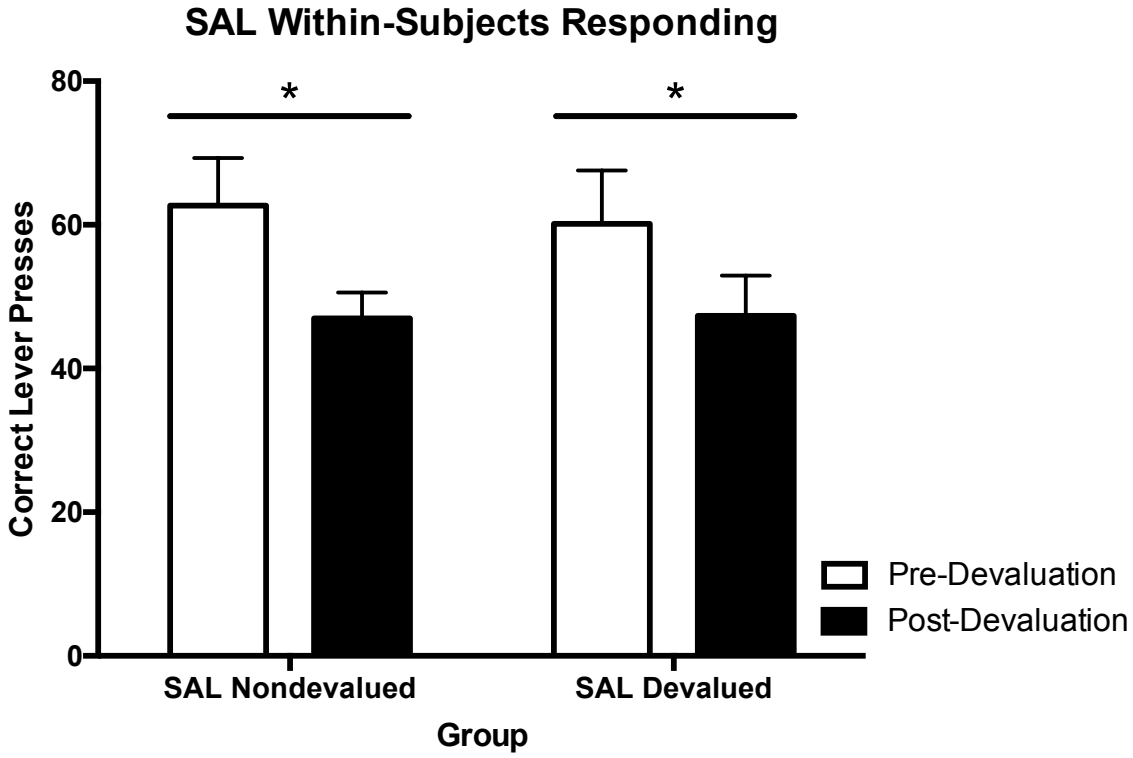


Figure 9: Experiment 3 Saline Mice

Comparison of pre- and post-devaluation tests of SAL animals only. Both devalued and non-devalued mice showed a significant reduction of responding. (*$p < 0.03$)

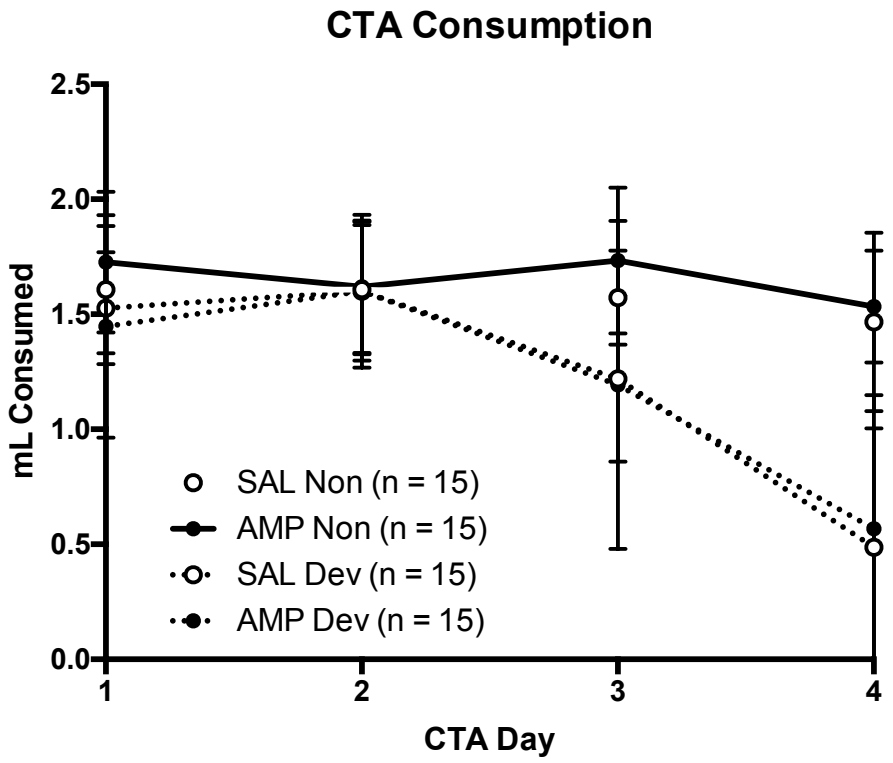


Figure 10: Experiment 3 CTA Intake:

Amount consumed during CTA devaluation in Experiment 3. There was an overall decrease in consumption over the four days of CTA, as well as a group x day interaction, indicating that the non-devalued groups were unaffected by the procedure on days 3 and 4.

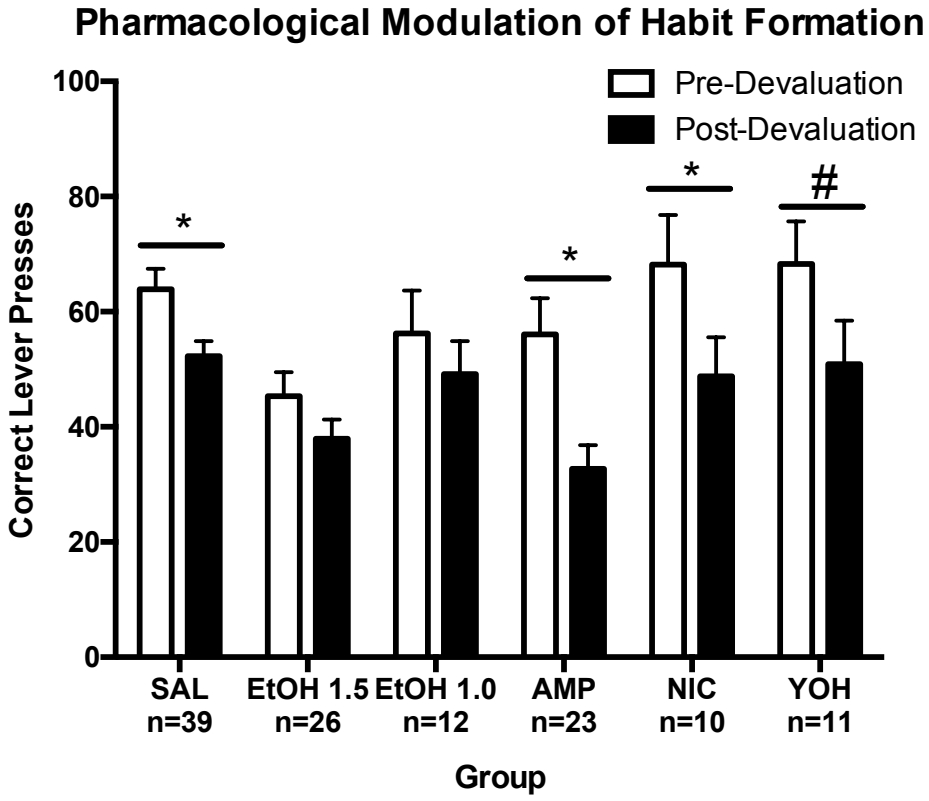


Figure 11: Omnibus Results

Collapsed results from Experiments 1 & 2 and EtOH pilot. Significant devaluation effects were observed in mice pretreated with SAL, AMP, NIC, and YOH. EtOH (1.5 g/kg) and LoEtOH (1.0 g/kg) animals behaved habitually. (*$p < 0.015$, #$p = 0.058$)

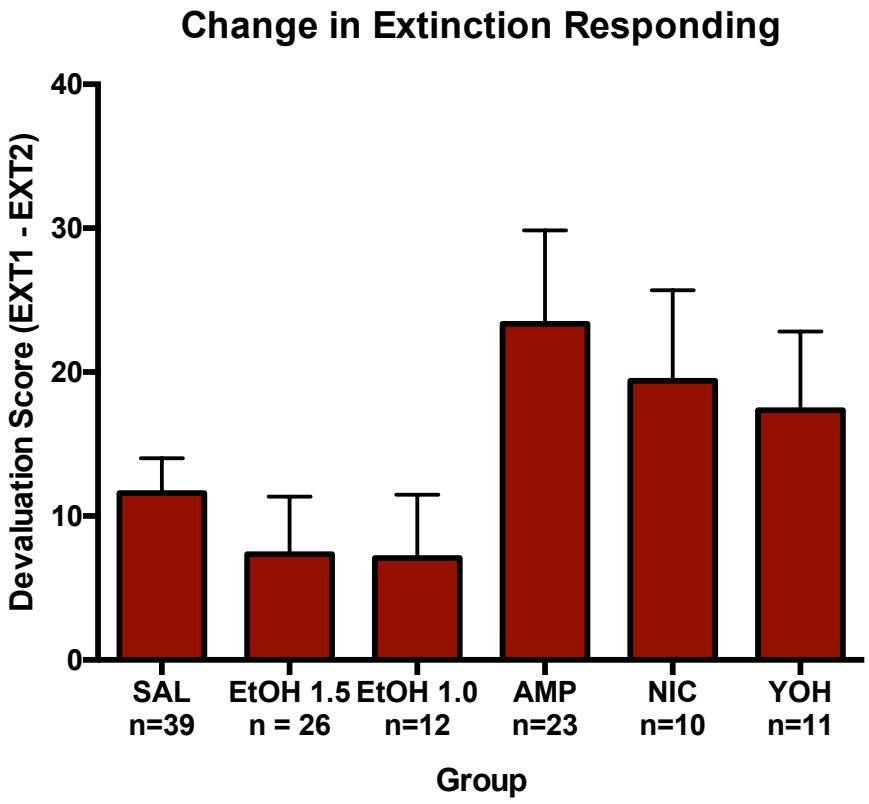


Figure 12: Omnibus Devaluation Score

Devaluation score for individual drug pretreatment group was calculated by subtracting EXT2 responding from EXT1. There was no significant main effect of group.

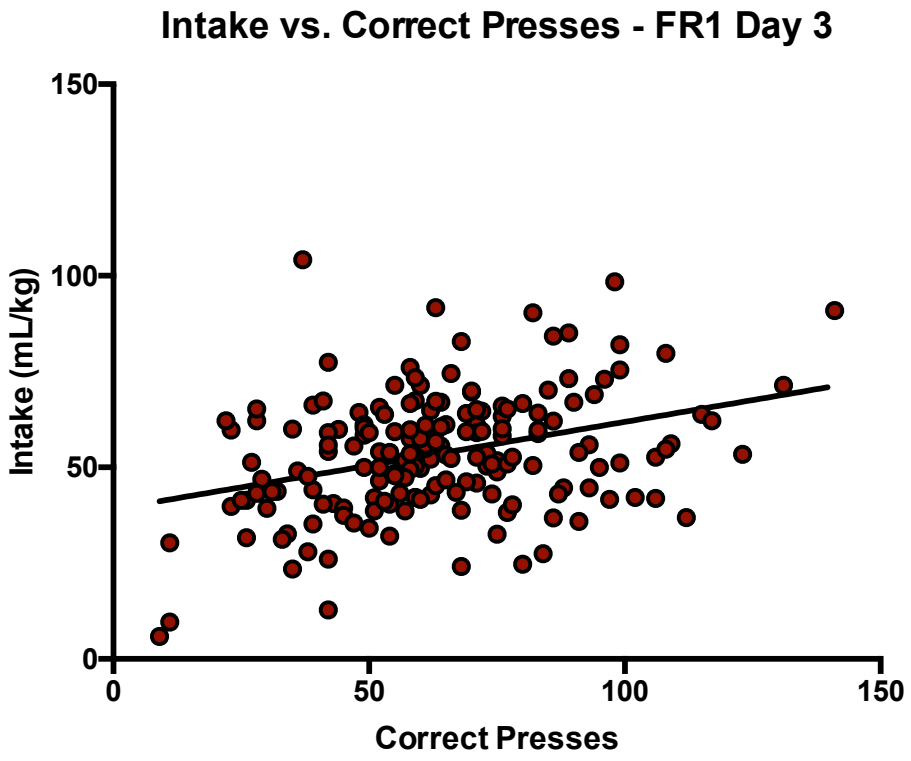


Figure 13: Omnibus Intake vs. FR1 Correct Lever Presses

The relationship between correct lever presses and consumption of banana was analyzed during training across all four experiments on the final day of FR training. There was a strong, positive relationship between correct lever presses and amount of banana consumed.

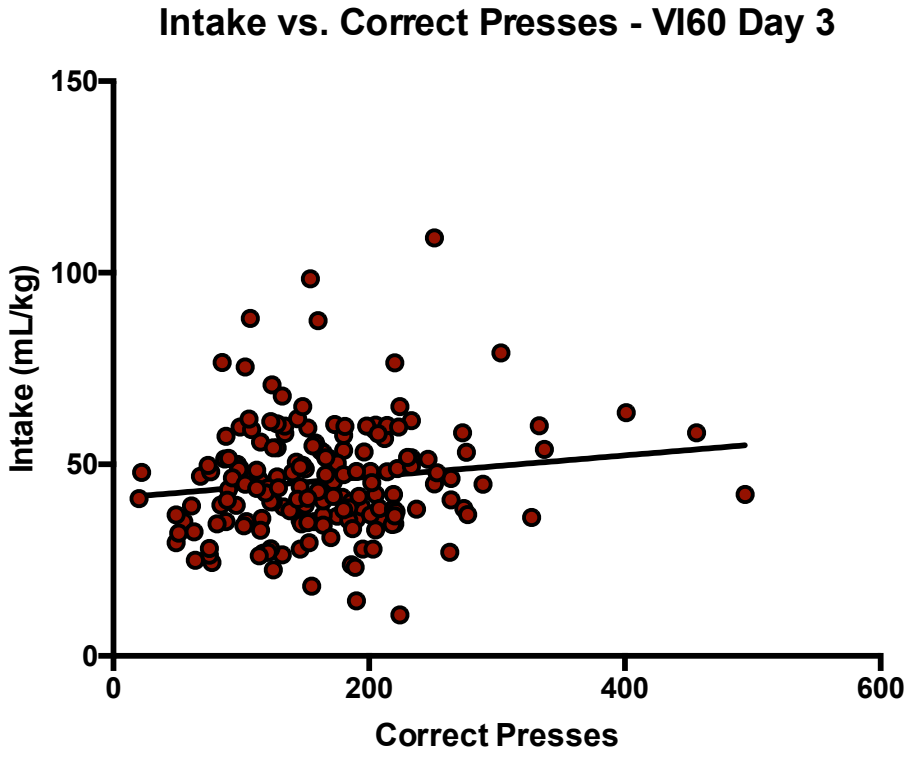


Figure 14: Omnibus Intake vs. VI60 Correct Lever Presses

The relationship between correct lever presses and consumption of banana was analyzed during training across all four experiments on the final day of VI training. There was a positive relationship between correct lever presses and amount of banana consumed.

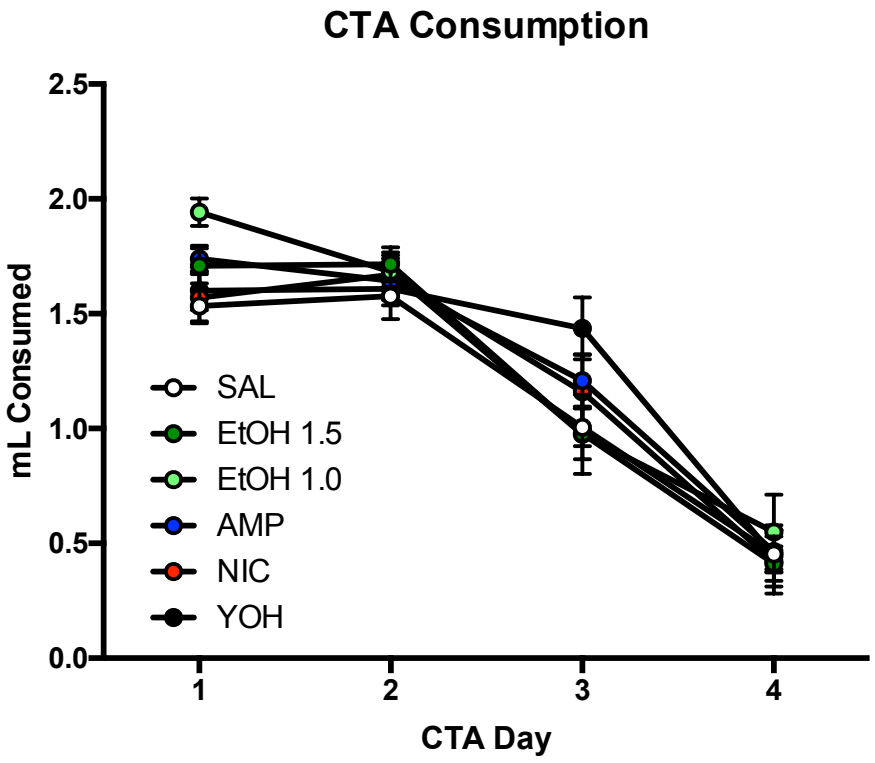


Figure 15: Omnibus CTA Intake

Average banana intake during each day of CTA was calculated within each group. There was a significant effect of day, but no group differences.

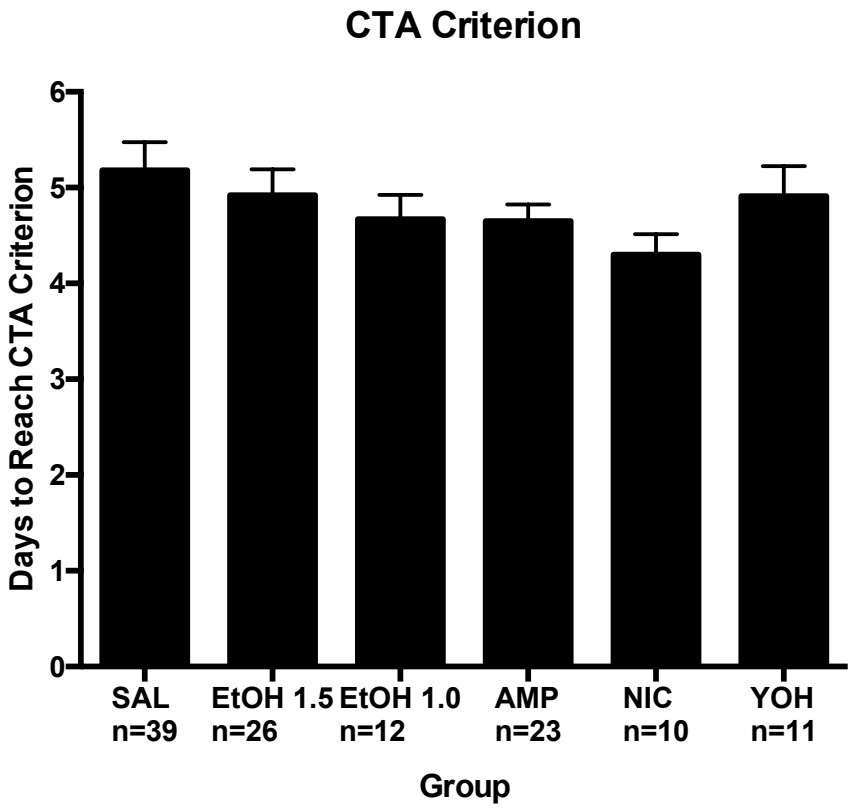
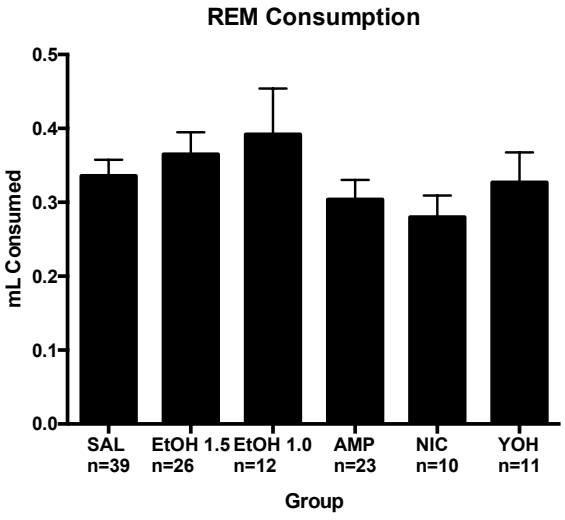
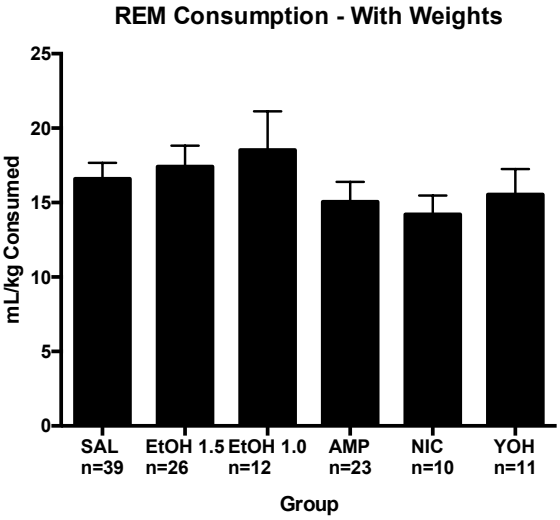


Figure 16: Omnibus Days to Reach CTA Criterion

There were no significant differences in time to reach devaluation criterion (less than 0.5 mL consumed) among the drug pretreatment groups.



A.



B.

Figure 17: Omnibus Reminder Intake

(A) Animals within each drug pretreatment group showed no difference in amount consumed during the reminder day. (B) This lack of significance persisted even when weight was considered. Mice were removed from the study if they did not consume at least 0.2 mL of banana solution.

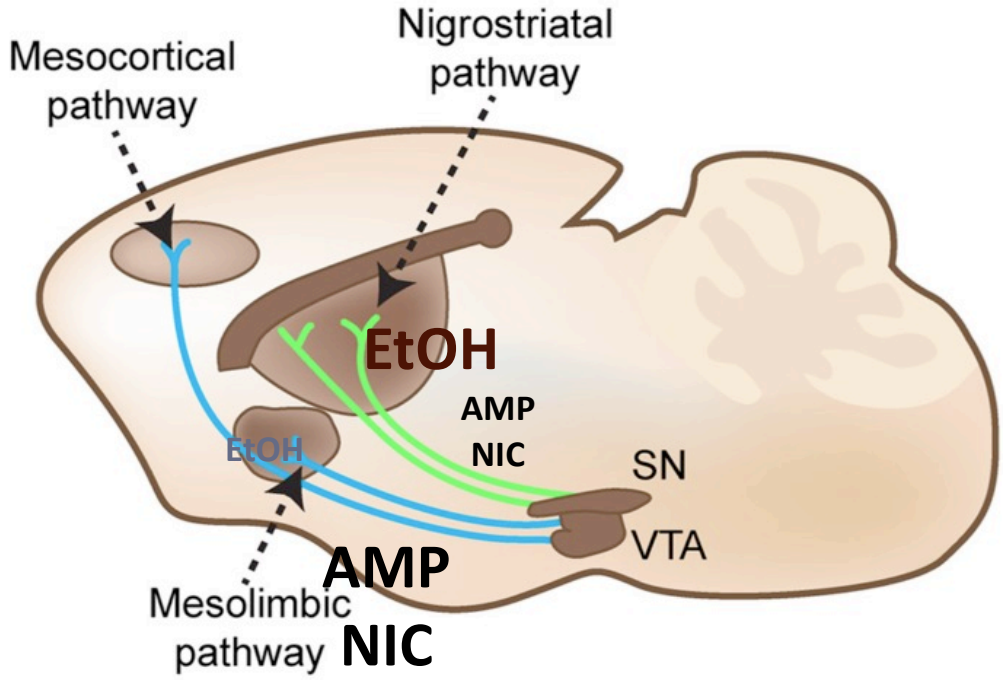


Figure 18: Proposed Dopamine Mechanism

Schematic of differential DA release in the striatum following acute administration of amphetamine, nicotine, and ethanol. Adapted from Money & Stanwood (2013).